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Outdoor scale-up of *Leptolyngbya* sp.: Effect of light intensity and inoculum volume on photoinhibition and -oxidation

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Abstract

The effect of light intensity and inoculum volume on the occurrence of photo-oxidation for *Leptolyngbya* sp. QUCCCM 56 was investigated, to facilitate the transition from small-scale laboratory experiments to large-scale outdoor cultivation. Indoor, the strain was capable of growing at light intensities of up to 5600 µmol photons/m^2/s, at inoculation densities as low as 0.1 g/L (10% inoculation volume vol/vol). Levels of chlorophyll and phycocyanin showed a significant decrease within the first 24 h, indicating some level of photooxidation, however, both were able to recover within 72 h. When cultivated under outdoor conditions in Qatar during summer, with average peak light intensities 1981 ± 41 µmol photons/m^2/s, the strain had difficulties growing. The culture recovered after an initial adaptation period, and clear morphological differences were observed, such as an increase in trichome length, as well as coiling of multiple trichomes in tightly packed strands. It was hypothesized that the morphological changes were induced by UV-radiation as an adaptation mechanism for increased self-shading. Furthermore, the presence of contaminating ciliates could have also affected the outdoor culture. Both UV and contaminants are generally not simulated under laboratory environments, causing a mismatch between indoor optimizations and outdoor realizations.

KEYWORDS
cyanobacteria, light intensity, outdoor cultivation, photoinhibition, phycocyanin

1 | INTRODUCTION

Successful outdoor cultivation of microalgae and cyanobacteria is the first step towards commercialization of microalgae value-chains. The transition from indoor to outdoor is not always straightforward (da Silva & Reis, 2015; Grobbelaar, 2009; Schoepp et al., 2014). Outdoor cultivation conditions are considerably different compared to those which are applied indoor — not only in absolute values but also in diurnal and seasonal fluctuations. In tropical and desert regions, one of the main issues associated with outdoor cultivation is the susceptibility of strains to photoinhibition and photooxidation, caused by high-light intensities (Singh et al., 1995; Tredici, 2010; Vonshak, 1997).

Photoinhibition is a reversible phenomenon during which the photosynthetic capacity of cells is reduced, induced by overexposure

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to visible light and subsequent oversaturation of the cells' photosystems. During photoinhibition, no gross changes in pigment concentrations are generally observed, however, biomass productivities can be reduced significantly (Jensen & Knutsen, 1993; Vonshak & Richmond, 1988). Photoinhibition is mainly a regulatory response, and it is possible for the photosynthetic rate to return to pre-photoinhibition levels almost immediately after reducing the light intensity to non-saturating levels (Tyystjärvi, 2013). Long-term exposure to high irradiances, however, can lead to photooxidation; a reduction in the number of active PSII centers, coupled to the photodestruction of photosynthetic pigments, such as chlorophyll and phycobiliproteins. A simultaneous biosynthetic repair can restore the number of active PSII centers, but if repair mechanisms are not able to keep up with the level of photo-oxidative stress, ultimately, cell-death will occur (Powles, 1984). Sub-optimal cultivation conditions, such as low temperatures, can cause a reduction in the rate of biosynthetic repair, which is why the onset of photooxidation has also been found to be temperature-dependent (Roos & Vincent, 1998; Suggett et al., 2010; Vonshak, 1997).

The sensitivity of cells to high irradiances is strain-dependent, with certain strains being more susceptible to photoinhibition and photo-oxidation than others (Eloff et al., 1976). The sensitivity can also be wave-length dependent, whereas ultraviolet radiation (UVR) can be the most inhibitory region of the spectrum (Castenholz & Garcia-Pichel, 2000). Selection of strains, capable of withstanding high irradiances with limited photoinhibition and oxidation, is key for successful outdoor cultivation with high productivities (Tredici, 2010). Furthermore, strategies can be applied which reduce the impact of photoinhibition, such as utilizing the self-shading effect of dense algal cultures to (partially) protect against photooxidative effects, or shading outdoor cultures to reduce the received irradiance (Borowitzka & Vonshak, 2017; Vonshak & Guy, 1992). Both strategies are especially important at the time of inoculation when biomass densities are lowest. Regardless, strains' susceptibilities to photoinhibition not only impact productivities but also pose limitations for commercial-scale production and facility design.

It is also possible for cells to acclimatize to higher irradiances, known as photoadaptation, which decreases the effect of photoinhibition and photo-oxidation on the growth and survivability of the strain (Vonshak et al., 1996). In the case of UVR, some cyanobacteria are capable of producing UV-protective compounds, such as scytonemin and mycosporine-like compounds, that partially or completely avoid the damage caused by UVR. Nonetheless, such photoadaptation does result in changes both to physiology and biochemicals composition, such as the reduction of pigments associated with the light-harvesting complexes (chlorophyll and phycobiliproteins), and an increase in photoprotective pigments (Grobbelaar, 2007; Han, 2002; Kirilovsky, 2007; Pathak et al., 2019).

Through the isolation and characterization of novel strains isolated from high-irradiance environments, strains can be selected, which exhibit limited photoinhibition. This would, in turn, improve the photosynthetic efficiency of the cultivation process, allowing for maximizing outdoor biomass productivities through deployment in areas with the highest production potential, such as the Middle East and North Africa region (Tredici, 2010). Qatar, a peninsula located in the Arabian Gulf, is one such location, and a number of high-potential strains have been isolated from the region (Das et al., 2019, Saadaoui et al., 2016, Schipper et al., 2019). One strain, in particular, has already been investigated for its potential to produce phycocyanin-rich biomass under simulated desert climate conditions, and only limited photoinhibition was observed at high light intensities of 1800 μmol photons/m^2/s (Schipper et al., 2020). This study, therefore, focuses on the scale-up and outdoor cultivation of *Leptolyngbya* sp. QUCCCM 56, and investigation of the effect of inoculum volume, light intensity, and temperature on the occurrence of photoinhibition and -oxidation.

2 | MATERIALS AND METHODS

2.1 | Strains, media, and basic culture conditions

Leptolyngbya sp. QUCCCM 56 was obtained from the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM). Stock-cultures were maintained in 250 ml conical flasks with a working volume of 100 ml and incubated in an illuminated Innova 44 Shaker Incubator (New Brunswick Scientific) at 150 RPM, 30°C, 70 μmol photons/m^2/s and 12:12 h light:dark cycle. Media for all indoor experiments and inoculum preparation was prepared using locally sourced seawater, with a salinity of 40.0 ppt, filtered (VWR 0.45 μm PES), autoclaved and supplemented with: $NaNO_3$, 4.71 mM; KH_2PO_4, 0.23 mM; $NaHCO_3$, 4.8 mM; Na_2EDTA, 2.56×10^{-2} mM; $FeSO_4 \cdot 7H_2O$, 1.44×10^{-3} mM; $MnCl_2 \cdot 4H_2O$, 1.41×10^{-4} mM; $ZnSO_4 \cdot 7H_2O$, 3.06×10^{-5} mM; $Co(NO_3)_2 \cdot 6H_2O$, 3.21×10^{-6} mM; $CuSO_4 \cdot 5H_2O$, 1.28×10^{-6} mM; and $Na_2MoO_4 \cdot 2H_2O$, 1.33×10^{-5} mM.

2.2 | Indoor cultivation conditions

The inoculum was cultivated in 2.0 L Duran bottles sparged with air, at a light intensity of 200 μmol photons/m^2/s (block, 12:12 h light:dark), with a total illuminated surface area of 163.2 cm^2 with a 13.6 cm light path, and a temperature of 28.0 ± 2.0°C was maintained. When biomass densities of 1.0 g/L were reached, the biomass was used to inoculate a series of conical benchtop photobioreactors (PBR101; Phenometrics Inc.), with an operating volume of 500 ml. The culture temperature was controlled using a temperature control jacket surrounding the reactor, and illumination was provided by a high-power cool-white LED, and the culture light path was 20 cm to simulate similar light curves as compared to the outdoor raceway ponds (Lucker et al., 2014). The total illuminated surface area was 29 cm^2. The culture was sparged with air, and pH (noncontrolled) ranged between 8.4 ± 0.2 and 9.6 ± 0.4 for night and day, respectively. The reactors were inoculated with various inoculum volumes (10%, 20%, and 50% vol/vol, equivalent to initial biomass concentrations of 0.1, 0.2, and 0.5 g/L, respectively). Peak incident light intensities investigated were 2800, 4200, or 5600 μmol photons/m^2/s (sinusoidal, 12:12 h light:dark), and temperatures of 20, 25, or 30°C (constant) or a sinusoidal night:day cycle ranging from 24°C to 31°C, as is

common during summer cultivation conditions in Qatar (Pruvost et al., 2019). Inoculation occurred at the same time for all conditions (10:00 a.m.), one hour before the peak light intensity. All experiments were performed in duplicate ($n = 2$). The theoretical light intensity at any given depth of the reactor at the time of inoculation for the different conditions was calculated using the Lambert-Beer Law as per Equation 1 (Blanken et al., 2016).

$$I_z = I_0 \bullet e^{(-a_X \bullet C_X \bullet z)} \quad (1)$$

In which I_0 and I_z are the light intensities (μmol photons/m^2/s) at depth 0 and z (m) of the reactor, a_x is the the wavelength dependent dryweight specific absorption coefficient measured for low-light acclimatized cells of *Leptolyngbya* sp. QUCCCM 56, using the method described by Vejrazka et al. (2011) in m^2/kg, and C_x is the biomass concentration in g/L.

2.3 | Outdoor cultivation conditions

Inoculum for outdoor trials was cultivated indoor in 10 L photobioreactors (24 cm diameter), sparged with air. Illumination was provided by white fluorescent lighting at 350 μmol photons/m^2/s (block, 12:12 h light:dark), and cultures were maintained at 25°C. Upon reaching a density of 1.0 g_X/L, the culture was used to inoculate outdoor 200 L raceway tanks, with a surface area of 1.0 m^2, containing locally sourced seawater supplemented with $NaNO_3$, 3.44 mM; KH_2PO_4, 0.16 mM; and $NaHCO_3$, 4.8 mM, in addition to the trace minerals identical to the ones added in the indoor experiments. The $NaNO_3$ concentration was selected to be able to support a biomass concentration of 0.5 g_X/L, based on assuming a nitrogen content of 9.6% in the biomass, as was found by Schipper et al. (2019). Inoculation occurred at 4:00 p.m. (afternoon), 2 h before sunset. The water level was maintained at 20 cm using freshwater to maintain the salinity at 40 ppt. Agitation was achieved through a four-blade paddle system rotating at 32 rpm, resulting in an average linear liquid velocity of 26.2 cm/s (Das et al., 2019). Inoculation volumes of 10% and 20% (vol/vol) were tested, and all experiments were performed in duplicate ($n = 2$). Outdoor light intensities were monitored during the experiments, as well as over the course of 2019, using a PAR quantum flux meter located on-site (25°48'06.4"N 51°21'06.4"E). Water temperature and light intensity in the culture at the bottom of the open pond were monitored with a submerged and weighted HOBO Pendant® MX Temperature/Light Data Logger (MX2202; Onset Computer Corporation). Once the stationary phase was reached (after 8 days of cultivation), the cultures were supplemented with additional nutrients at identical concentrations as at inoculation.

2.4 | Biomass analysis

Biomass densities were monitored through optical density measurements at 680 and 750 nm, as well as through gravimetric analysis (Zhu & Lee, 1997). The growth rate was determined over the initial 72 h of cultivation, using Equation 2.

$$\mu = \frac{\ln\left(C_{X,t}/C_{X,0}\right)}{t - t_0}. \quad (2)$$

In which μ is the growth rate (d^{-1}), and $C_{X,t}$ and $C_{X,0}$ are the biomass densities (g/L) at times t and t_0 (d), respectively. For both the indoor and outdoor experiments, phycocyanin and chlorophyll contents in the biomass were monitored. The chlorophyll content was determined through methanol extraction, followed by spectrophotometric analysis; samples were centrifuged for 8 min at 4500 rpm, after which the pellet was resuspended in 100% methanol. The methanol suspension was incubated in an ultrasound bath for 5 min, followed by subsequent incubations at 60°C and 0°C for 50 and 15 min, respectively. After centrifugation (8 min, 4500 rpm), the pellet was discarded and the absorbance of the solution was measured at 652 and 665 nm using a quartz cuvette and a DR3900 VIS-Spectrophotometer (Hach-Lange). Arnon's equation was used to determine the chlorophyll concentration as per Lichtenthaler (1987), see Equations 3, 4, and 5 for chlorophyll *a*, *b*, and total chlorophyll, respectively:

$$C_{Chl_a} = \left(16.72 \times Abs_{665} - 9.16 \times Abs_{652}\right) \times \frac{V_{\text{buffer}}}{V_{\text{sample}}}, \quad (3)$$

$$C_{Chl_b} = \left(34.09 \times Abs_{652} - 15.28 \times Abs_{665}\right) \times \frac{V_{\text{buffer}}}{V_{\text{sample}}}, \quad (4)$$

$$C_{Chl_{tot}} = Chl_a + Chl_b. \quad (5)$$

In which C_{Chl_a}, C_{Chl_b}, and $C_{Chl_{tot}}$ are the concentrations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll, respectively, in mg_{Chl}/L. Abs_{665} and Abs_{652} are the absorptions measured at 665 and 652 nm, respectively, V_{buffer} and V_{sample} are the methanol and sample volumes, respectively. Chlorophyll content (X_{Chl}, mg_{Chl}/g_X) was determined by dividing the chlorophyll concentration with the biomass concentration (C_x, g_X/L). Phycocyanin extraction was performed using phosphate buffer and bead beating, followed by 24 h incubation at 4°C, and spectrophotometric analysis, according to the method described by Schipper et al., 2020.

2.5 | Statistical analysis

The reported values are the means of individual samples, whilst the error bars represent the range. One-way analysis of varaince was used to determine whether the different light, temperature, and inoculum volumes significantly influenced the growth rate and/or chlorophyll and phycocyanin content. Variable effects were deemed significant if $p < 0.05$, in which case, post hoc Tukey HSD analysis was used to perform multiple comparisons between the individual means. All statistical analyses were performed using SPSS 26 (SPSS).

3 | RESULTS & DISCUSSION

3.1 | Initial outdoor scale-up cultivation trials

Leptolygnbya sp. QUCCCM 56, a cyanobacteria isolated from the Qatar desert (Schipper et al., 2019) was previously studied under a

temperature range of 20–45°C and high light intensities of up to 1800 μmol photons/m^2/s, respectively (Schipper et al., 2020). Due to its promising maximum growth rate (1.09 ± 0.03 g_X/L^1/d at 40°C), and potential for production of high-purity phycocyanin, the potential for scale-up under outdoor conditions was investigated. Multiple outdoor trials, located in Qatar, in 200 L raceway tanks were initiated over the course of 2016–2018, during multiple seasons (October 2016, April 2018, and September 2018). Regardless of the season, within 48–72 h of inoculation, bleaching and subsequent culture crash occurred on all occasions, suspected to be related to photooxidation (Figure S1). Average peak light-intensities during these months, as calculated from onsite measurements, were 1812 ± 111, 2278 ± 236, 1757 ± 98 μmol photons/m^2/s for October, April and September, respectively. Peaks of up to 2871 μmol photons/m^2/s were found to occur (Figure 1). Overall, the highest average peak light intensities observed were around 2250 μmol photons/m^2/s for 4 months of the year, with lowest averages of approx. 1500 μmol photons/m^2/s for the winter months.

The occurrence of suspected photooxidation during the outdoor trials immediately after inoculation would suggest that the strain is light-sensitive, and the transition from a low-light/high-density inoculum to a high-light/low-density culture caused photooxidative cell death. Similar results have been found for certain strains of *Arthrospira*, which is why it is recommended to scale up with a factor 5 (20% inoculum volume) rather than the factor 10 (10% inoculum volume) industry standard (Borowitzka & Vonshak, 2017; Singh et al., 1995; Vonshak, 1997). Nevertheless, the occurrence of photooxidation during the outdoor trials was unexpected. In previous work, the strain was shown to be able to grow under high incident light intensities of up to 1800 μmol photons/m^2/s in flat panel photobioreactors (14 mm light path) (Schipper et al., 2020). It should be noted that the referenced indoor experiments were operated in turbidostat mode, during which the light intensity was gradually increased over time, and biomass concentrations were kept constant. This could have led to a gradual acclimatization of the strain to the higher light intensities, as well as maintained lower light:biomass ratios as compared to outdoor cultures immediately after inoculation. Therefore, it is hypothesized that it is not only the strain's ability to grow under high irradiance levels, but the light:-biomass ratio at the moment of inoculation, which are crucial for outdoor scale-up of the culture.

3.2 | The effect of light intensity, inoculum volume, and temperature in simulated laboratory environments

To further study the occurrence of photooxidation, and to propose methods to mitigate its onset during outdoor scale-up, *Leptolyngbya* QUCCCM 56 was cultivated indoor using inoculum volumes ranging from 10% to 50% (vol/vol), high light intensities, and various temperatures. The aim was to simulate the outdoor cultivation conditions as much as possible. The growth rate of the strain under the different conditions is shown in Figure 2a.

Contrary to the preliminary outdoor experiments, *Leptolyngbya* sp. grew well under all conditions tested. Even at the highest light intensities and lowest inoculum volumes, growth rates were positive, and no visible photooxidation was observed. Over the initial 48 h after inoculation, the maximum growth rate of 0.519 ± 0.015/day was obtained for the cultures inoculated at the lowest biomass concentration (10% inoculum volume, equivalent to 0.11 ± 0.02 g_X/L), and a light intensity of 2800 μmol photons/m^2/s at 30°C. At higher light intensities (4200 and 5600 μmol photons/m^2/s) and similar inoculum volumes (10%), the growth rates were as much as 35% lower compared to 2800 μmol photons/m^2/s, despite the increased light availability. This slight reduction in growth rate would suggest that photoinhibition occurred to some degree, although not sufficient to cause a culture crash. This, as well as the decrease in growth rate with decreasing temperatures, is concurrent with previous results (Schipper et al., 2020).

Besides temperature, increasing inoculum volumes also caused a decrease in growth rates within the first 48 h. This is easily understood by analyzing the overall light availability over the reactor depth, which decreases with increasing biomass densities, as is

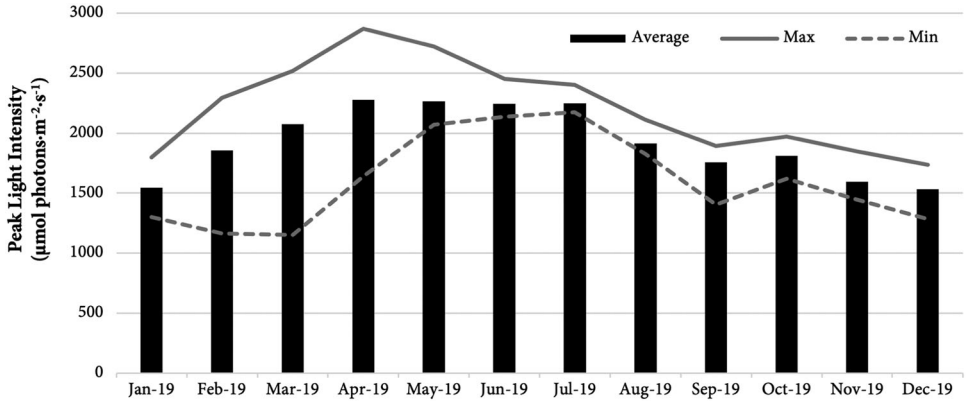

FIGURE 1 Monthly average, absolute maximum, and absolute minimum peak light intensity (μmol photons/m^2/s) as recorded in Qatar (25°48'06.4"N 51°21'06.4"E) over the course of 2019

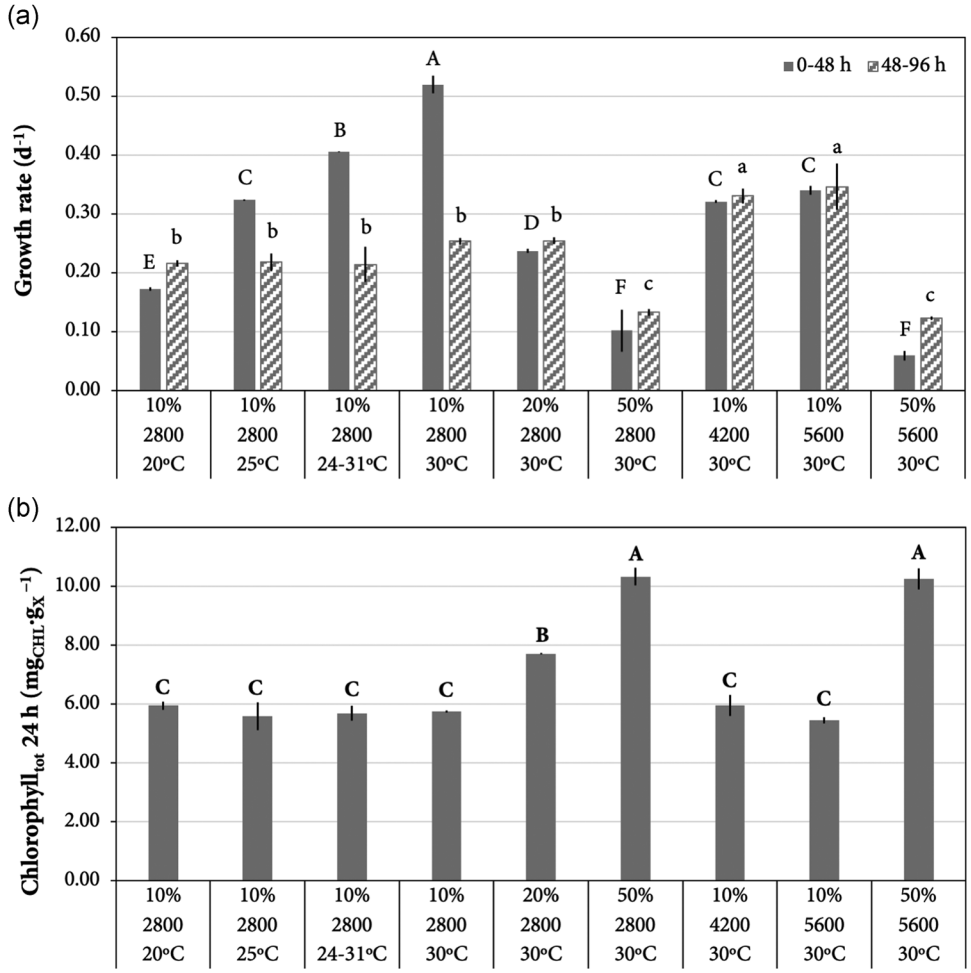

FIGURE 2 (a) Growth rate, μ (h^{-1}) calculated over initial 0–48 h and 48–96 h of cultivation; and (b) Chlorophyll content (mg_{CHL}/g_X) after 24 h of cultivation, of indoor cultivated *Leptolyngbya* sp. QUCCCM 56 under different inoculum volumes (10%, 20%, and 50% vol/vol), light intensities (2800, 4200, and 5600 μmol photons/m^2/s) and temperatures (20, 25, and 30°C and 24–31°C night:day cycle). Values are mean ± range, $n = 2$, capital and small letters indicating significant differences ($p < .05$) between the individual means for 0–48 h and 48–96 h, respectively

shown in Figure 3. The theoretical light penetration in the reactors inoculated with low inoculum volumes (10%) was 100% at the time of inoculation, with light available over the entire reactor depth. This would indicate that growth was not light limited at the time of inoculation, and maximum growth rates could occur provided no other factors were limiting or inhibiting. For 20% and 50% inoculum volumes with 2800 μmol photons/m^2/s, and 50% inoculum volume with 5600 μmol photons/m^2/s, light penetration was 89%, 32%, and 42% of the reactor depths, respectively. This indicates that light could be limited, which is suspected to be the reason for the lower growth rates at higher inoculum volumes, and not necessarily photoinhibition.

As the growth progressed (48–96 h), no significant differences were found anymore between the growthrates for the different temperatures and 10% and 20% inoculum volumes. Furthermore, the growth rates for 25, 24–31, and 30°C at 10% inoculum volume and 2800 μmol photons/m^2/s were lower as compared to those found over the initial 48 h, suggesting that growth during this time was limited by factors not at play within the initial 48 h of cultivation. The growth rates under the higher light intensities and low inoculum densities, however, showed no significant difference with those recorded over the initial 48 h of cultivation. This suggests that light becomes a limiting factor, rather than a stressor, in the 2800 μmol photons/m^2/s and 10% inoculum volume culture after the initial 48 h, when biomass concentrations increase. Only for the higher light intensity cultures (4200 and 5600 μmol photons/m^2/s), the similar growth rates in all phases of the culture, suggested that light is not (yet) limiting within 96 h of inoculation.

3.3 | Pigments as indicators of photoinhibition and photo-oxidation

Even though no visible photooxidation occurred, at inoculum volumes of 10% and 20%, the chlorophyll content in the biomass decreased significantly within the first 24 h of cultivation, returning

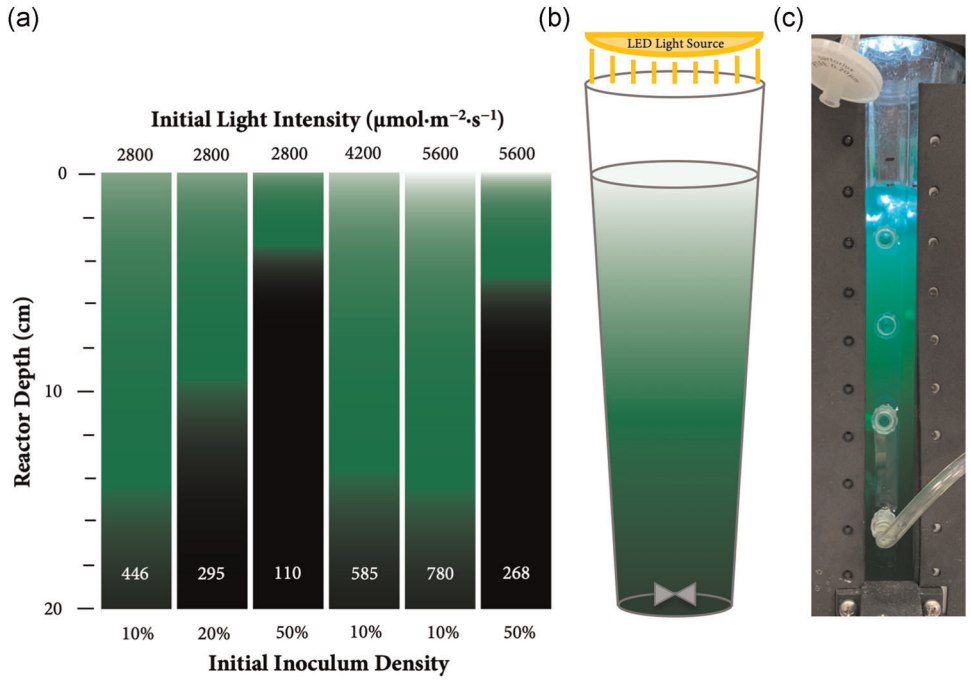

FIGURE 3 (a) Illustration of theoretical light intensities calcualted over the reactor depth (cm) using Equation 1, for the various inoculum volumes and light intensities tested indoor, (b) schematic illustration of photobioreactor configuration, (c) actual light gradient in the reactor for 2800 µmol photons/m^2/s and 20% inoculum volume. In schematic illustrations (a and b), white represents the highest light intensity and black the lowest (5600 and 0 µmol photons/m^2/s, respectively), and green 50th percentile; scale is uniform across all conditions. Values in white represent the average light intensity (µmol photons/m^2/s) received by the cells, assuming aan ideally mixed reactor [Color figure can be viewed at wileyonlinelibrary.com]

to similar ($p > 0.05$) values for all conditions within 72 h (Figure 4a). When analyzing the chlorophyll content after 24 h (Figure 2b), it becomes evident that inoculum volume had a significant effect on the chlorophyll content, with all 10% inoculum volume experiments having the lowest, similar chlorophyll contents of 5.73 ± 0.29 mg_{Chl}/g_X, regardless of temperature and light intensity. Increasing inoculum volumes showed a significant increase in chlorophyll content, which was 10.28 ± 0.27 mg_{Chl}/g_X for the highest inoculum volumes.

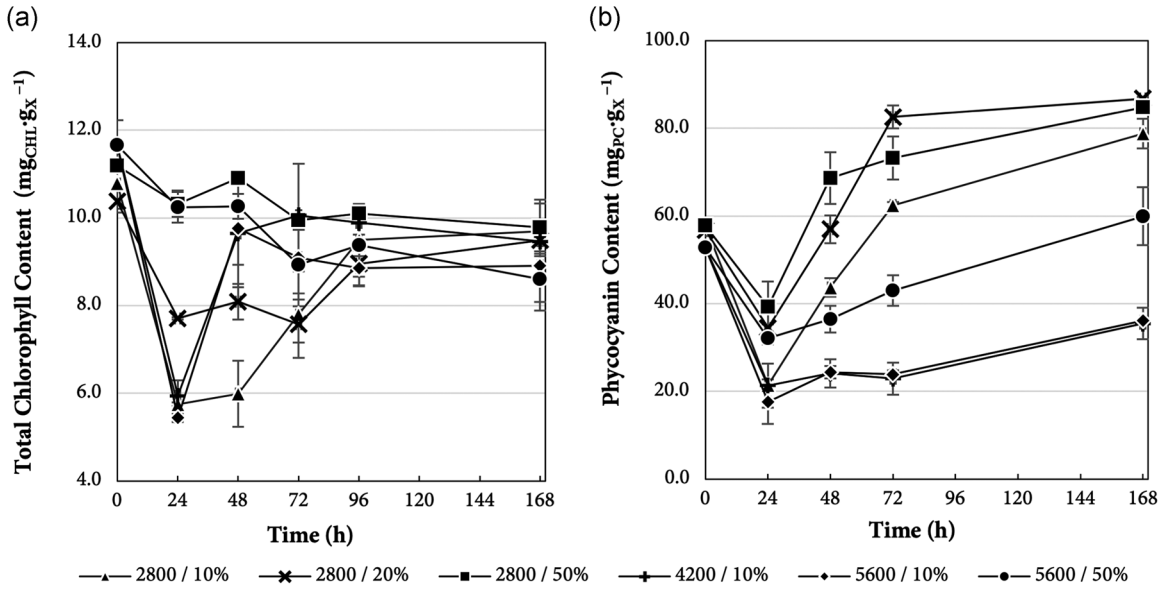

FIGURE 4 (a) Total chlorophyll content (mg_{Chl}/g_X) and (b) phycocyanin content (mg_{PC}/g_X) over time for *Leptolyngbya* sp. QUCCCM 56 cultivated with different inoculum dilutions (10%, 20%, and 50%), and light intensities (2800, 4200, and 5600 µmol photons/m^2/s) at 30°C. Values are mean ± range ($n = 2$)

Furthermore, higher inoculum volumes also showed the least decline within the first 24 h as compared to the chlorophyll content of the inoculum.

Similar to chlorophyll, phycocyanin showed a decline within the first 24 h of inoculation compared to inoculation levels, which was strongest for the cultures inoculated with the lowest densities (10%) (Figure 4b). The recovery, however, occurred in a very different pattern, with higher phycocyanin values for low light intensity cultures (2800 μmol photons/m^2/s) as compared to the phycocyanin levels found for higher light intensities (4200 and 5600 μmol photons/m^2/s). It is quite common for phycocyanin contents in cyanobacteria to decrease with increasing light intensities, as was found previously for *Leptolyngbya* sp. as well as other cyanobacteria (Pagels et al., 2019; Schipper et al., 2020). Furthermore, high-inoculum volume cultures had a higher final phycocyanin content, as compared to the same light intensity with lower inoculum volumes. Thus, contrary to chlorophyll, the phycocyanin content, in particular, that after 168 h, seems dependent on both light intensity and biomass density.

The different recovery of chlorophyll and phycocyanin is thought to be related to their different roles in photosynthesis. The light harvesting system of cyanobacteria is classified by thylakoid membranes containing two types of photosystems, photosystem I and II (PSI and PSII). Both photosystems contain chlorophyll *a*, although, unlike other photosynthetic organisms, PSII contains unique multimolecular structures called phycobilisomes. Phycobiliproteins, such as phycocyanin, make up the major component of the phycobilisomes, and these structures are able to harvest light and transfer energy at close to 100% efficiency. It is mainly PSII, which is influenced by increasing light intensities, with the ratio of PSII:PSI found to decrease for various cyanobacterial strains (de Marsac & Houmard, 1993). This phenomenon would explain the different ratios between chlorophyll and phycocyanin found for the different light intensities and biomass densities.

Even though no extreme photo-oxidation nor cell death occurred, the decrease in photosynthesis-associated pigments (chlorophyll and phycocyanin) suggests that the high-light/low-biomass density conditions induced a stress response in the culture. Furthermore, at low inoculum volumes, increasing the light intensity did not further affect the reduction in chlorophyll content in the first 24 h, nor the time required for the cells to recover. This could indicate that inoculum volume is the most important factor affecting photo-oxidation, regardless of light intensity. It is, however, unclear whether this is only the case at high light intensities as applied (≥2800 μmol photons/m^2/s), which could have already been oversaturating, or if lower light intensities would induce a similar response. Furthermore, the recovery of the chlorophyll content within 72 h, indicated that either the cells were able to adapt to the high light cultivation conditions, or could be related to the increasing biomass:light ratios returning to subsaturation levels. All in all, the results would suggest that the culture crash outdoor is more likely due to the suboptimal inoculum volumes, rather than high light intensities, and higher inoculum volumes could prove beneficial.

3.4 | Outdoor cultivation with varying inoculum volumes

To verify that outdoor culture crash occurrences for *Leptolyngbya* sp. QUCCCM 56 are related to photo-induced stress, and to test the hypothesis that increased inoculum volumes could prevent photooxidation, the cultures were scaled-up under outdoor conditions, whilst applying two different inoculum volumes of 10% and 20% (vol/vol). Cultivation occurred in August, 2020, and average peak light intensities of 1981 ± 41 μmol photons/m^2/s were recorded over the course of the experiment. Culture temperature varied between 25°C and 39°C, with an average high and low of 36.1 ± 1.7°C and 27.1 ± 1.6°C, respectively (Figure 5b). Initially, biomass densities and chlorophyll content increased, and no significant difference in growth rate was observed for the two inoculum volumes (0.61 ± 0.00 and 0.58 ± 0.02/day for 10% and 20%, respectively). During the first 2 days of cultivation, the cultures were not light limited (Figure 5b, yellow line), which would explain the similar growth rates for both inoculum volumes. The differences between the theoretical light gradients which are shown in Figure 3a, and the recorded values in Figure 5b can be attributed to variations in the biomass specific absorption coefficient. Absorption coeffecients are not constant, and can vary depending on culture conditions and strain adaptation (de Vree et al. 2016). Light-stressed cells generally have lower absorption coefficients compared to non-stresses cells (Vejrazka et al., 2011), and therefor light-penetration would be higher. This can also be linked to a decrease in concentrations of pigments which is shown to occur in the days following inoculation. Notably, the phycocyanin content of the strain dropped significantly from 32.2 ± 1.2 mg/g_X in the inoculum to 2.7 ± 0.4 and 4.5 ± 0.8 mg/g_X within 24 h of inoculation, for 10% and 20% inoculum volumes, respectively (Figure 5e). A slight recovery, to 4.0 ± 1.7 and 6.3 ± 0.7 mg_{PC}/g_X for 10% and 20% inoculum volumes was seen after 48 h. The difference between the phycocyanin content of the two inoculum volumes was significant, indicating that the higher biomass density did result in (limited) protection against the high light intensities.

Nevertheless, on the 3rd and 4th day, a drastic reduction in chlorophyll concentration, phycocyanin content, and light absorption by the culture was observed, and visual observations showed a change in culture color (Figures 5a, d, and e). There was no significant difference in response between the two inoculum volumes. Microscopic observations on Day 4, when chlorophyll concentrations had dropped to the lowest recorded levels, showed only a sparse number of trichomes related to *Leptolyngbya* sp., which were short in length (Figure 6b), in addition to some contamination from other species, most obvious being a fast moving ciliated protozoa of approximately 16–19 μm in length (Figure 6c).

Nevertheless, on Day 5, the chlorophyll concentration and phycocyanin content started to increase again for all conditions, reaching a peak on Day 7. Microscopic observations on Day 6

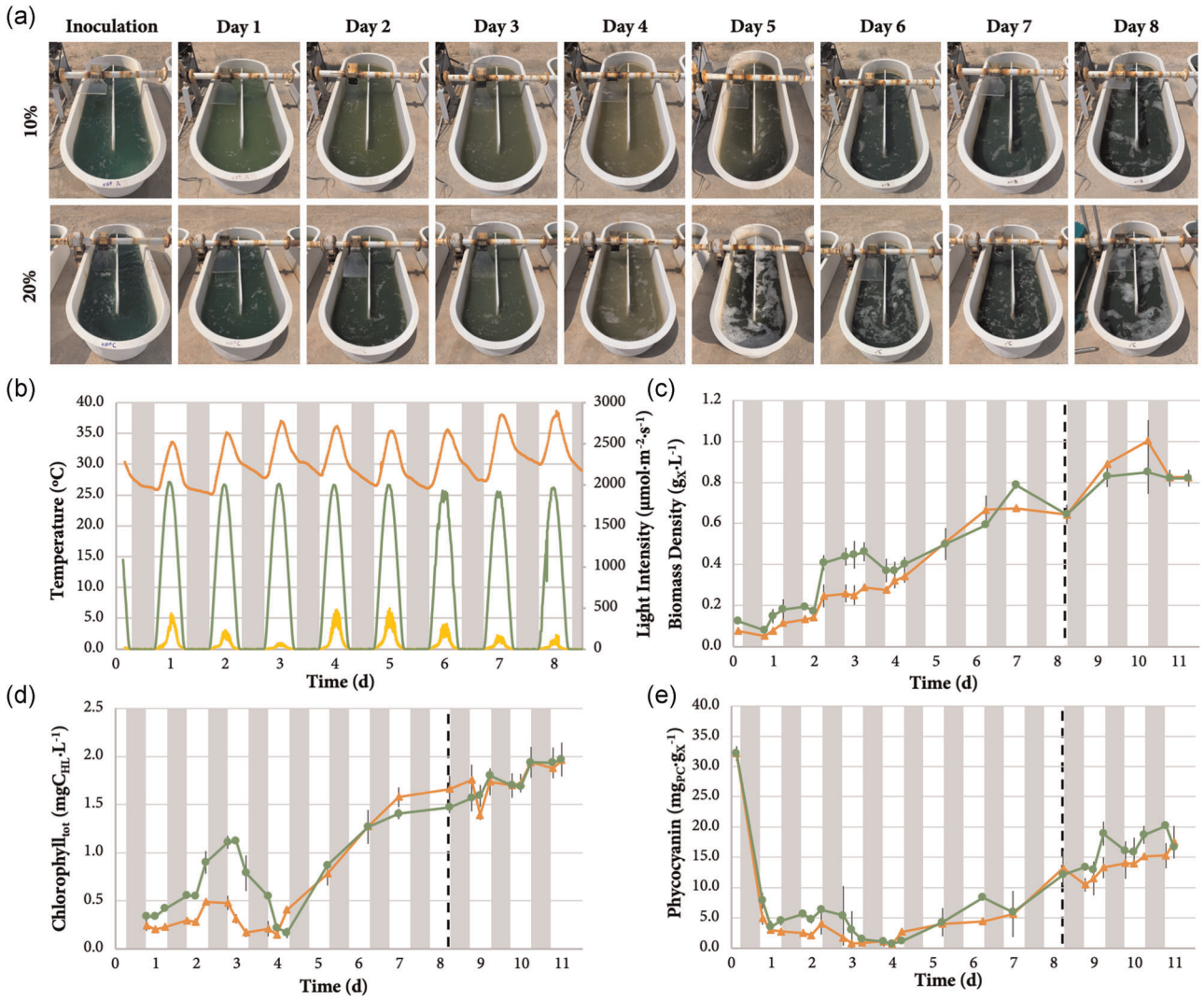

FIGURE 5 Outdoor growth trials of *Leptolyngbya* sp. QUCCCM 56 performed in August 2020, inoculated at 10% and 20% (vol/vol) inoculum volumes. Gray bars represent night-time. (a) Daily photos of 10% (top) and 20% (bottom) 200 L raceway tanks; (b) water temperature (orange line, °C), PAR light intensity at water surface (green line, µmol photon/m^2/s), and PAR light intensity at 20 cm culture depth in the 20% inoculum volume culture (yellow line, µmol photons/m^2/s); (c–e) biomass density (g_X/L), chlorophyll$_{tot}$ concentration (mg_{CHL}/L) and phycocyanin content (mg_{PC}/g_X), respectively, with orange line representing 10% inoculum volumes, and the green line 20% inoculum volumes. Black dashed line indicates supply of additional nutrients on Day 8 (more details in Section 2). Data shown in the mean ± range (n = 2). Culture turbidity (OD750) and phycocyanin concentration (mg_{PC}/L) are provided in Figure S2 [Color figure can be viewed at wileyonlinelibrary.com]

showed an increase in trichomes, both in number and in length, and very few contaminating species (Figure 6d–f). The trichomes were longer as compared to those found during cultivation in aerated photobioreactors (Figure 6a). Furthermore, the coiling of multiple trichomes, a phenomenon not observed before during laboratory experiments either, was observed (Figure 6e,f).

Upon reaching the stationary phase (Day 8), additional nutrients were supplemented to the cultures, which lead to a further increase in biomass density for another 4 days, with growth rates of 0.19 ± 0.0 and 0.18 ± 0.0 for the cultures initially inoculated with 10% and 20% inoculum volumes, respectively. Final biomass densities up to 1.00 ± 0.10 g_X/L were reached, with phycocyanin contents up to 20.2 ± 0.4 mg_{PC}/g_X.

3.5 | Biological contaminants and UV radiation as possible reasons for culture crash

The initial growth rate found during the outdoor growth-trials was 15% higher as compared to those found during the indoor experiments under a light intensity of 2800 µmol photons/m^2/s. This would suggest that the cultures indoor were more photoinhibited, as compared to the outdoor experiments, where light intensities peaked at 2034 µmol/m^2/s. Both the higher growth rate, as well as the insignificant differences in response between the two inoculum volumes during the outdoor experiments, would suggest that the culture crash on Days 3 and 4 is not related to the culture's response to (PAR) light intensity.

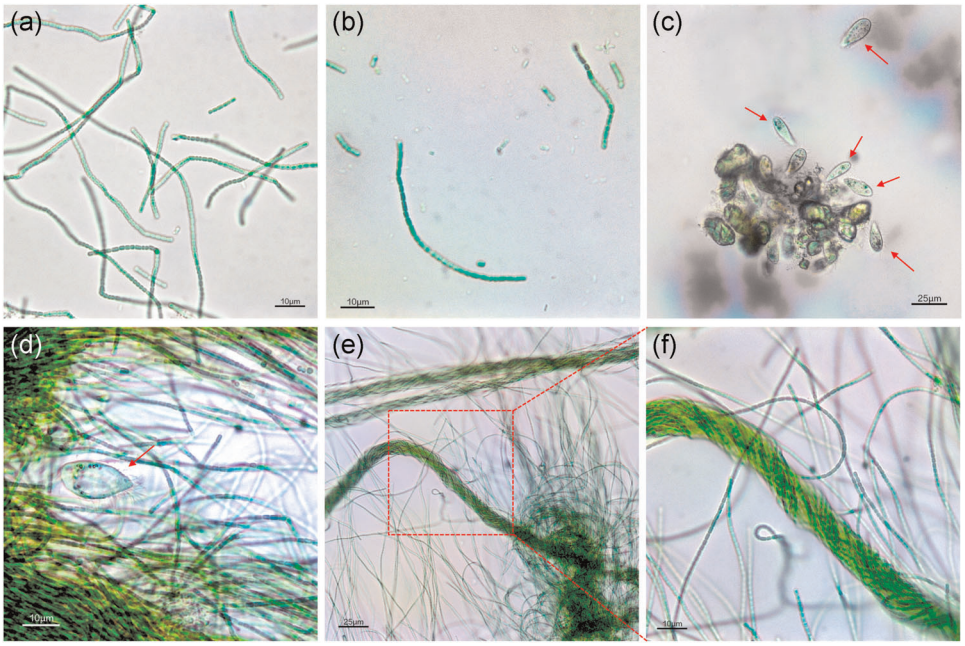

FIGURE 6 Light microscope images of *Leptolyngbya* sp. QUCCCM 56 during indoor and outdoor cultivation. (a) Indoor cultivation in photobioreactors. (b, c) Outdoor culture on Day 4, (d–f) outdoor culture on Day 6. Red arrows indicating ciliates; scale bar representing 10 µm for (a), (b), (d), and (f), and 25 µm for (c and e) [Color figure can be viewed at wileyonlinelibrary.com]

The initial response of the culture strongly resembles the description of culture crash occurrence found by Troschl et al. (2017) in cultures of cyanobacteria *Synechocystis* sp., which was caused by contamination by the ciliate *Colpoda steinii*. Ciliates are protozoa which feed on smaller organisms, such as bacteria and algae, and can rapidly wipe out algae cultures. Nevertheless, the gradual reduction of the amount of ciliates after Day 4, as well as the recovery of *Leptolyngbya* sp. QUCCCM 56, suggest that the ciliate was unable to consume *Leptolyngbya* sp., due to the size and filamentous nature of the strain. This is also suggested by microscopic observations (Figure 6d), which show the relative size of the ciliate compared to *Leptolyngbya* sp. It is further hypothesized that the ciliate was possibly able to graze upon the smaller *Leptolyngbya* sp. cells which were present at time of inoculation, although adaptation of the strain, seen through an increase in trichome length and trichome coiling, could have aided in preventing full culture crash. Finally, removal of smaller contaminant microorganisms by the ciliate could have perhaps even benefited the recovery of *Leptolyngbya* sp. QUCCCM 56 after the initial adaptation period. Contaminants are one of the major drawbacks for cultivation at industrial scale, not only in open race-way ponds, but also in closed photobioreactors, as the scale of operations can limit the possibilities for sterile cultivation, both from operational and economic perspectives. Having a strain which can resist to grazing is beneficial to the process, as it can reduce the requirement for alternative strategies to attempt to deal with contaminants, such as increase salinities, pH values, and/or CO_2 concentrations (Troschl et al., 2017).

Another possible reason for the experienced culture crash could be the presence of damaging UVR. Especially in summer months, the UV-index, which indicates the amount of UVR, is in the range of 10–12 in Qatar. UVR consists of 5%–7% of the total global horizontal irradiation in Qatar, and in August, average values of 49 and 1.8 W/m^2, for UVA and UVB, respectively, have been found (Roshan et al., 2020). Exposure of cyanobacteria to UVR can cause DNA damage, and negatively affect photosynthesis, growth, motility, and other cellular processes, including cell differentiation. UVA (320–400 nm) is primarily associated with the production of reactive oxygen species, which in turn can cause chlorophyll photobleaching, and phycobiliprotein degradation (Castenholz & Garcia-Pichel, 2000). Simulated light conditions in the laboratory generally do not emit light in the UV-wavelength range (280–400 nm), thus thereby can give an unrealistic growth environment. This could be one of the reasons as to why the phycocyanin content during the outdoor experiments ($20.2 \pm 0.4\ mg_{PC}/g_X$) was significantly lower compared to the indoor work—despite the high PAR applied during indoor experiments.

Cyanobacteria have however developed ways to cope with UVR, through different methods of photoprotection, including changes in gene regulation, production of non-photosynthetic pigments and enzymes, such as scytonemin and mycosporine-like compounds, as well as changes in morphology (Larkum et al., 2003). In the case of morphology, it has been found that larger cells are less susceptible to physical damage caused by ionizing radiation (Jeffrey & Mitchell, 1997). For example, Wu et al. (2005) found that the spiral structure of a long-term (adapted) outdoor-grown strain of *Arthrospira platensis* was much tighter as compared to the indoor-grown strain. Both strain-types witnessed a decrease in trichome length in response to UVR, but only for the indoor strain did this ultimately lead to cell

death. For the outdoor strain, the trichome length increased again after 6 days of exposure to UVR. Similarly, *Leptolyngbya* sp. QUCCCM 56 showed an initial decrease in trichome length during outdoor cultivation, yet it was able to adapt, forming the tightly packed trichome coils of increased length. This compressed structure is hypothesized to be able to protect against UVR, and most likely also high PAR-intensities, through self-shading, thereby providing an advantage for the strain to survive and thrive under outdoor conditions. Indoor however, these morphological changes were not witnessed, despite high PAR-intensities applied (5600 µmol photons/m^2/s), which would suggest that the adaptation mechanism is UVR dependent, and/or related to the reactor configuration. Furthermore, *Leptolyngbya* sp. has also been reported to produce mycosporine-like amino acids under UVR stress (Joshi et al., 2017). Further investigation into the capabilities of *Leptolyngbya* sp. QUCCCM 56 to produce similar UVR-protectants would aid in better understanding the strain's adaptation-mechanisms necessary for stable outdoor cultivation.

The additional growth of the strain after the addition of extra nutrients, further contributes towards the theory that the strain was able to adapt to the outdoor conditions, including suspected stressors, such as UVR and biological contaminants. Nevertheless, the outcomes of the outdoor cultivation trials, were not as expected based on the outcomes of the indoor experiments. Discrepancies between laboratory and outdoor cultivation are unfortunately very common, as the full spectrum of outdoor conditions, inlcuding light and temperature variations, contaminants, and full light-spectra, are exceedingly difficult to replicate under laboratory settings. It is clear that the strain required an adaptation period to allow for stable cultivation outdoors, and the change in morphology was one of the obvious adaptations, although other molecular adaptations could have also been at play. PAR-intensity levels did not seem to be the main culprit of the culture crash, and eventhough they did cause limited levels of photoinhibition and loss of pigmentation, indoor cultures showed adaptation within 72 h. More investigation is necessary to further understand the effects of UVR on the strain, as well as to determine whether the adapted strain can be cultivated without initial relapse under outdoor conditions, for example under (semi-) continuous conditions.

4 | CONCLUSION

Indoor to outdoor transitions of algae cultivation is a challenging process, mainly due to the inability to recreate the full spectrum of outdoor conditions. Indoor, *Leptolyngbya* sp. was able to grow under extreme light-conditions of up to 5600 µmol photons/m^2/s, with limited photooxidation occurring even at low inoculum volumes. Nevertheless, during outdoor cultivation trials, a culture crash occurred within 3 days of inoculation, irrespective of inoculum volume. The culture was able to recover, although an adaptation period of multiple days was required. The suspected acclimatization of the cells to the outdoor cultivation conditions was characterized by changes in pigment concentration and cell morphology. Biological contaminants and UV-radiation, both conditions which are generally not simulated under laboratory environments, were hypothesized to be the most probably reasons for the long adaptation period.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of iterests.

AUTHOR CONTRIBUTIONS

Kira Schipper contributed to conceptualization, methodology, formal analysis, validation, visualization, project administration, funding acquisition, writing—original draft preparation and reviewing and editing. Probir Das contributed to conceptualization, methodology, supervision, writing—reviewing and editing. Mariam Al Muraikhi, Mohammed AbdulQuadir, and Mahmoud Ibrahim Thaher contributed to methodology, investigation, data curation and writing—reviewing and editing. Hareb Mohammed S. J. Al Jabri contributed to conceptualization, funding acquisition, project administration, supervision and writing—reviewing and editing. René H. Wijffels and Maria J. Barbosa contributed to conceptualization, methodology, supervision and writing—reviewing and editing.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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