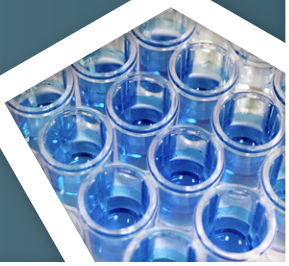








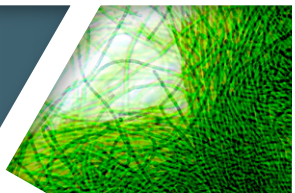

# Producing algae in the Qatari desert:

*from strain to process*

Kira Schipper

# Propositions

1. The biggest limitation for industrial bioprospecting is the inability to mimic relevant cultivation conditions. (this thesis)

2. Sustainable algae cultivation should be inspired by nature. (this thesis)

3. Publishing failed experiments would enhance the rate of scientific breakthroughs.

4. In scientific research, intuition is a necessary supplement to intellect.

5. Hiring women for their gender undermines their credibility as a professional.

6. Working mothers benefit more from reduced working hours than they do from extended maternity leaves.

Propositions belonging to the thesis, entitled:

**Producing algae in the Qatari desert: *from strain to process***

Kira Schipper

Wageningen, June 8th 2021

# Producing algae in the Qatari desert:

## *from strain to process*

Kira Schipper

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This research was conducted under the auspices of the Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences)

# Producing algae in the Qatari desert:

## *from strain to process*

Kira Schipper

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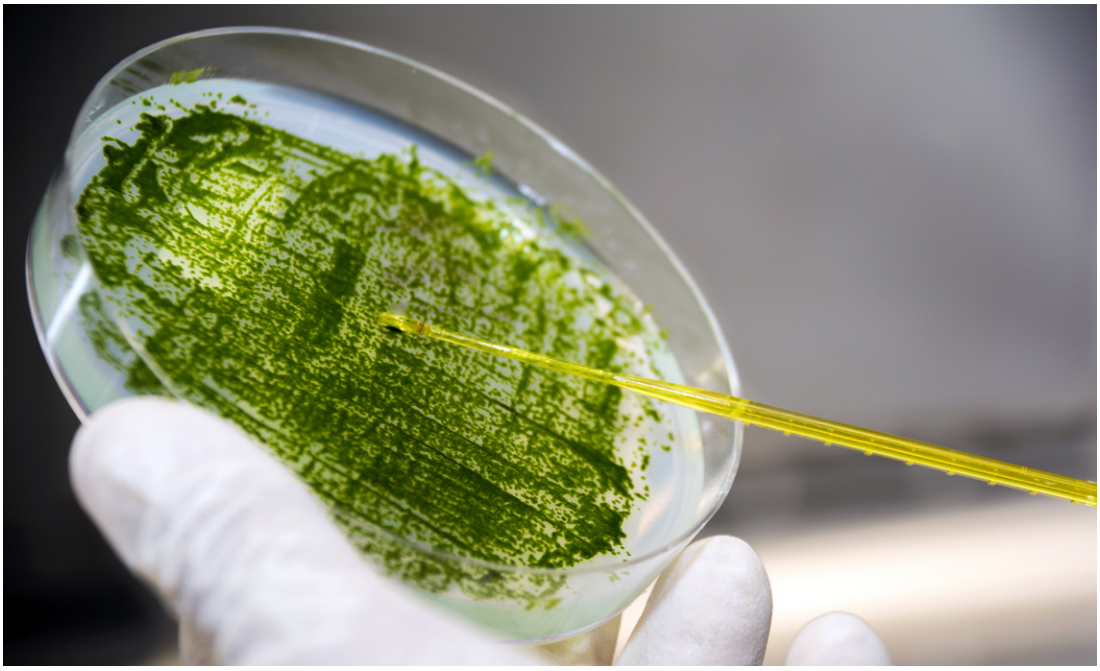

# Chapter 1

## General Introduction

## 1.1 MICROALGAE AS A SUSTAINABLE FEEDSTOCK

The world is in search of more sustainable feedstocks; population increase and associated ever growing food demand, the call for decarbonization and energy transitions, as well as the critically required rationalization of unsustainable ecological footprints are all drivers for change [1]. Qatar, a peninsula located in the Arabian Gulf and a member state of the Gulf Cooperation Council (GCC), is one of the richest countries in the world in terms of GDP per capita due to its large reserves of natural gas. It is however also one of the most scarce in terms of renewable natural resources, such as agricultural land and fresh water. This leads to a large demand and dependency on (food) imports and desalinated water capacity to sustain its growing population [2]. The region is characterized by a desert climate, with hot arid conditions and limited precipitation, further complicating the applicability of conventional agricultural activities. These challenges are however being met head on, with a drive for economic diversification and the development of a more sustainable society [3].

Both globally and regionally, algae have been recognized as a possible cornerstone for the sustainable production of food, feed, fuel, and chemical feedstocks [4-6]. Algae are a large and diverse group of organisms that grow in various aquatic environments, ranging from freshwater to oceans, brackish water and wastewater. They range in size from small microscopic 'microalgae' – either eukaryotic (green algae, red algae and diatoms) or prokaryotic (cyanobacteria) – to more complex multicellular forms also known as macro-algae (i.e. seaweed). Algae can be described as lower plants, growing phototrophically using $CO_2$, (sun-) light and water as main carbon and energy source (Figure 1.1).

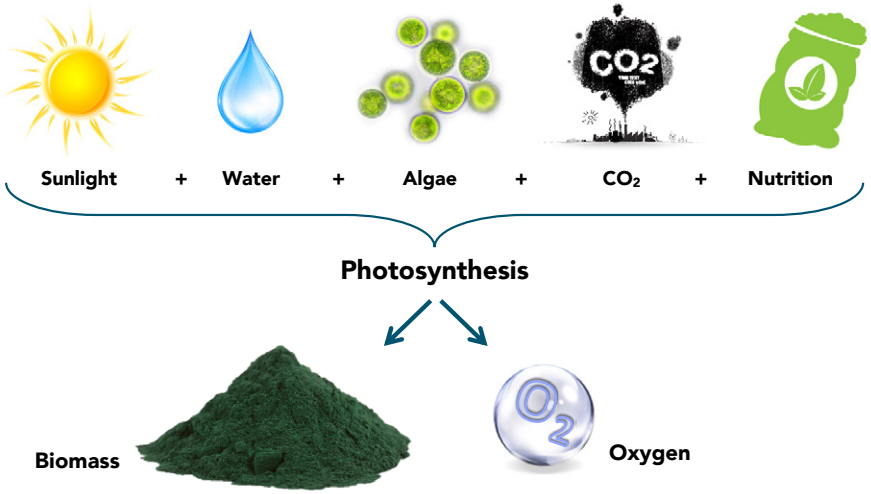


**Figure 1.1** - The principle of algae; algae require (sun-) light, water and $CO_2$, and through photosynthesis can produce oxygen and biomass, the latter of which can be used for a multitude of applications.

Advantages of algae over conventional terrestrial crops include higher areal productivities, the possibility of using non-arable land and non-fresh water sources for cultivation, potential for year-round production as well as higher efficiency carbon capture. Furthermore, the large biodiversity of algae – including strains which have adapted to the more extreme environments on our planet – allows for a potentially significantly broader range of cultivation conditions compared to traditional agricultural crops [7,8].

### 1.1.1 ALGAE-BASED VALUE-CHAINS: SIGNIFICANCE FOR QATAR

The typical algae production process is subject to many location-dependent factors, the most important of which are shown in Figure 1.1: sunlight, water, algae strain selection, $CO_2$, and nutrients. In terms of sunlight availability, the Arabian Peninsula (including Qatar) is one of the top most productive regions for algae biomass production [9]. With over 3200 sunlight hours per year and an average temperature of 32°C, Qatar's climate can support year-round algae production [10]. The product market potential in the region is also diverse, with opportunities to link into value chains ranging from feed and foods, to fuels, and high-value metabolites.

#### *FEED & FOOD*

The largest current global market for algae-based products is the food and feed market. Species such as *Chlorella* and *Spirulina* (*Arthrospira*) have been produced as human food supplement since the 1960's [4]. With a 30% share of the globally produced algae biomass, *Spirulina* is the most commercially cultivated alga. Its popularity is mainly driven by its overall nutritional qualities: high protein content, vitamin content, and other high-value added components such as chlorophylls, carotenoids and phycobiliproteins [11]. Furthermore, the use of algae for aquaculture and animal feed, as either a feed supplement or substitute of conventional protein sources, is a market exhibiting significant growth [12,13]. Qatar currently is heavily reliant on food imports to sustain both its human- and animal (agricultural and aquacultural) populations, which imposes a food-security exposure and associated risk [14]. Local production of algae-based food and feeds could contribute towards reducing Qatar's dependence on imports, thereby increasing the autonomy as well as sustainability of Qatar's food supply-chain.

#### *BIOFUELS*

Microalgae have long been investigated as a potential biofuel resource. The low current energy-equivalent market price of fossil-based fuels has however suppressed the techno-economic potential of such biofuels thus far. Nonetheless, against the backdrop of the global drive for energy transition and decarbonization, the potential for algae-based biofuels, and therefore both academic and industrial interest, remains. Various types of biofuels can be produced from microalgae, through either thermochemical or biochemical conversion processes of whole

algae biomass, or biomass fractions. The most commonly investigated methods therein are the transesterification of algal-lipids to biodiesel, the fermentation of carbohydrates to ethanol, and the hydrothermal liquefaction (HTL) of whole algae biomass to biocrude oil [15]. Developments in the efficient downstream processing of algal biomass to fuels, including the application of a biorefinery concept in which high-value products are co-produced, could enhance the commercial viability [16]. Finally, the availability of existing refinery infrastructures, and the continuing push for decarbonization of the energy market could lead to increased opportunities for such biofuels in Qatar and the region [17].

### *HIGH-VALUE METABOLITES*

Microalgae can produce a plethora of secondary metabolites and pigments, including chlorophylls, carotenoids, and phycobiliproteins. These compounds have been found to have various promising pharmaceutical-attributes, such as anticancer, antiviral, antibacterial and antioxidant activities [18]. It is therefore not surprising that microalgae have been identified as one of the most promising groups of organisms for the isolation of novel bioactive compounds [19]. The most developed high-value products in this area are astaxanthin and β-carotene, carotenoids which are used as a pigment, dietary supplement, or food additive, and also have strong antioxidant activities [20]. Phycobiliproteins, such as phycocyanin, phycoerythrin and allophycocyanin, are also gaining interest as a high-value natural product from cyanobacteria, with applications in nutraceutical, pharmaceutical, food, feed and cosmetics industries [21]. The desert climate and geography of Qatar has led to interesting natural environments with high biodiversity, for the bioprospecting of novel strains [22]. Such novel strains could possess (novel) secondary metabolites of nutraceutical and pharmaceutical value, opening doors towards locally produced high-value products for a global market.

### *BIOREMEDIATION*

Besides food and feed, algae can also play an important role in bioremediation efforts. Carbon dioxide ($CO_2$) emissions from industry are prevalent throughout Qatar, and $CO_2$ emissions have been linked to global climate change [23,24]. As microalgae require $CO_2$ to grow – over 1.8 ton $CO_2$ per ton of algal biomass produced – bio-sequestration of $CO_2$ using microalgal processes is a promising route for recycling $CO_2$ and potentially producing value-added products at the same time [25,26]. As such, algae hold the potential as differentiators in a sustainable economic diversification strategy, using a biological approach to converting carbon dioxide into useful fuels, chemicals and feed. Such a development could support sustaining the Qatari and global environment for future generations, whilst also directly contributing to international efforts at carbon capture, utilization and sequestration.

## 1.2 AIM AND THESIS OUTLINE: FROM STRAIN TO PROCESS

The potential of Qatar as a location for commercially viable algae production is promising. Limited research has been performed in Qatar and other countries in Arabian Peninsula, into the isolation of novel strains and their associated products, while a small number of outdoor scale-up studies have demonstrated feasibility of the technical concept [10,27-29]. To date, an Arabian Peninsula region-centered end-to-end assessment of the potential commercialization of algae production, to map out overall feasibility, enabling factors and associated challenges, has not yet been performed.

In this writing, we aim to assess the entire development process (Figure 1.2), starting from the isolation and screening of potential algae strains, until techno-economic modeling and projections of large-scale productivities and production costs for commercial algae production in the State of Qatar. The intent is that this can be used as a blue-print for developing an algae-based industry in the region, as well as other desert-locations which share similar characteristics to the Arabian Peninsula.

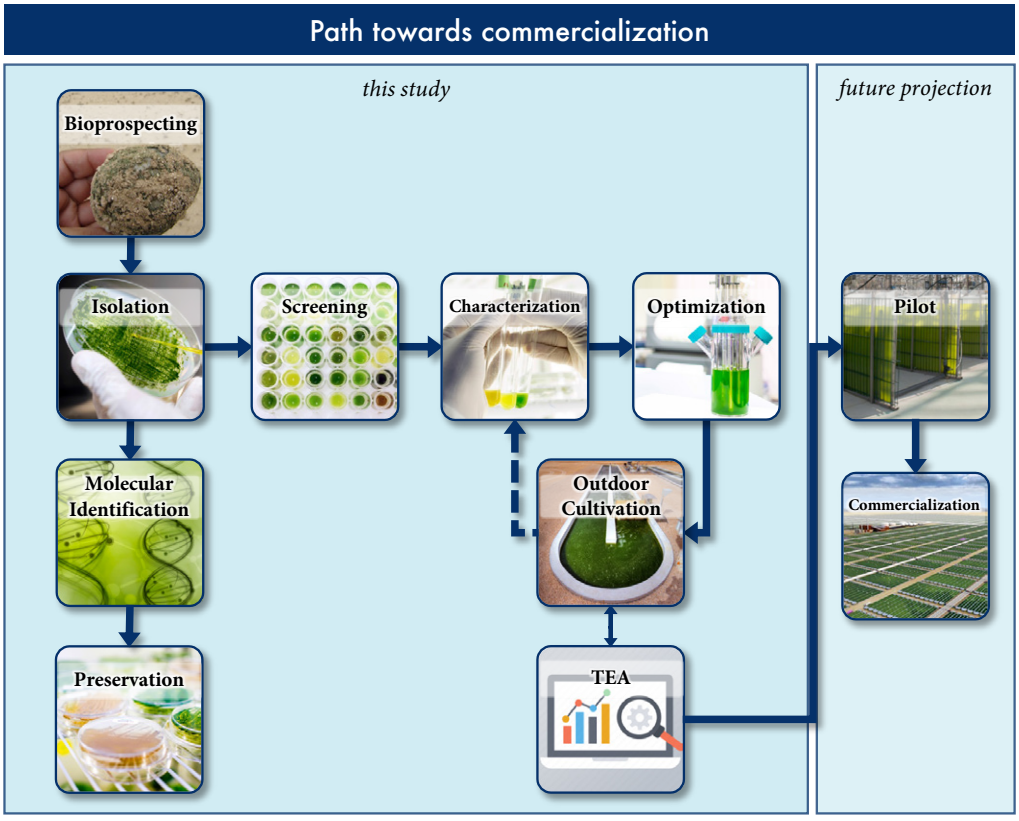


**Figure 1.2** – Projected steps required for the development of an algae-based commercial process, starting from strain selection, and finishing with pilot demonstration and finally commercialization

The isolation and identification of novel strains is necessary to funnel into the algae-value chain development process. The best strain for a process will differ based on cultivation locations as well as on desired end-products. In **Chapter 2**, the screening of four novel microalgae and cyanobacteria isolated from the Qatar environment is described, focusing on their biomass productivities and carbon capture potential. The novel isolates' tolerance to industrially relevant conditions – more specifically high temperatures and $CO_2$ concentrations – are studied. Such conditions are to be expected when coupling an algae production process to industrial waste streams such as flue-gas $CO_2$. General composition in terms of lipid, protein, carbohydrates and secondary metabolites is assessed in order to make recommendations as to strain selection for further optimization.

The further optimization of one such isolate, *Leptolyngbya* sp. for its potential to produce phycocyanin under prevalent desert climate conditions is investigated in **Chapter 3**. High temperatures and light intensities are studied for their effect on biomass and phycocyanin productivities. Furthermore, the extraction methods of phycocyanin from the isolate are optimized, with the aim of enabling maximum product yields and product quality.

Following the indoor optimization, the transition to outdoor cultures is described in **Chapter 4**. A successful transition from indoor to outdoor cultures is one of the most crucial stages towards the development of commercial algae-based processes. Here, the optimization of the outdoor cultivation is preceded by an investigation into the effect of light intensity and initial biomass densities on the occurrence of photo-oxidation, which can be detrimental for outdoor cultures, and followed by outdoor cultivation trials.

In **Chapter 5**, a Techno-Economic Analysis (TEA) is performed to generate projections regarding the commercial potential of algae production processes in the Arabian Peninsula (incl. Qatar). Production in four different cultivation systems is modelled, allowing for cost and productivity case comparisons. A sensitivity analysis is included in order to assess the most impactful process-aspects on the production costs, in order to make recommendations for future regional development.

Finally, in **Chapter 6**, a perspective on the potential of algae cultivation as a sustainable resource for the Arabian Peninsula is given. Technical and non-technical aspects are interwoven into a SWOT-analysis to visualize the opportunities of algae in the region, but equally also to address the anticipated challenges ahead, for the establishment of a successful algae-based value chain in the region.

# Chapter 2

# Potential of novel desert microalgae and cyanobacteria for commercial applications and $CO_2$ sequestration

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## ABSTRACT

$CO_2$ fixation by phototrophic microalgae and cyanobacteria is seen as a possible global carbon emissions reducer. Novel strains, with tolerance to elevated temperatures and $CO_2$ concentrations, are however essential for further development of algae-based carbon capture. Four novel microalgae and cyanobacteria, isolated from the Arabian Gulf, were investigated for their thermotolerance and $CO_2$-tolerance, as well as their carbon capture capability. Two strains, *Leptolyngbya* sp. and *Picochlorum* sp., grew well at high temperatures up to 40 ˚C, with productivities of 106.6±10.0 and 87.5±2.1 mg biomass·$L^{-1}$·$d^{-1}$, respectively. *Tetraselmis* sp. was not able to grow at 40 ˚C, but at lower temperatures (30 ˚C) did show the highest biomass productivity and carbon capture rate of 157.7±10.3 mg biomass·$L^{-1}$·$d^{-1}$ and 270.8±23.9 mg $CO_2$·$L^{-1}$·$d^{-1}$, respectively. *Leptolyngbya* sp., *Tetraselmis* sp. and *Picochlorum* sp. were all able to tolerate $CO_2$ concentrations of up to 30%, with the latter two showing significant increases in growth rate under 20% $CO_2$ levels to 333.8±41.1 and 244.7±29.5 mg biomass·$L^{-1}$·$d^{-1}$, respectively. Both microalgae also presented significant amounts of lipids, up to 25.6±0.9% and 28.0±2.0% (w/w), as well as presence of EPA and DHA. Both cyanobacteria, *Leptolyngbya* sp. and *Chroococcidiopsis* sp., showed presence of phycobiliproteins. The isolated strains all showed high potential in various aspects, from thermo-tolerance to $CO_2$-tolerance, as well as the production of high-value metabolites, making them valuable strains for further investigation towards commercial applications and $CO_2$ capture.

## 2.1 INTRODUCTION



Microalgae have long been investigated for various commercial applications, however the size of the microalgae industry is still very limited, in the range of 10-20 thousand tons of dry biomass per year [20,30]. This can be partly contributed to the small number of algae species that are currently applied in large-scale economically feasible processes [31-33]. The most commonly cultivated strains are *Spirulina* sp., *Chlorella* sp., *Dunaliela salina* and *Haematococcus* sp., which are being applied mainly for human food supplements, health food, β-carotene, and astaxanthin production respectively [20,34]. One of the major constraints in applying novel strains is not only whether they produce economically viable products, but also their ability to grow under industrially relevant outdoor conditions, with seasonal and diurnal fluctuations in temperature and light, and their resistance to contamination [33,35,36]. Additional cultivation stresses to commercially cultivated strains can include local water conditions, such as high salinities and/or pH, as well as increased $CO_2$ levels and temperatures when coupled directly to an industrial flue gas source, or when cultivated in closed systems [37]. All in all, robust strains are required for commercial application, and ideally those that can handle a wide range of cultivation conditions.

Furthermore, productivity, which is both strain and location dependent, is an important driving factor for the development of a commercially feasible algae industry, as it has a large impact on the economics of the process [9,38]. Tredici (2010) analyzed the global potential productivities for algae biomass, as a function of the availability of photosynthetic energy, and concluded that a limited number of global locations, including the Arabian Peninsula, can support high theoretical biomass productivities of over 200 $t \cdot ha^{-1} \cdot y^{-1}$. Ruiz *et al.* (2016) also concluded from a techno-economic perspective, that the Arabian Peninsula (Saudi Arabia in particular) is a very attractive location for algae production from a commercial perspective, as it had one of the lowest algae production and biorefinery costs as compared to 5 other analyzed locations across the world [39].

The large availability of non-arable land, easy access to seawater and easy availability of industrial flue gas rich in $CO_2$, make the Arabian Peninsula one of the most promising regions for commercial algae production. As of such, isolation and characterization of strains capable of coping with the local conditions, such as high temperatures and high solar irradiances, is essential to support the further development of this algae industry. This is not only the case for regional production, but also for algae production in other locations; strains which can cope with increased temperatures require less cooling in closed cultivation systems [39], could be coupled directly to industrial flue gas with elevated temperatures [15], and are less susceptible to contamination in locations where most strains have optima in the range of 20-30 ˚C [37]. Ruiz *et al.* (2016) even concluded that using strains adapted to 45 ˚C would have a higher impact on cost reduction, as

compared to improvements in harvesting or nutrient recycle, and could reduce overall biomass production costs by 0.3 - 1.2 €·kg$^{-1}$, depending on the cultivation system [39].

Unique strains are expected to be found in the environments of the Arabian Peninsula, however only a limited number have been reported so far [10,40,41]. One of the most comprehensive resources in the region is the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM), which houses cyanobacteria and microalgae isolated from Qatar [10]. This culture collection shows a large diversity of strains, the potential of which still remains largely unexplored.

As isolation and characterization of novel strains, in particular from extreme desert environments, is paramount to improving the overall feasibility of commercial algal processes, this research focused on the characterization of four novel marine strains from the Arabian Gulf. The strains were evaluated for biomass productivity under elevated temperatures and $CO_2$ concentrations, as well as for their carbon capture and commercial potential, through biomass analysis, with as aim to identify a promising strain for commercial application in tropical and sub-tropical regions.

## 2.2 MATERIALS AND METHODS

### 2.2.1 STRAIN ISOLATION & MOLECULAR IDENTIFICATION

Environmental samples (water, rock and sand) were collected between 2010-2012 from various marine environments around the Qatar Peninsula, as per Table 2.1. Isolation and molecular identification occurred as per the methods described previously by Saadaoui *et al.* (2016) [10]. After isolation, all strains were maintained on BG11 supplemented agar seawater media in environmental test chambers (Sanyo, Japan) at 28 °C, a photon flux density of 100 µmol photons·m$^{-2}$·s$^{-1}$ and a 12:12h light:dark cycle.

For QUCCCM 51 and QUCCCM 127, protocols and primers for molecular identification were applied as per Saadaoui *et al.* (2016) [10], however for strains QUCCCM 26 and

**Table 2.1** - Isolated strain collection date, location, and environmental characteristics

| Strain No. | Collection Date | Collection Location | Environment Characteristics | Average Temp. | GenBank Accession No. |
|---|---|---|---|---|---|
| QUCCCM 26 | 28-Mar-2011 | N25 59 167 E51 01 293 | Sandy beach | 22.2 °C | MG022133 |
| QUCCCM 51 | 8-Sep-2012 | N25 24 152 E050 45 237 | Sandy beach | 34.3 °C | MG022131 |
| QUCCCM 56 | 2-Oct-2010 | N25 24 882 E050 45 237 | Mangrove | 31.0 °C | MG049742 |
| QUCCCM 127 | 20-Dec-2013 | N24 47 175 E050 51 562 | Sandy Beach | 18.9 °C | MG022132 |

QUCCCM 56 (cyanobacteria), some modifications to the molecular identification protocol were made: increased duration of both the cell lysis step (from 30 min to 4 h), and the DNA precipitation step (from 10 min to overnight incubation at -20 ˚C). For strains QUCCCM 26 and 56, the 16SrDNA gene was amplified via PCR using primers BS1F 5' GATCCTKGCTCAGGATKAACGCTGGC3' and CPL10R 5' GCCGGCTCTTCAAC3'. The internal primers 5' TTTGCGGCCGCTCTGTGTGCCTAGGTATCC3'; 63F 5' CAGGCCTAACACATGCAAGTC-3' and 1389R 5'-ACGGGCGGTGTGTACAAG-3' were applied for sequencing of the cyanobacterial strains. The assembled sequences corresponding to the four strains under investigation were submitted in Genbank (Bankit) and the accession numbers are cited in Table 2.1. The phylogenetic analysis was performed using Clustal X 2.0 [42].



## 2.2.2 TOXICITY ANALYSIS

The 2 cyanobacterial strains were subjected to molecular toxicity investigation via PCR amplification using 3 primer couples to determine the presence or absence of genes specific to cyanotoxins microcystin (MC), cylindrospermopsin (CYN) and saxitoxin, followed by agarose gel electrophoreses of the PCR products, as per Yilmaz and Phlips (2011) [43]. Furthermore, freeze-dried biomass samples were also extracted for paralytic shellfish toxins (PSTs), microcystins (MCs), nodularins, anatoxins and cylindrospermopsins (CYNs), and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS, Cawthron Institute, New Zealand).

## 2.2.3 GROWTH CHARACTERIZATION

### *GROWTH MEDIA*

Seawater supplemented growth media was used for all experiments. Media was prepared using locally sourced seawater, with a salinity of 40.0 ppt, filtered (VWR 0.45µm PES), autoclaved and supplemented with: $NaNO_3$, 4.71 mM; $KH_2PO_4$, 0.23 mM; $NaHCO_3$, 2.4 mM; $Na_2EDTA$, $2.56 \cdot 10^{-2}$ mM; $FeSO_4 \cdot 7H_2O$, $1.44 \cdot 10^{-3}$ mM; $MnCl_2 \cdot 4H_2O$, $1.41 \cdot 10^{-4}$ mM; $ZnSO_4 \cdot 7H_2O$, $3.06 \cdot 10^{-5}$ mM; $Co(NO_3)_2 \cdot 6H_2O$, $3.21 \cdot 10^{-6}$ mM; $CuSO_4 \cdot 5H_2O$, $1.28 \cdot 10^{-6}$ mM; and $Na_2MoO_4 \cdot 2H_2O$, $1.33 \cdot 10^{-5}$ mM. Media composition was designed as such that the amount of nitrogen added was sufficient to support growth of an estimated 1.0 $g \cdot L^{-1}$ of biomass based on the conventional biomass composition of $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ [25]. For the $CO_2$ tolerance experiments, no inorganic carbon ($NaHCO_3$) was added to the media.

### *TEMPERATURE OPTIMA*

Strains were sub-cultured in flasks (250 mL working volume), and incubated in an illuminated Innova 44 Shaker Incubator (New Brunswick Scientific) at 150 rpm, 30°C, 60 µmol photons$\cdot m^{-2} \cdot s^{-1}$ and 12:12h light:dark cycle. After two weeks, cultures were used to inoculate 6 photobioreactors

(DASGIP parallel bioreactor system, Eppendorf, Inc., USA), with a culture volume of 1.2 L, to an initial biomass concentration of 60-90 mg·$L^{-1}$. Strains were cultivated at 30 ˚C, 35 ˚C, 40 ˚C and 45 ˚C. Illumination was provided by 3 internal DASGIP LED Sticks, with a 3-channel emission-spectrum (Channel A, 660,780 nm; Channel B, 572,625,640 nm; Channel C, 453 nm). Set-points were 2.00, 1.244 and 2.00 µmol·$s^{-1}$ for channels A, B and C respectively, which is equivalent to approximately 150-242 µmol photons·$m^{-2}$·$s^{-1}$, under a 12:12h light:dark cycle. The coupled DASGIP MX4/4 gas mixing system provided air blended with $CO_2$ at a gas-flow rate of 3 standard L·$h^{-1}$, with the $CO_2$ concentration controlled to maintain a pH of 8.0. Mixing was set to 200 rpm (pitch-blade impeller). Bi-daily optical density measurements at 680 and 750 nm were used to monitor the growth of the culture, and the reactors were stopped once the stationary phase was reached ($t_{end}$). QUCCCM 26 did not reach a stationary phase, and $t_{end}$ was set at 17 days. Dry weight determinations were performed at time of inoculation ($t_0$) and at $t_{end}$ to determine biomass productivities. Each experiment was performed in biological duplicate. Total nitrogen in the media ($C_N$) was monitored (LCK138 Total Nitrogen 1-16 mg TN·$L^{-1}$ and DR3900 VIS Spectrophotometer, Hach-Lange, CO, USA) for QUCCCM 26 isolate, as it showed interesting characteristics in regards to nitrogen consumption.

### 2.2.4 $CO_2$ TOLERANCE SCREENING

The $CO_2$ screening was performed in 24 micro-well plates (Corning® Costar® CLS3526, 2 mL culture volume), covered with transparent sterile gas-permeable membranes (Breathe Easy, Diversified Biotech, MA, USA) and incubated in clear zip-lock bags filled with air mixed with $CO_2$ at concentrations of 0.004 (air only), 5, 10, 20 or 30% $CO_2$ (v/v) [20,21]. Strains were incubated in environmental test chambers (Sanyo, Japan) at 30 °C, 200 µmol photons·$m^{-2}$·$s^{-1}$ and 12:12h light:dark cycles. Each strain was cultivated in quadruplicate for each $CO_2$ concentration; three wells were used for daily optical density measurements ($OD_{750}$, Synergy H4 Hybrid Reader, VT, USA), one well was used for weekly pH measurements (Orion Star A325, Thermo Scientific, MA, USA). The pH was not controlled. The gas in the zip-lock bag was refreshed daily with the initial $CO_2$ concentration. Dry weight was determined for the inoculum ($t_0$) as well as for the final biomass in the wells upon reaching the stationary growth phase ($t_{end}$).

### 2.2.5 BIOMASS DRY WEIGHT DETERMINATION

The biomass concentration was determined according to Zhu and Lee (1997) [44]. Duplicate samples (unless stated otherwise) of between 2 – 15 mL (depending on biomass density) were filtered through and pre-dried (24 h, 95 °C) and pre-weighed glass microfiber filters (Whatman GF/C™ Ø 47 mm) under a constant vacuum. The filters were then washed with a double volume of 0.5 M Ammonium Formate, followed by DI water, dried (24 h, 95 °C), cooled in a desiccator

(>30 min) and weighed. The biomass dry weight was determined as the difference between the weight of the dried filters prior to and after biomass filtration and drying.

### 2.2.6 KINETIC PARAMETERS

Volumetric biomass productivity, $P_X$ (mg·L$^{-1}$·d$^{-1}$) was obtained using equation 2.1:

$$P_X = \frac{C_{X_{end}} - C_{X_0}}{t_{end} - t_0} \qquad \text{(eq. 2.1)}$$

where $C_{X_{end}}$ is the biomass concentration (mg·L$^{-1}$) at $t_{end}$, and $C_{X_0}$ at $t_0$ [45]. $t_0$ and $t_{end}$ were defined as the time of inoculation and the time at which the growth rate decreased respectively. The Carbon Capture Rate, $R_{CO_2}$ (mg $CO_2$·L$^{-1}$·d$^{-1}$) was determined using equation 2.2:

$$R_{CO_2} = X_C \cdot P_X \left(\frac{M_{CO_2}}{M_C}\right) \qquad \text{(eq. 2.2)}$$

where $X_C$ is the carbon content of the strain (%, w/w), and $M_{CO_2}$ and $M_C$ the molecular weights of $CO_2$ and carbon respectively [46].

### 2.2.7 BIOMASS HARVESTING & COMPOSITION ANALYSIS

Biomass was harvested by centrifugation (5000 rpm for 15 min), washed with 0.5 M Ammonium Formate solution, followed by a subsequent centrifugation step. The pellet was freeze-dried over 48 h (FreeZone 12, Labconco, MO, USA), and stored at -80 ˚C until further analysis. Total lipids were extracted using a modified Folch Method and Fatty Acid Methyl Esters (FAMEs) were extracted using a one-step trans-esterification method, both as described by Saadaoui *et al.* (2016) [10,47]. Carbohydrates were extracted and quantified according to Dubois *et al.* (1956), and protein content was estimated according to Lourenço *et al.* (2004) [48,49]. Preliminary investigation on the presence of phycobiliproteins was performed on both cyanobacteria, through freeze-thawing of washed biomass, resuspended in MQ-water. Biomass samples were analyzed for Total Nitrogen ($X_N$) and Carbon ($X_C$) content using a Thermo Scientific Flash 2000 Organic Elemental Analyzer coupled to a CHNS-O analyzer (Bremen, Germany). Aspartic Acid STD (Thermo Scientific, Bremen, Germany) was used as a standard (C=36.09%, N=10.52%).

### 2.2.8 STATISTICAL ANALYSIS

All experiments were performed in duplicate, unless stated otherwise. The reported values are the mean of the individual samples, while the error bars represent the range. One-way ANOVA was used to determine significance difference (α = 0.05%) between the means of independent conditions.

## 2.3 RESULTS & DISCUSSION

### 2.3.1 STRAIN IDENTIFICATION & TOXICITY ANALYSIS

16SrDNA and 18SrDNA sequencing for the Cyanobacterial and Microalgal strains respectively, followed by phylogenetic analysis, showed that microalgae isolates QUCCCM 51 and QUCCCM 127 most closely corresponded to *Tetraselmis subcordiformis* and *Picochlorum maculatum* respectively, and cyanobacteria isolates QUCCCM 26 and QUCCCM 56 corresponded to *Chroococcidiopsis* sp. and *Leptolyngbya* sp. respectively (Figure 2.1). These findings were confirmed by morphological analysis (Figure 2.2).

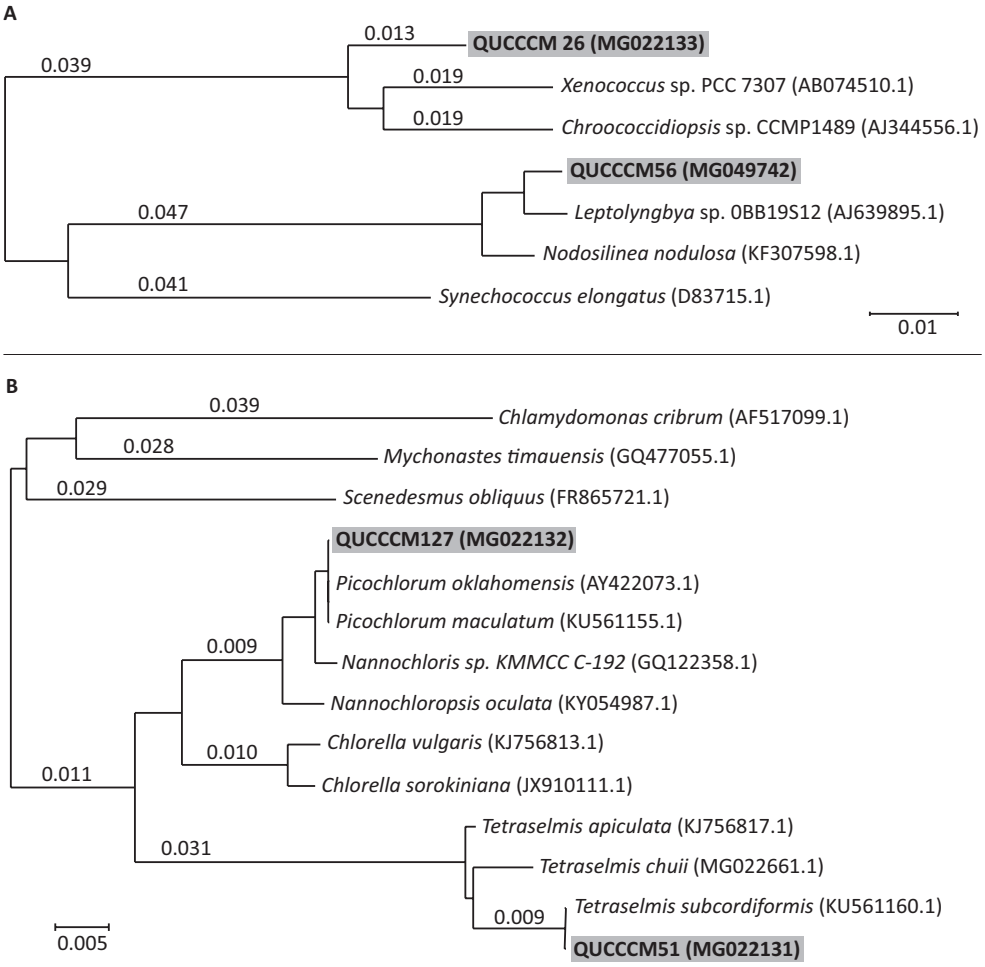


**Figure 2.1** – Phylogenetic trees of investigated QUCCCM isolates; **(A)** Cyanobacteria, including QUCCCM 26 and QUCCCM 56, and **(B)** Microalgae including QUCCCM 51 and QUCCCM 127. Distances within the tree were constructed using neighbor joining method with ClustalW. Horizontal lengths are proportional to the evolutionary distance

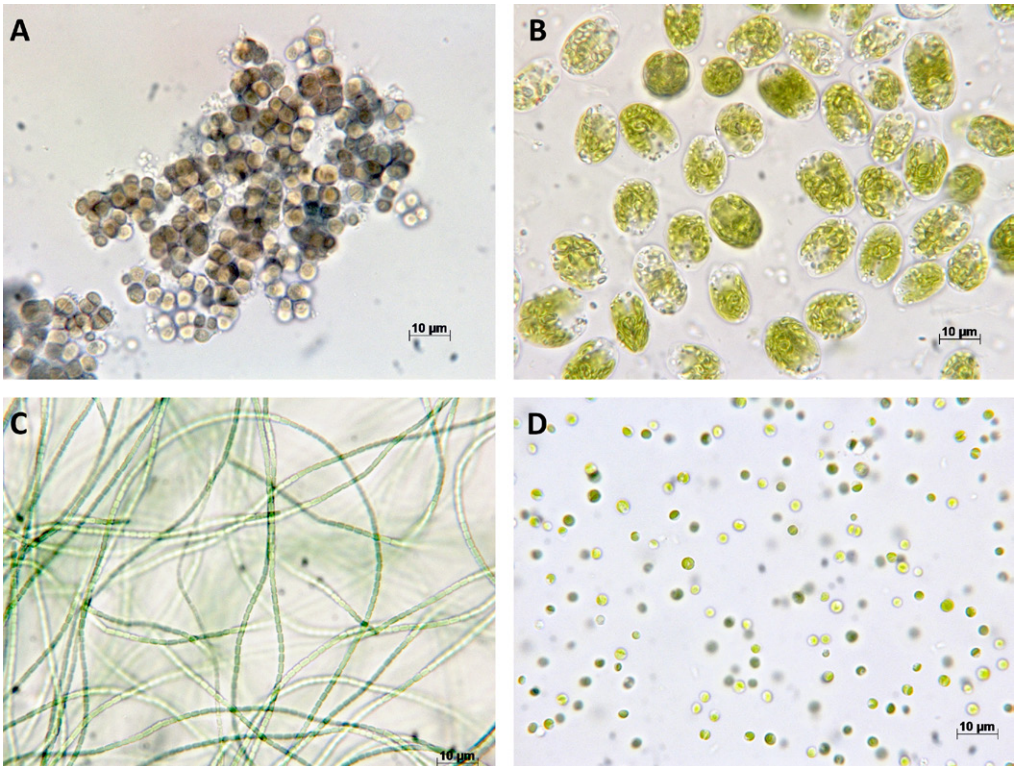


**Figure 2.2** – Morphological study of QUCCCM isolates by light microscope; (A) *Chroococcidiopsis* sp. QUCCCM 26; (B) *Tetraselmis subcordiformis* QUCCCM 51; (C) *Leptolyngbya* sp. QUCCCM 56; (D) *Picochlorum maculatum* QUCCCM 127

Molecular investigation of the toxicity of isolates QUCCCM 26 and 56 showed no presence of putative genes responsible of the biosynthesis of microcystin, cylindrospermopsin and saxitoxin. Furthermore, no presence of paralytic shellfish toxins, microcystins, nodularin or anatoxins was found though LC-MS/MS (supplemental materials Table S.2.1).

### 2.3.2 EFFECTS OF TEMPERATURE ON BIOMASS PRODUCTIVITY AND COMPOSITION

All four strains were subjected to productivity screening under various temperatures, ranging from 30 ˚C to 45 ˚C (Figure 2.3). The highest productive strain was *T. subcordiformis* QUCCCM 51, with a biomass productivity of 157.7±10.3 $mg{\cdot}L^{-1}{\cdot}d^{-1}$ at 30 ˚C, an insignificant decrease in productivity for increasing temperature ($p > 0.05$), and no growth at 40 ˚C or 45 ˚C. The same was observed for *Chroococcidiopsis* sp. QUCCCM 26, with biomass productivities of 112.2±10.8 and 96.0±0.24 $mg{\cdot}L^{-1}{\cdot}d^{-1}$ at 30 ˚C and 35 ˚C respectively. Both *Leptolyngbya* sp. QUCCCM 56 and *P. maculatum* QUCCCM 127 were able to grow at temperatures up to 40 ˚C, with similar productivities ($p > 0.05$) over the different conditions, ranging from 91.4±9.6 to 106.6±10.0 mg

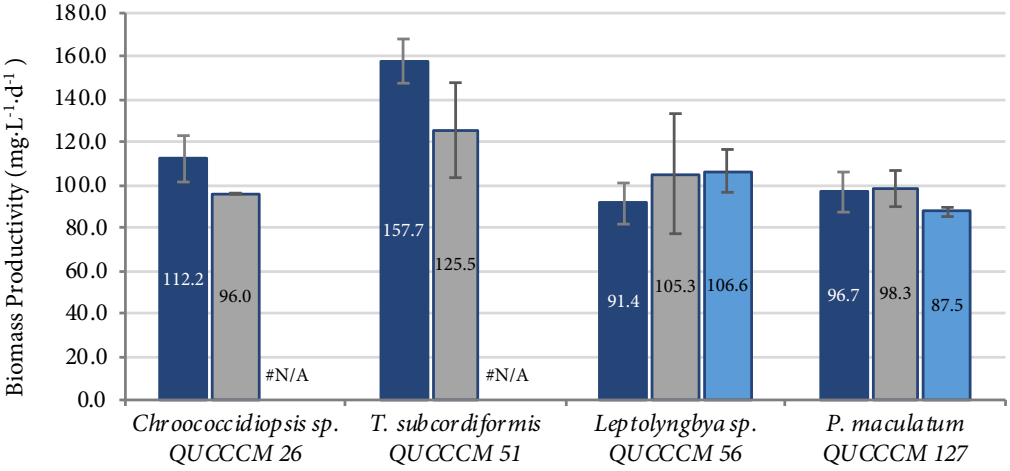


**Figure 2.3** – Biomass Productivities (mg·L$^{-1}$·d$^{-1}$) of the investigated strains at 30 ˚C (■), 35 ˚C (■), and 40˚C (■). Data shown is the mean±range, n=2

biomass·L$^{-1}$·d$^{-1}$ and 87.5±2.1 to 98.3±8.4 mg biomass·L$^{-1}$·d$^{-1}$ for both strains respectively. None of the strains were able to grow at 45 ˚C, however it should be noted that under this temperature, the biomass density ($OD_{750}$) of *Leptolyngbya* sp. QUCCCM 56 did increase over the first 24 h, and was constant between 24 h and 48 h of cultivation, prior to decreasing (data not shown).

Both *Leptolyngbya* sp. QUCCCM 56 and *P. maculatum* QUCCCM 127 were able to grow at 40˚C, making them interesting strains from a thermotolerance perspective. Singh *et al.* (2014) reported a *Leptolyngbya* strain which was capable of growing at temperatures up to 45 ˚C, however 30 ˚C was reported as the optimum temperature [50]. Regardless, the biomass productivity of QUCCCM 56 was found to be 35% higher at 40 ˚C, as compared to the highest productivities found by Singh *et al.* (2014) at 30 ˚C, and 18% higher compared to Choix *et al.* (2017) at 28 ˚C [51]. One consideration which should also be taken into account is that in non-temperature-controlled cultivation systems (open and closed), the temperature will fluctuate over the course of a diurnal cycle, and even under extreme conditions, continuous temperatures of 45 ˚C are not expected. *Leptolyngbya* sp. QUCCCM 56 was able to grow over the first 24 h, and survive over the first 48 h, of exposure to a constant temperature of 45 ˚C. As of such, it is expected that the strain would be resistant to temporary temperature peaks of 45 ˚C and perhaps higher, however the duration of these temperature peaks and subsequent productivities would need to be investigated.

## BIOMASS COMPOSITION

The biomass composition of each strain, cultivated at 30 ˚C, 35 ˚C and 40 ˚C, was analyzed for total lipids, carbohydrates and protein, data of which is shown in Figure 2.4. Both microalgae strains showed the highest amounts of total lipids, at 28.0±2.0% and 25.6±0.9% (w/w) for *P. maculatum* QUCCCM 127 and *T. subcordiformis* QUCCCM 51, respectively. The lipid content

variation of these strains roughly followed the biomass productivity, with the highest total lipid amounts found where biomass productivity was the highest. This is consistent with the results found by Wei *et al.* (2014), where the lipid content of *T. subcordiformis* first increased and then decreased with increasing temperature – displaying the highest lipid content at 20 ˚C, which was consistent with the optimal temperature for growth [52]. The highest total lipid amount found by Wei *et al.* (2014) was 22.25% at 20 ˚C, compared to 25.6% found in the current study. The total lipid content found for *T. subcordiformis* QUCCCM 51 was also significantly higher as compared to the 12.97-17.57% found by Das *et al.* (2016) for a different *Tetraselmis* sp. also isolated from Qatar [27].



*Chroococcidiopsis* sp. QUCCCM 26 showed an opposite trend, with increasing values of lipids, carbohydrates and proteins, for decreasing productivities and increasing temperature. *Leptolyngbya* sp. QUCCCM 56 showed a stable total lipid content for all temperatures, of around 15%. There was however a clear correlation between the carbohydrate content and temperature, with increasing carbohydrate values of 14.8±4.5, 17.5±3.0 and 22.5±2.3% for increasing temperatures of 30 ˚C, 35 ˚C and 40 ˚C respectively.

When comparing the biomass compositions found for *Leptolyngbya* sp. QUCCCM 56 to other *Leptolyngbya* sp. described in literature, differences were found especially in regards to protein contents. Choix *et al.* (2017) and Kim *et al.* (2015) found protein values of 11.4% and 52.6% for *Leptolyngbya sp.* CChF1 and *Leptolyngbya* sp. *KIOST-1* respectively, compared to 44-46% found in the current work [51,53]. It is thought that the large differences are mainly due to different protein extraction and analysis methods applied [49,54,55]. In terms and lipids and carbohydrates, the results found in the current work are similar to the two studies. The minor differences in

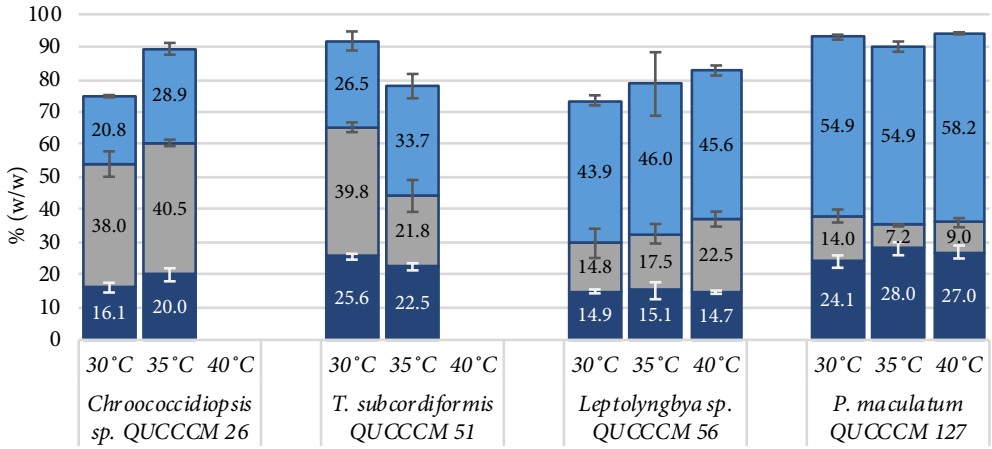


**Figure 2.4** – Lipid (■), carbohydrate (■) and protein (■) content (% w/w) of the 4 investigated strains cultivated at 30, 35 and 40 ˚C. Data shown is the mean±range, n=2

carbohydrate and lipid contents are thought to be linked to temperature and salinity respectively, as increased temperatures were found to increase the carbohydrate content (current study), and increased lipid contents were found for decreased salinities [50]. As of such, if higher lipid contents would be desired for *Leptolyngbya* sp. QUCCCM 56, it could be considered to investigate the effect of salinity.

Furthermore, preliminary investigations of water-extracts of *Chroococcidiopsis* sp. QUCCCM 26 and *Leptolyngbya* sp. QUCCCM 56 also showed absorption peaks 560 nm and 620 nm respectively, suggesting the presence of phycoerythrin and phycocyanin, two phycobiliproteins of high commercial value [21] (supplemental materials Figure S.2.1).

### *FAME ANALYSIS*

The FAME profile for the tested strains at the different temperatures is shown in Table 2.2. For *Chroococcidiopsis* sp. QUCCCM 26 over 85% of all FAMEs were C16. Increasing temperature showed a decrease in saturated fatty acids (SFAs), and an increase in monounsaturated fatty acids (MUFAs). In *T. subcordiformis* QUCCCM 51 the FAME profile showed that 50.79±3.22% and 44.45±2.37% of FAMEs were C20:1, with the second most significant FAME being C16:0 at 22.41±0.92% and 25.21±0.53%, both for 30 and 35°C respectively. Furthermore, presence of both EPA (C20:5) and DHA (C22:6) was found for *T. subcordiformis* QUCCCM 51, with highest concentrations of 0.53±0.09% and 2.29±0.46% respectively, for cultivation at 30 ˚C. Compared to Wei *et al.* (2014), who also studied a *Tetraselmis subcordiformis* strain, QUCCCM 51 showed significantly higher amounts of 20C FAMEs (52% and 45% as compared to 17-20%), and lower concentrations of both 16C and 18C FAMEs [52].

Furthermore Wei *et al.* (2014) did not find any DHA, but the amounts of EPA were higher as compared to the results found in this study. Wei *et al.* (2014) did find that the EPA concentration increased significantly (almost 2-fold) with decreasing temperature, and similar observations were made for *T. subcordiformis* QUCCCM 51 for both EPA and DHA. More investigation is necessary to determine whether a further decrease in temperature could increase the omega-3 content of this strain.

The fatty acid composition of *Leptolyngbya* sp. QUCCCM 56 showed clear trends with regards to temperature and subsequent productivities: increasing temperatures caused a shift from C20 FAMEs to C16 and C18. Furthermore, SFAs increased with increasing temperature, while MUFAs and PUFAs decreased, which is consistent with conclusions from Hu *et al.* (2008). *P. maculatum* QUCCCM 127 showed an opposite trend to productivity (not temperature), with decreased levels of C16 and C18 FAMEs and SFAs for increased productivities [56]. This is in accordance

**Table 2.2** – FAME Profiles for the 4 strains, as the average of the relative percentage of total FAMEs (wt%) of duplicate experiments. FAMEs with values below 1.0% (C18:3, C20:0, C20:2, C20:3, C20:4, and C22:0) are not shown. Data shown is the mean±range (n=2)

| Lipid Class | Strain | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Chroococcidiopsis* sp. QUCCCM 26 | | *T. subcordiformis* QUCCCM 51 | | *Leptolyngbya* sp. QUCCCM 56 | | | *P. maculatum* QUCCCM 127 | | |
| | 30°C | 35°C | 30°C | 35°C | 30°C | 35°C | 40°C | 30°C | 35°C | 40°C |
| C14:0 | 3.34±0.05 | 2.59±0.06 | 0.43±0.07 | 0.57±0.08 | 0.44±0.00 | 0.47±0.07 | 0.59±0.04 | 0.59±0.01 | 0.4±0.02 | 0.44±0.03 |
| C16:0 | 42.95±0.13 | 41.7±1.01 | 22.41±0.92 | 25.21±0.53 | 34.25±1.18 | 39.45±1.77 | 43.66±1.95 | 20.52±0.35 | 17.6±0.59 | 22.03±1.39 |
| C16:1 | 42.38±0.02 | 46.42±1.94 | 0.31±0.02 | 0.43±0.13 | 1.71±0.34 | 1.84±0.46 | 1.86±0.61 | 0.19±0.00 | 0.21±0.01 | 0.26±0.02 |
| C18:0 | 6.67±0.53 | 5.71±0.8 | 1.5±0.01 | 1.53±0.03 | 2.83±0.29 | 2.55±1.87 | 3.78±1.30 | 1.68±0.27 | 1.39±0.1 | 1.64±0.16 |
| C18:1[a] | | | 6.84±2.46 | 6.59±1.08 | 27.51±3.21 | 38.95±1.69 | 44.06±1.79 | 2.22±0.43 | 1.03±0.25 | 1.08±0.01 |
| C18:1[b] | 2.67±0.20 | 2.16±0.18 | 1.65±0.18 | 1.72±0.11 | 0.44±0.14 | 0.45±0.21 | 0.21±0.09 | 0.16±0.02 | 0.11±0.00 | 0.07±0.03 |
| C18:2 | 0.51±0.26 | 0.87±0.03 | 10.64±1.29 | 15.29±2.84 | 14.18±0.62 | 9.37±2.85 | 4.08±1.76 | 21.86±1.11 | 17.45±1.44 | 24.09±2.73 |
| C20:1 | | | 50.79±3.22 | 44.45±2.37 | 18.64±4.93 | 6.91±1.85 | 1.6±0.97 | 50.44±1.54 | 59.94±2.71 | 48.55±2.90 |
| C20:5 | | | 0.53±0.09 | 0.12±0.48 | | | | 1.14±0.06 | 0.89±0.16 | 0.58±1.17 |
| C22:1 | | | 1.15±0.19 | 1.31±0.25 | | | | 1.11±0.01 | 0.86±0.15 | 0.94±0.09 |
| C22:6 | | | 2.29±0.46 | 1.47±0.12 | | | | | | 0.04±0.07 |
| | | | | | | | | | | |
| Σ 16C | 85.33±0.15 | 88.13±2.96 | 22.71±0.65 | 25.64±0.66 | 35.96±1.52 | 41.29±2.23 | 45.52±2.56 | 20.71±0.35 | 17.81±0.60 | 22.3±1.40 |
| Σ 18C | 10.6±0.98 | 8.73±1.01 | 20.73±1.97 | 25.13±4.06 | 44.95±4.26 | 51.32±6.63 | 52.27±5.21 | 25.92±1.84 | 19.99±1.79 | 26.92±3.01 |
| Σ 20C | | | 52.1±2.28 | 45.04±2.98 | 18.64±4.93 | 6.92±1.88 | 1.63±0.99 | 51.68±1.61 | 60.94±2.89 | 49.36±4.14 |
| Σ SFA | 52.97±0.7 | 50.01±1.88 | 25.02±0.66 | 28.23±0.77 | 37.52±1.48 | 42.48±3.73 | 48.06±3.3 | 22.8±0.63 | 19.4±0.71 | 24.13±1.58 |
| Σ MUFAs | 45.78±0.24 | 49.13±2.14 | 60.74±2.87 | 54.51±3.93 | 48.3±8.62 | 48.15±4.22 | 47.73±3.47 | 54.12±2.01 | 62.15±3.12 | 50.9±3.04 |
| Σ PUFAs | 1.25±0.26 | 0.87±0.03 | 14.24±5.77 | 17.26±3.56 | 14.18±0.62 | 9.37±2.85 | 4.21±2.02 | 23.09±1.17 | 18.45±1.61 | 24.98±4.12 |

[a] C18:1 Methyl oleate (112-62-9)
[b] C18:1 Methyl vaccenate (1937-63-9)

to previous observations by Roleda *et al.* (2013), who concluded that cultivation under optimal conditions resulted in a decrease in saturation levels, and an increase in fatty acid chain length, however the opposite was found for *Leptolyngbya* sp. QUCCCM 56, suggesting that the effect is strain dependent [57].

According to Ramos *et al.* (2009), who created a predictive model for which FAME compositions would satisfy European standards when converted to biodiesel, all 4 of the tested strains would satisfy the limit of cetane number and iodine value [58]. However, Only *P. maculatum* QUCCCM 127 cultivated at 35 ˚C had sufficient levels of monounsaturated fatty acids (MUFAs) to satisfy the cold filter plugging point (CFPP) requirements as well, making this strain optimally suitable for biodiesel purposes. *T. subcordiformis* QUCCCM 51, being second best, lacked sufficient MUFAs to satisfy all requirements, however as decreasing temperatures showed an increase in MUFAs, this could be further investigated.

### 2.3.3 BIOMASS NITROGEN REQUIREMENTS

Total nitrogen content ($X_N$) analysis of the strains showed that both *T. subcordiformis* QUCCCM 51 and *Chroococcidiopsis* sp. QUCCCM 26 had the lowest total nitrogen content at 5.54±0.61% and 4.36±0.07% respectively, both at 30 ˚C (Table 2.3). The total nitrogen content of these two strains was inversely correlated to the biomass productivity, with higher productivities coupled to lower total nitrogen levels. The low total nitrogen content is promising from a commercial perspective, as it requires less nutrients per unit of biomass produced, which in turn can reduce the biomass production costs.

**Table 2.3** – Total Carbon ($X_C$) and Nitrogen ($X_N$) content (% w/w) of the 4 investigated strains cultivated at 30, 35 and 40 ˚C. Data shown is the mean±range (n=2)

| Strain | Temperature | Nitrogen Content | Carbon Content |
|---|---|---|---|
| | | *% (w/w)* | *% (w/w)* |
| *Chroococcidiopsis* sp. QUCCCM 26 | 30˚C | 4.36 ± 0.07 | 35.86 ± 0.77 |
| | 35˚C | 6.05 ± 0.38 | 39.68 ± 0.80 |
| *T. subcordiformis* QUCCCM 51 | 30˚C | 5.54 ± 0.61 | 46.85 ± 1.08 |
| | 35˚C | 7.06 ± 0.78 | 47.56 ± 1.20 |
| *Leptolyngbya* sp. QUCCCM 56 | 30˚C | 9.19 ± 0.32 | 44.15 ± 0.53 |
| | 35˚C | 9.62 ± 2.04 | 47.00 ± 1.08 |
| | 40˚C | 9.55 ± 0.32 | 47.79 ± 0.37 |
| *P. maculatum* QUCCCM 127 | 30˚C | 11.49 ± 0.16 | 50.58 ± 1.19 |
| | 35˚C | 11.49 ± 0.33 | 51.24 ± 0.36 |
| | 40˚C | 12.17 ± 0.07 | 50.95 ± 1.29 |

**Table 2.4** – Total Nitrogen content in media ($C_N$) and biomass ($X_N$) and biomass concentration ($C_X$) for *Chroococcidiopsis* sp. QUCCCM 26 for cultivation at 35˚C. Data shown is the mean±range (n=2)

| Time | Total Nitrogen in Media | Biomass Concentration | Total Nitrogen in Biomass |
|---|---|---|---|
| *day* | *mg N·L$^{-1}$* | *mg·L$^{-1}$* | *% (w/w)* |
| 0 | 59.5 ± 5.0 | 74.34 ± 3.33 | - |
| 8 | 0[a] ± 0 | 953.50 ± 23.00 | 6.03 ± 0.23 |
| 17 | - | 1719.31 ± 7.37 | 6.04 ± 0.38 |

[a] measurement below detection limit of 1 mg TN·L$^{-1}$



Both *Leptolyngbya* sp. QUCCCM 56 and *P. maculatum* QUCCCM 127 showed high total nitrogen contents, ranging from 9.2-9.6% and 11.5-12.2% for the two strains respectively. The total nitrogen content of *P. maculatum* QUCCCM 127 in particular was significantly higher than the standard assumed 6.6% (w/w) derived from the molecular formula for microalgae biomass generally applied [25], and should be taken into account when customizing a growth media for this strain.

For the analysis, all strains were harvested once the stationary phase was reached. *Chroococcidiopsis* sp. QUCCCM 26 however, did not reach the stationary phase (Figure 2.5 for cultivation at 35 ˚C). In the first 8 days of cultivation exponential growth was observed, and after the nitrogen was depleted (day 8), growth continued linearly. This could indicate that the first growth-limiting factor was nitrogen, and not light. The total nitrogen content in the biomass was measured at $t_{N=0}$ and $t_{end}$, as well as the total nitrogen concentration in the media, and the biomass concentration over time (Table 2.4).

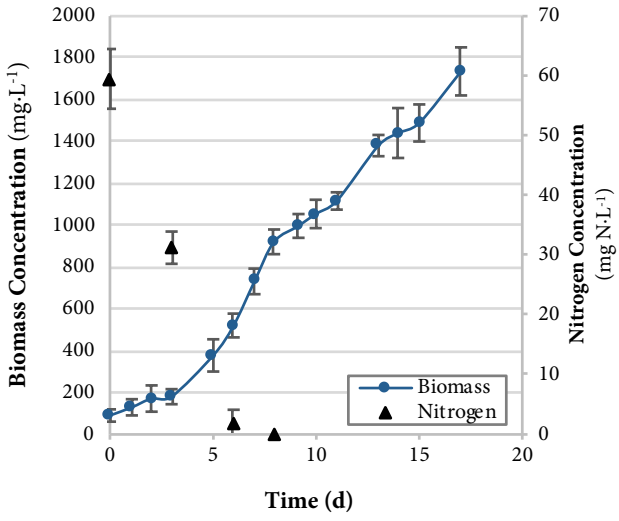


**Figure 2.5** – *Chroococcidiopsis* sp. QUCCCM26 Biomass Concentration (● - mg·L$^{-1}$) and nitrogen concentration (▲ - mg N·L$^{-1}$) over time at 35˚C. Data shown is the mean±range (n=2)

Results show that the nitrogen content of the biomass remained stable at 6.0% for cultivation under nitrogen-rich and nitrogen depleted conditions. Performing a nitrogen mass balance showed that 99.4±4.4 mg $N{\cdot}L^{-1}$ was incorporated into the biomass at the end of the cultivation, which was 39.9±5.6 mg $N{\cdot}L^{-1}$ more than the 59.5±3.5 mg $N{\cdot}L^{-1}$ provided in the media at the time of inoculation. This indicates that the strain was able to incorporate nitrogen from an alternative source, presumably $N_2$. *Chroococcidiopsis*, which is a non-heterocystous cyanobacteria from the Pleurocapsalean group, has previously been reported be able to synthesize nitrogenase [59]. Nitrogenase however, is sensitive to oxygen, and generally nitrogen fixation in non-heterocystous bacteria occurs under stringent anaerobic conditions [60].

Rippka and Waterbury (1977) as well as Billi and Caiola (1996) reported that the *Chroococcidiopsis* strains tested were unable to grow photosynthetically under aerobic conditions in the absence of a combined nitrogen source [59,61]. Hayashi *et al.* (1994) however did report a *Chroococcidiopsis* isolate to be capable of nitrogen fixation under aerobic conditions [62]. As the current results were obtained under conditions with 0.21 $pO_2$, and only an inorganic carbon source, this indicates that the strain could be capable of simultaneous oxygen evolving photosynthesis and oxygen-sensitive nitrogen fixation, under aerobic conditions.

### 2.3.4 CARBON CAPTURE RATE

Total carbon analysis of the strains showed that *P. maculatum* QUCCCM 127 had the highest carbon content of all strains at 51.2%, with no significant variation ($p > 0.05$) over the different temperatures (Table 2.3). The carbon content of *Leptolyngbya* sp. QUCCCM 56 showed a significant increase in carbon content for increasing temperatures ($p < 0.05$). Determination of the Carbon Capture Rate ($R_{CO_2}$) showed that *T. subcordiformis* QUCCCM 51 had the highest $R_{CO_2}$ at 270.8±23.9 mg $CO_2{\cdot}L^{-1}{\cdot}d^{-1}$ at 30 ˚C (Table 2.5). This value is significantly higher compared to the maximum value of 111.26 mg $CO_2{\cdot}L^{-1}{\cdot}d^{-1}$ found by Kassim and Meng (2017) for *Tetraselmis suecica*, a strain which has been researched for its capability in carbon capture [63,64]. *Leptolyngbya* sp. QUCCCM 56 showed the second-best carbon capture potential with 186.8±16.0

**Table 2.5 –** Carbon capture rate, $R_{CO2}$ (mg $CO_2{\cdot}L^{-1}{\cdot}d^{-1}$) for the investigated strains at 30 ˚C, 35 ˚C, and 40 ˚C. Data shown is the mean±range (n=2)

| **Strain** | **Carbon Capture Rate** $mg{\cdot}L^{-1}{\cdot}d^{-1}$ | | |
|---|---|---|---|
| | 30 ˚C | 35 ˚C | 40 ˚C |
| *Chroococcidiopsis* sp. QUCCCM 26 | 147.4 ± 11.0 | 139.7 ± 3.1 | - |
| *T. subcordiformis* QUCCCM 51 | 270.9 ± 23.9 | 219.1 ± 44.1 | - |
| *Leptolyngbya* sp. QUCCCM 56 | 148.0 ± 17.3 | 181.1 ± 44.0 | 186.8 ± 16.0 |
| *P. maculatum* QUCCCM 127 | 179.5 ± 21.5 | 184.8 ± 14.5 | 163.4 ± 8.2 |

mg $CO_2 \cdot L^{-1} \cdot d^{-1}$ at 40 °C. This is higher than the carbon capture rate found by Choix *et al.* (2017) for *Leptolyngbya* sp. CChF1 159 ± 40 mg $CO_2 \cdot L^{-1} \cdot d^{-1}$[51]. Besides, Choix *et al.* (2017) based the carbon capture rate on the hypothetical carbon content of 51.4% [25] which is an overestimation according to the findings of the current study, where total carbon values of 44-47% were found for *Leptolyngbya* sp. QUCCCM 56.



Overall, the carbon capture rates found in this work were on par with similar research on other strains, for example Tang *et al.* (2011) [46] found values of 288 and 260 mg $CO_2 \cdot L^{-1} \cdot d^{-1}$ for *Scenedesmus obliquus* SJTU-3 and *Chlorella pyrenoidosa* SJTU-2 respectively, and Sydney *et al.* (2010) [65] found 252, 318 and 272 mg $CO_2 \cdot L^{-1} \cdot d^{-1}$ for *Chlorella vulgaris*, *Spirulina platensis*, and *Dunaliela tertiolecta* respectively [66]. It is expected that the $R_{CO2}$ of the investigated strains could be further improved, for example, Tang *et al.* (2011) [46] showed that varying $CO_2$ concentrations (10% $CO_2$ compared to air) could increase the carbon capture rate as much as 92%. This improvement was however mainly related to an increase of biomass productivity, presumably due to an increase in $CO_2$ mass transfer rate, rather than an increase in carbon content of the strain, indicating that biomass productivity improvement is the main determining factor in regards to improving the carbon capture rate.

### 2.3.5 $CO_2$ TOLERANCE

Biomass productivities and tolerance of the four strains, under increased concentrations of $CO_2$ in the gas phase, was studied for $CO_2$ concentrations ranging from 5% to 30% (v/v). For certain strains, *Chroococcidiopsis* sp. QUCCCM 26 and *Leptolyngbya* sp. QUCCCM 56 in particular, the optical density measurements showed to be unreliable as large variances occurred within the triplicate samples, which is thought to be due to the nature of these strains, which exhibited aggregation and/or biofilm formation in the wells due to the lack of agitation during cultivation (data not shown). For this reason, optical density measurements were used solely for growth-phase monitoring, and productivities were based on biomass dry weights. The dry-weight based biomass productivities of the four strains under various $CO_2$ concentrations are shown in Figure 2.6.

With productivities of 53.7±16.1, 162.2±7.6, 333.8±41.1 and 312.8±18.1 mg$\cdot L^{-1} \cdot d^{-1}$ for 5, 10, 20 and 30% $CO_2$ respectively, *T. subcordiformis* QUCCCM 51 showed that significant productivity increases could be obtained with increased $CO_2$ concentrations ($p < 0.05$). The maximum productivity was obtained at 20% $CO_2$. Assuming the same $C_C$ as reported above (Table 2.3), the estimated $R_{CO2}$ for *T. subcordiformis* QUCCCM 51 under 20% $CO_2$ was calculated at 573.4±16.5 mg $CO_2 \cdot L^{-1} \cdot d^{-1}$, which is an 112% increase compared to the $R_{CO2}$ found above (Table 2.5). *P. maculatum* QUCCCM 127 showed a similar trend of increasing productivities for increasing $CO_2$ concentrations up to 20%, with productivities of 67.1±0.3, 160.5±30.9, 244.1±29.5 and 219.9±22.1

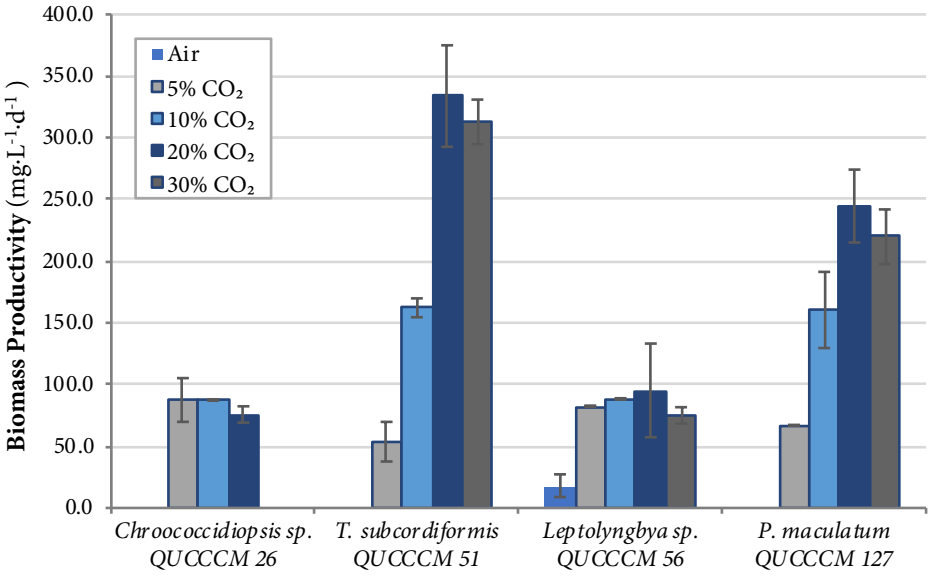


**Figure 2.6 –** Productivities in mg·L$^{-1}$·d$^{-1}$ of various Qatari Marine strains under $CO_2$ concentrations of 0.004 (■), 5 (■), 10 (■), 20 (■) and 30% (■). Data shown is the mean±range (n=2)

mg·L$^{-1}$·d$^{-1}$ for 5, 10, 20 and 30% $CO_2$ respectively. The estimated $R_{CO2}$ for *P. maculatum* QUCCCM 127 under the optimal $CO_2$ concentration of 20%, assuming the same $X_C$ as reported in Table 2.3, was 388.9±1.9 mg $CO_2$·L$^{-1}$·d$^{-1}$.

Both cyanobacteria, *Leptolyngbya* sp. QUCCCM 56 and *Chroococcidiopsis* sp. QUCCCM 26, did not show a similar productivity increase when exposed to higher $CO_2$ concentrations. *Leptolyngbya* sp. QUCCCM 56 even showed no significant difference in productivities for $CO_2$ concentrations ranging from 5-30% ($p > 0.05$), and *Chroococcidiopsis* sp. QUCCCM 26 showed stable productivities at 5, 10 and 20% $CO_2$ ($p > 0.05$), and no growth at 30% $CO_2$. The latter is similar to what was found by Hayashi *et al.* (1995), who showed that high $CO_2$ concentrations inhibited growth of *Chroococcidiopsis* [67].

A possible reason for the varying $CO_2$-incited responses of the strains could be the difference in the $CO_2$ Concentrating Mechanisms (CCM) of the prokaryotic cyanobacteria versus the eukaryotic microalgae. Generally speaking, cyanobacteria possess high-affinity CCMs, well adapted to environments with low low-inorganic carbon concentrations, and high pH, such as algal-blooms. Microalgae on the other hand, are generally known to possess low-affinity but high-flux CCMs, and are expected to out-compete cyanobacteria in high inorganic carbon environments [68]. The possible presence of a high-affinity low-flux CCM in the cyanobacteria in this study could have limited the rate at which inorganic carbon was transferred over the

cell-membrane, which could explain why the productivity of these strains did not improve with increased $CO_2$ concentrations. Even under elevated $CO_2$ conditions, where intracellular $CO_2$ concentrations are high due to passive diffusion over the cell-membrane, the conversion of this $CO_2$ into $HCO_3^-$ by NADPH-dependent reactions facilitated by NDH-1 complexes can be rate limiting in the supply of $HCO_3^-$ to Rubisco [69]. An additional theory which would explain the difference in behavior is the pH. Under the experimental conditions, for both cyanobacteria the pH decreased from 9.5 to 6.6 for $CO_2$ concentrations of 0.04 – 30% respectively. For both microalgae, the pH difference was significantly smaller, decreasing from 9.6 to 9.2 and 8.9 to 8.0 for 0.04-20% $CO_2$ for *T. subcordiformis* QUCCCM 51 and *P. maculatum* QUCCCM 127 respectively. The pH is considered an important factor in competitiveness of cyanobacteria over microalgae, as it influences the $CO_2/HCO_3^-$ ratio, and cyanobacteria generally have been found to prefer high pH environments [68,70]. As here the high $CO_2$ concentrations lead to a reduction in pH, it should be further investigated whether higher productivities could be obtained under high pH conditions concurrent with high inorganic carbon concentrations.



## 2.4 CONCLUSION

Four novel isolates from the Arabian Gulf were characterized for productivity under elevated temperatures and $CO_2$ concentrations, two industrially relevant conditions. Both *Picochlorum* sp. and *Leptolyngbya* sp. grew well under elevated temperatures up to 40 ˚C, with the latter able to survive short durations of exposure to even higher temperatures. Both microalgae investigated were found to be rich in lipids, with FAME profiles indicating suitability for biodiesel and omega-3 fatty acid production. The cyanobacteria investigated were less suitable for biofuel production due to their lower lipid content, however the presence of high-value phycobiliproteins could be further investigated. *Tetraselmis* sp., *Picochlorum* sp. and *Leptolyngbya* sp. were all able to grow under elevated $CO_2$ concentrations of up to 30%. Further investigation into these strains is necessary to identify conditions which increase product productivity, and the capability of direct coupling to industrial flue gas.

## 2.5 SUPPLEMENTAL MATERIALS

### SUPPLEMENT A BIOTOXIN ANALYSIS

**Table S.2.1** – Biotoxin analysis results for cyanobacterial strains QUCCCM 26 and QUCCCM 56, as analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

| **Biotoxins Analyzed** | | **QUCCCM 26** | **QUCCCM 56** |
|---|---|---|---|
| | | $\mu g \cdot g^{-1}$ | $\mu g \cdot g^{-1}$ |
| Paralytic Shellfish Toxins | C1 | < 0.2 | < 0.2 |
| | C2 | < 0.2 | < 0.2 |
| | C3 | < 0.2 | < 0.2 |
| | C4 | < 0.2 | < 0.2 |
| | dcGTX2 | < 0.2 | < 0.2 |
| | dcGTX3 | < 0.2 | < 0.2 |
| | GTX2 | < 0.2 | < 0.2 |
| | GTX3 | < 0.2 | < 0.2 |
| | GTX1 | < 0.2 | < 0.2 |
| | GTX4 | < 0.2 | < 0.2 |
| | GTX5 | < 0.2 | < 0.2 |
| | GTX6 | < 0.2 | < 0.2 |
| | doSTX | < 0.05 | < 0.05 |
| | dcSTX | < 0.05 | < 0.05 |
| | dcNEO | < 0.05 | < 0.05 |
| | STX | < 0.05 | < 0.05 |
| | NEO | < 0.05 | < 0.05 |
| Anatoxin/Cylindrospermopsin | ATX | < 0.03 | < 0.03 |
| | HTX | < 0.03 | < 0.03 |
| | dhATX | < 0.03 | < 0.03 |
| | dhATX | < 0.03 | < 0.03 |
| | CYN | < 0.04 | < 0.04 |
| | DO-CYN | < 0.04 | < 0.04 |
| Microcystin/Nodularin | MC-RR | < 0.001 | < 0.001 |
| | dmMC-RR | < 0.001 | < 0.001 |
| | didmMC-RR | < 0.001 | < 0.001 |
| | MC-YR | < 0.001 | < 0.001 |
| | MC-LR | < 0.001 | < 0.001 |
| | dmMC-LR | < 0.001 | < 0.001 |
| | MC-AR | < 0.001 | < 0.001 |
| | MC-FR | < 0.001 | < 0.001 |
| | MC-WR | < 0.001 | < 0.001 |
| | MC-RA | < 0.001 | < 0.001 |
| | MC-RAba | < 0.001 | < 0.001 |
| | MC-LA | < 0.001 | < 0.001 |
| | MC-FA | < 0.001 | < 0.001 |
| | MC-WA | < 0.001 | < 0.001 |
| | MC-LAba | < 0.001 | < 0.001 |
| | MC-FAba | < 0.001 | < 0.001 |
| | MC-WAba | < 0.001 | < 0.001 |
| | MC-LY | < 0.001 | < 0.001 |
| | MC-LF | < 0.001 | < 0.001 |
| | MC-LW | < 0.001 | < 0.001 |
| | Nodularin-R | < 0.001 | < 0.001 |

## SUPPLEMENT B PHYCOBILIPROTEIN ANALYSIS

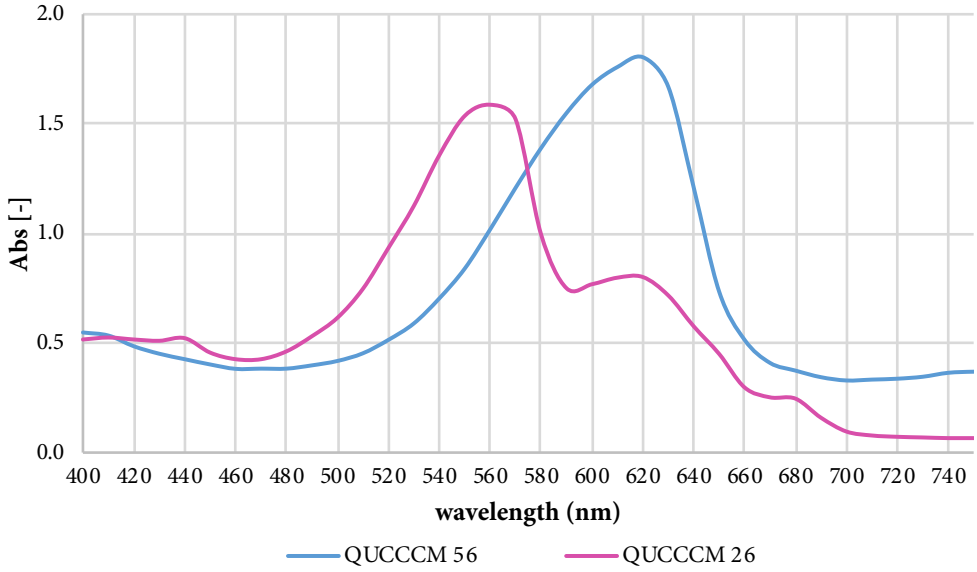


**Figure S.2.1** – Absorption spectrum of water extracts from QUCCCM 26 and QUCCCM 56, cultuvated at 30 °C

**Table S.2.2 –** Growth rates, Productivities, Final Biomass Concentrations and Biomass Compositions of the investigated strains at 30 °C, 35 °C, and 40°C. Data shown is the mean±range, n=2

| Strain | Temp. | Max. Specific Growth rate | Biomass Productivity | Final Biomass Concentration | Lipid Content | Carbohydrate Content | Protein Content | Nitrogen Content | Carbon Content |
|---|---|---|---|---|---|---|---|---|---|
| | | $d^{-1}$ | $mg\ L^{-1}\ d^{-1}$ | $mg\ L^{-1}$ | % (w/w) | % (w/w) | % (w/w) | % (w/w) | % (w/w) |
| *Chroococcidiopsis* sp. QUCCCM 26 | 30°C | 0.315±0.004 | 112.20±10.79 | 2400.0±229.0 | 16.05±1.50 | 37.95±3.90 | 20.85±0.34 | 4.36±0.07 | 35.86±0.77 |
| | 35°C | 0.354±0.008 | 96.00±0.24 | 1719.3±7.4 | 20.00±2.00 | 40.50±1.00 | 28.90±1.81 | 6.05±0.38 | 39.68±0.80 |
| *Tetraselmis* sp. QUCCCM 51 | 30°C | 0.472±0.074 | 157.67±10.29 | 1493.2±109.7 | 25.55±0.90 | 39.75±1.50 | 26.50±2.93 | 5.54±0.61 | 46.85±1.08 |
| | 35°C | 0.283±0.017 | 125.53±22.10 | 1204.5±209.0 | 22.45±1.10 | 21.75±4.90 | 33.74±3.73 | 7.06±0.78 | 47.56±1.20 |
| *Leptolyngbya* sp. QUCCCM 56 | 30°C | 0.377±0.004 | 91.38±9.57 | 728.0±59.0 | 14.90±0.60 | 14.75±4.50 | 43.91±1.55 | 9.19±0.32 | 44.15±0.53 |
| | 35°C | 0.393±0.091 | 105.28±27.94 | 842.8±183.5 | 15.10±2.60 | 17.50±3.00 | 45.99±9.76 | 9.62±2.04 | 47.00±1.08 |
| | 40°C | 0.382±0.005 | 106.59±9.97 | 827.8±66.5 | 14.65±0.50 | 22.45±2.30 | 45.63±1.55 | 9.55±0.32 | 47.79±0.37 |
| *Picochlorum* sp. QUCCCM 127 | 30°C | 0.340±0.020 | 96.72±9.34 | 836.8±78.4 | 24.05±1.90 | 14.00±2.00 | 54.92±0.77 | 11.49±0.16 | 50.58±1.19 |
| | 35°C | 0.372±0.004 | 98.35±8.41 | 832.5±67.0 | 28.00±2.00 | 7.15±0.30 | 54.92±1.57 | 11.49±0.33 | 51.24±0.36 |
| | 40°C | 0.373±0.055 | 87.46±2.14 | 757.0±16.0 | 27.00±2.00 | 9.00±1.40 | 58.16±0.32 | 12.17±0.07 | 50.95±1.29 |

## SUPPLEMENT D PRODUCTIVITY DATA UNDER VARIOUS $CO_2$ CONCENTRATIONS



**Table S.2.3 –** Biomass Productivities of the of the investigated strains under $CO_2$ concentrations of 0.004, 5, 10, 20 and 30%. Data shown is the mean±range, n=2

| **Strain** | **Biomass Productivity** *mg $L^{-1}$ $d^{-1}$* | | | | |
|---|---|---|---|---|---|
| | **Air** | **5% $CO_2$** | **10% $CO_2$** | **20% $CO_2$** | **30% $CO_2$** |
| *Chroococcidiopsis* sp. QUCCCM 26 | nd ± nd | 87.5 ± 17.7 | 87.5 ± 0.5 | 75.8 ± 6.6 | nd ± nd |
| *T. subcordiformis* QUCCCM 51 | nd ± nd | 53.7 ± 16.1 | 162.2 ± 7.6 | 333.8 ± 41.1 | 312.8 ± 18.1 |
| *Leptolyngbya* sp. QUCCCM 56 | 18.0 ± 9.20 | 82.4 ± 0.5 | 91.5 ± 0.6 | 98.4 ± 38.0 | 78.2 ± 6.6 |
| *Picochlorum* sp. QUCCCM 127 | nd ± nd | 95.4 ± 0.3 | 181.4 ± 30.9 | 207.0 ± 29.5 | 170.2 ± 22.1 |

nd: not detected

# Chapter 3

# Production of phycocyanin by *Leptolyngbya* sp. in desert environments

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## ABSTRACT

*Leptolyngbya* sp. QUCCCM 56 was investigated as a possible alternative to *A. platensis*, for the production of phycocyanin-rich biomass under desert conditions. Under elevated temperatures and light intensities, of up to 40 ˚C and 1800 µmol photons·$m^{-2}$·$s^{-1}$, the strain's biomass productivity was up to 45% higher as compared to reported productivities for *A. platensis*, with comparable phycocyanin content. Increasing temperatures were found to improve the biomass productivity and phycocyanin content, which, at 40 ˚C, were 1.09±0.03 $g_X$·$L^{-1}$·$d^{-1}$ and 72.12±3.52 $mg_{PC}$·$g_X^{-1}$, respectively. The optimum biomass productivity was found at a light intensity of 300 µmol photons·$m^{-2}$·$s^{-1}$, with higher light intensities causing a decrease of 15%. Furthermore, of the various phycocyanin extraction methods tested, bead-beating in phosphate buffer had the highest combined phycocyanin yield (169.9±3.6 $mg_{PC}$·$g_X$) and purity (7.37±0.16) for *Leptolyngbya* sp.. For *A. platensis*, this extraction method also resulted in the highest extract purities (3.78±0.04). The extract purities obtained for *Leptolyngbya* sp. are considerably higher than other reported phycocyanin purities, and further investigation is recommended to study the scale-up of both *Leptolyngbya* sp. and bead-beating for commercial scale high-grade phycocyanin production under desert conditions.

## 3.1 INTRODUCTION

Phycocyanin is a water-soluble pigment-protein complex unique to cyanobacteria and eukaryotic algae, which functions as light-harvesting complex that absorbs light in regions of the visible spectrum that are poorly absorbed by chlorophyll. Applications of phycocyanin in biotechnological processes, as well as in the food and pharmaceutical industries, are increasing as it is a natural source of bioactive-pigment, with antioxidant, anticancer, and anti-inflammatory effects [18,71,72].

Presently, the main source of commercial phycocyanin is *Arthrospira platensis*, which generally contains around 7% phycocyanin (dry weight basis), however values of up to 18% have also been reported [73]. Furthermore, volumetric biomass productivities of *A. platensis* have been reported up to 0.32 and 1.59 $g_X \cdot L^{-1} \cdot d^{-1}$ for open raceway ponds and novel tubular reactors, respectively [74,75]. Even though *A. platensis* is widely cultivated, limitations still exist under elevated temperatures and light intensities [76]. This is especially a concern for production in desert climates, where temperatures and light intensities can reach extreme levels, but also in more temperate regions, when cultivating in closed reactors, in which temperatures can increase significantly. High light intensities and temperatures do not only have a negative effect on biomass productivity, but can also have a negative effect on the phycocyanin content of the strain [21].

*Leptolyngbya*, a member of the Oscillatoriales order, is one of the most common cyanoprokaryotic organisms, and has been found in an extreme diverse range of ecological habitats, ranging from desert environments to hot springs and even the coastal waters of Antarctica [77-79]. At present, 158 species have been taxonomically classified to the genus [80]. Despite its abundant global presence, which would signify the genera's highly competitive edge over other cyanoprokaryotic strains, there is limited research into the genus' commercial potential. The research on applications is limited to identification of the strain as an interesting candidate for bioremediation of $CO_2$ streams and biofuel production [50,51,77,81] and as a possible candidate for wastewater treatment [82,83]. Furthermore, the strain has been identified as a potential producer of phycobiliproteins (amongst which phycocyanin) [84,85], as well as a potential alternative to *Arthrospira,* possessing advantageous characteristics in terms of high biomass productivity, protein and lipid content, under a wide range of temperatures (10-40 ˚C) and salinities (0-80 ppt) [53,86]. Nonetheless, no studies have been found which look into the commercial application for the production of phycocyanin from *Leptolyngbya*.

The aim of this study is to quantify and optimize biomass and phycocyanin productivity of *Leptolyngbya* sp. QUCCCM 56, a thermotolerant marine cyanobacteria isolated from Qatar

[77]. Focus was specifically on assessing the strain's performance under desert conditions, with temperatures and light intensities of up to 45 ˚C and 1800 µmol photons·$m^{-2}$·$s^{-1}$ respectively. Furthermore, the optimal phycocyanin extraction protocol for *Leptolyngbya* sp. was investigated, to allow for rapid phycocyanin extraction with high yields and purities. The effectiveness of the various phycocyanin extraction protocols was compared with *A. platensis* in order to verify the methods as well as compare extract yields and purities between the two strains.

## 3.2 MATERIALS AND METHODS

### 3.2.1 CULTURES

*Leptolyngbya* sp. QUCCCM 56 was obtained from the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM, Doha, Qatar). *Arthrospira platensis* UTEX 1940 was obtained from the UTEX® Culture Collection of Algae (University of Texas, Austin, USA). Stock cultures were maintained in 250 mL conical flasks with a working volume of 100 mL, in Zarrouk medium [87], and pH was not controlled. Flasks were kept in an environmental incubator (Snijders Scientific®; Micro Clima-Series; Economic Lux Chamber) at 30 °C and 25 °C, for *Leptolyngbya* sp. QUCCCM 56 and *A. platensis*, respectively, under a 12:12h light:dark cycle with a light intensity of 85±5 µmol photons·$m^{-2}$·$s^{-1}$, and the culture was agitated using a flask shaker set at 150 rpm (Heidolph Instruments® Rotamax 120).

### 3.2.2 EFFECTS OF LIGHT INTENSITY AND TEMPERATURE ON BIOMASS PRODUCTIVITY, AND PHYCOCYANIN CONTENT AND PRODUCTIVITY

*Leptolyngbya* sp. QUCCCM 56 cultures were inoculated in flat-panel airlift photobioreactors (Algaemist, Technical Development Studio, Wageningen University, the Netherlands) with a working volume of 0.4 L ($V_R$), an optical depth of 14 mm, and one-sided illumination by six broad spectrum LEDs (BXRA W1200, Bridgelux, USA), over an illumination area of 0.028 $m^2$ under a 12:12h light:dark cycle [88]. Aeration was set at 200±20 ml·$min^{-1}$ with $CO_2$ added to maintain a pH of 9.0±0.1. Cultures were initiated as batch, and after reaching a biomass concentration of 1.0 $g_X$·$L^{-1}$ operation mode was set to turbidostat and a constant biomass concentration was maintained. The optical density (750 nm) and harvest volume ($F_H$, L·$d^{-1}$) were measured every 24 h, and biomass dry weight determinations were performed every 48 h. Biomass productivity and phycocyanin content were evaluated for six light intensities (80, 160, 300, 700, 1000 and 1800 µmol photons·$m^{-2}$·$s^{-1}$) and six temperatures (20, 25, 30, 35, 40, and 45 ˚C). When not under investigation, standard temperature and light intensity set-points of 30 ˚C and 300 µmol photons·$m^{-2}$·$s^{-1}$ were used, respectively. Samples were taken for 3 consecutive days once a steady state was reached (stable

biomass concentration, $C_X$, $g_X \cdot L^{-1}$, and harvest volume for 2 consecutive days) from duplicate reactors (n=6), and the harvest volume, biomass concentration, and phycocyanin content ($X_{PC}$, $mg_{PC} \cdot g_X^{-1}$) were determined. Biomass productivities ($P_X$, $g_X \cdot L^{-1} \cdot d^{-1}$) and phycocyanin productivities ($P_{PC}$, $mg_{PC} \cdot L^{-1} \cdot d^{-1}$) were calculated as per equations 3.1 and 3.2, respectively.

$$P_X = \frac{F_H \cdot C_X}{V_R} \quad \text{(eq. 3.1)}$$

$$P_{PC} = P_X \cdot X_{PC} \quad \text{(eq. 3.2)}$$

### 3.2.3 PHYCOCYANIN EXTRACTION OPTIMIZATION & MEASUREMENTS

Biomass aliquots containing 5 mg and 10 mg of biomass of *Leptolyngbya* sp. QUCCCM 56 and *A. platensis* were taken from batch cultures cultivated in flasks (as described in 3.3.1). Biomass was separated from the media by centrifugation (30 min at 4200 RCF at 4 ˚C), after which pellets were resuspended in 1.25 mL of either a) Phosphate Buffer (0.1 M 6.0 pH), b) Calcium Chloride ($10\ g \cdot L^{-1}$), or c) Milli-Q water. Samples were subjected to either i) freeze-thawing (incubated at -20 ˚C until solid, followed by thawing for 24 h at 4˚C in the dark), ii) bead-beating (3 cycles of 25 sec at 2500 rpm, Bertin® Precellys 24 and Lysing Matrix Tubes, Lysing Matrix E, 2 mL tubes, containing 1.4 mm ceramic spheres, 0.1 mm silica spheres, and one 4 mm glass bead, mpbio®), or iii) sonication (5 sec pulses of 8 W over 30 sec, on ice, Sonics® VCX 130 Ultrasonic processor). After all treatments, the samples were centrifuged (20238 RCF for 30 min at 4 ˚C), and the pellet and supernatant were separated. Phycocyanin was determined in the supernatant, and the pellet was resuspended in an equal volume of fresh extraction buffer and incubated for an additional 24 h at 4 ˚C in the dark. The process of centrifugation and resuspension was repeated twice more (48 h and 96 h), or until no significant amount of phycocyanin was extracted during subsequent incubation times. For freeze-thawing, no direct measurements were performed due to the nature of the treatment, requiring at least 24 h incubation time. Phycocyanin concentrations ($C_{PC}$, $mg_{PC} \cdot L^{-1}$) were determined as per Lawrenz *et al.* (2011, 2013) [89,90] (equation 3.3).

$$C_{PC} = \frac{Abs_{620} - Abs_{750}}{\varepsilon d} \cdot M_{PC} \cdot \frac{V_{buffer}}{V_{sample}} \cdot 10^6 \quad \text{(eq. 3.3)}$$

In which $Abs_{620}$ and $Abs_{750}$ are the measured absorbances of the phycocyanin extract at 620 and 750 nm, respectively, which were determined using a DR 6000 UV-VIS spectrophotometer (Hach-Lange, CO, USA). ε, $d$ and $M_{PC}$ are the molar extinction coefficient of phycocyanin ($1.9 \times 10^6$ $L \times mol^{-1} \times cm^{-1}$), path length of the cuvette (1 cm), and the molecular weight of phycocyanin (264,000 $g \times mol^{-1}$), respectively. $V_{buffer}$ and $V_{sample}$ are the volume of the buffer and sample. The phycocyanin content ($X_{PC}$, $mg_{PC} \cdot g_X^{-1}$) was then determined as per equation 3.4:

$$X_{PC} = \frac{C_{PC}}{C_X} \quad \text{(eq. 3.4)}$$

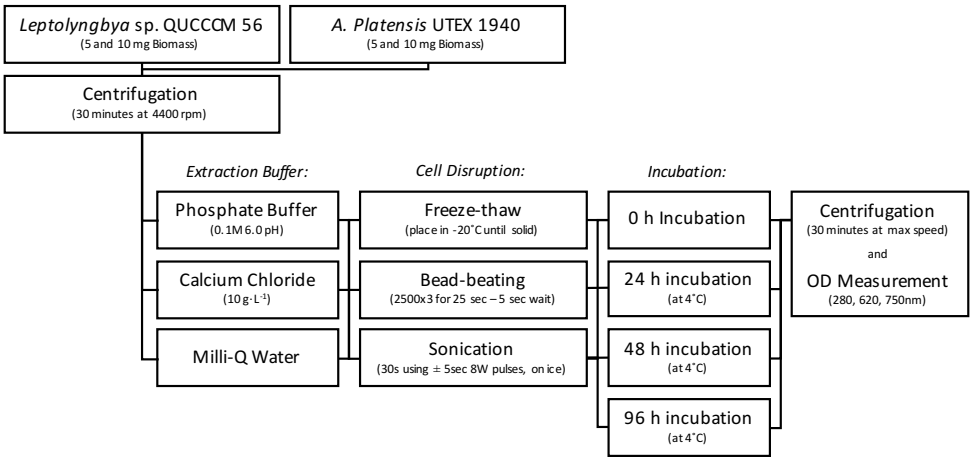


**Figure 3.1** – Overview of extraction buffers, cell disruption methods, and incubation times tested for phycocyanin extraction from *Leptolyngbya* sp. QUCCCM 56 and *A. platensis* UTEX 1940.

In which $C_X$ is the concentration of biomass in $g_X \cdot L^{-1}$. In addition to absorbance measurements at 620 nm and 750 nm, the absorbance at 280 nm was determined to calculate the extract purity (*EP*) as per equation 3.5 [91]:

$$EP = \frac{Abs_{620}}{Abs_{280}} \qquad \text{(eq. 3.5)}$$

In which $Abs_{620}$ and $Abs_{280}$ are the measured absorbance of the phycocyanin extract at 620 nm and 280 nm respectively. In Figure 3.1 an overview is given of the different extraction buffers, cell disruption techniques and incubation times tested. Each combination of biomass quantity, extraction buffer and cell disruption method was performed in triplicate (n=3).

As per the results of the phycocyanin extraction optimization, in all reactor experiments investigating the light and temperature effects on *Leptolyngbya* sp. QUCCCM 56 as described in 3.2.2, phycocyanin was determined using aliquots containing 5 mg biomass, phosphate buffer and bead-beating as the extraction buffer and cell-disruption method, respectively, followed by direct measurements of the extract (no incubation time).

### 3.2.4 BIOMASS DRY WEIGHT

For biomass dry weight determination, duplicate biomass samples of 2-15 mL were diluted 5 times with ammonium formate (0.5 M) prior to being filtered through pre-dried (24 h, 95 °C), pre-weighed, and washed with 0.5 M ammonium formate, glass microfiber filters (Whatman GF/F™ Ø 55 mm) under a constant vacuum. The filters were then washed with a double volume of 0.5 M Ammonium Formate, dried (24 h, 95 °C), cooled in a desiccator (>2 h) and weighed. The biomass dry weight was determined as the difference between the weight of the dried filters prior to and after biomass filtration and drying.

### 3.2.5 STATISTICAL ANALYSIS

The reported values are the mean of all individual samples, while the error bars represent the standard deviation. For the effect of light and temperature on the biomass productivity and phycocyanin content, one-way ANOVA was used to determine significance difference between the means of independent conditions (n=6). Variable effects were deemed significant if $p<0.05$. Furthermore, correlations between light, temperature, biomass productivity, phycocyanin content, and extract purity, were tested using Pearson Correlation Analysis. For the extraction protocol development, the effect of the different variables (biomass amount, extraction buffer and cell disruption method) on phycocyanin content and extract purity was analyzed using a General Linear Mixed Model with Gamma Regression and Linear Regression, respectively. The effect of the variables on both phycocyanin content and extract purity simultaneously was analyzed using a regression factor representing both values with equal weight. This factor was computed through a dimension reduction factor analysis applying Principle Components Analysis (PCA). Subsequently, a 3-way General Linear Mixed Model was applied with a Linear Regression. All statistical analyses were performed using SPSS 26 (SPSS, Chicago, IL, USA).



## 3.3 RESULTS & DISCUSSION

### 3.3.1 EFFECT OF TEMPERATURE AND LIGHT INTENSITY ON BIOMASS PRODUCTIVITY AND PHYCOCYANIN CONTENT

Under desert climate conditions, as can be found in Qatar, ambient temperatures and light intensities can reach up to 49.8 ˚C and over 2200 µmol photons·$m^{-2}$·$s^{-1}$ [92]. In open raceway ponds, average culture temperatures during summer are generally around 7-8 ˚C below ambient temperatures, which is still considerably higher than the 10-30 ˚C temperature range appropriate for most algal species [93]. In order to investigate the potential of *Leptolyngbya* sp. QUCCCM 56 under such desert conditions, the effects of light intensities up to 1800 µmol photons·$m^{-2}$·$s^{-1}$, and temperatures up to 45 ˚C on biomass productivity, and phycocyanin content, phycocyanin productivity, and extract purity, were investigated under continuous turbidostat cultivations. Results are shown in Figure 3.2.

Both temperature and light intensity were found to have a significantly effect on biomass productivity, phycocyanin content, phycocyanin productivity, and extract purity ($p<0.05$) (supplemental materials, Table S.3.1 and Table S.3.2). Increasing temperatures showed a strong positive correlation with the biomass productivity and phycocyanin content of the strain (r = 0.921 and 0.977, respectively), with the highest biomass productivity and phycocyanin content of 1.09±0.03 $g_X$·$L^{-1}$·$d^{-1}$ and 72.12±3.52 $mg_{PC}$·$g_X^{-1}$ found at 40 ˚C. This phycocyanin content is on

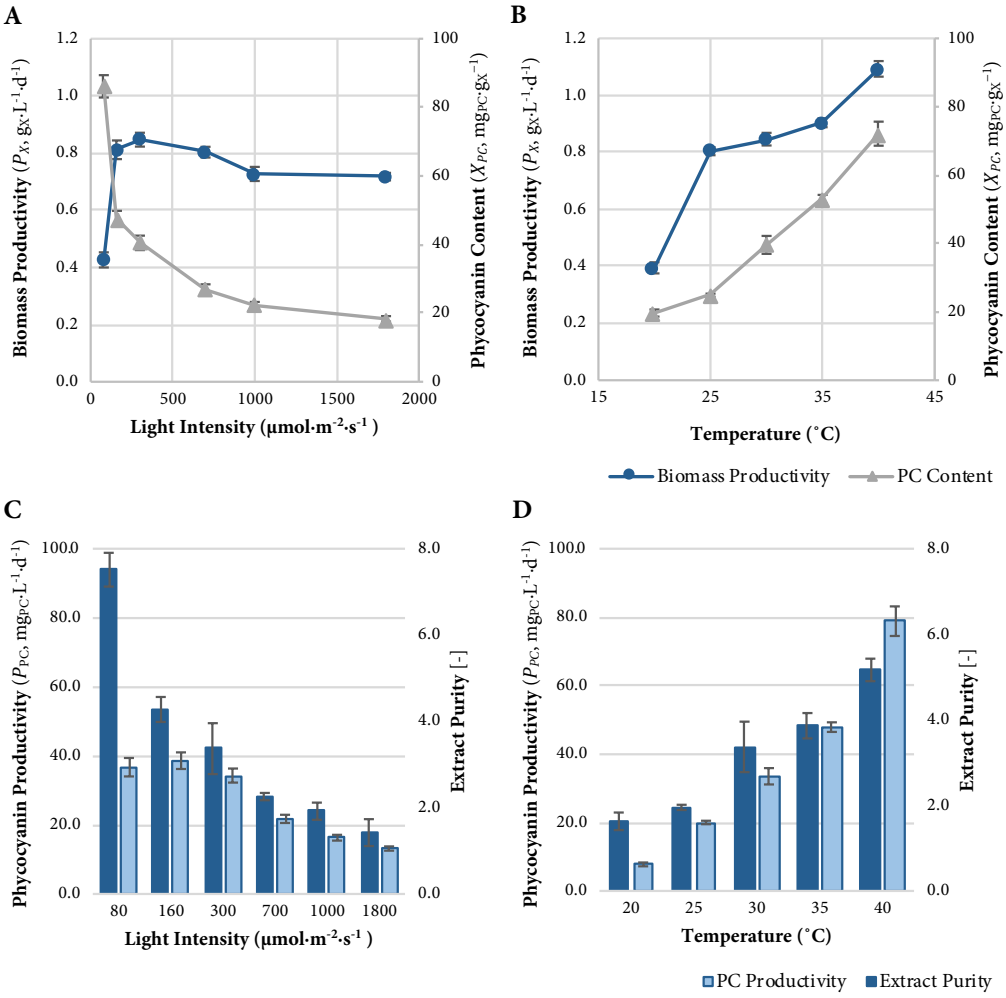


**Figure 3.2 A & B)** Biomass Productivities (● $P_X$, $g_X \cdot L^{-1} \cdot d^{-1}$) and Phycocyanin Content (▲ $X_{PC}$, $mg_{PC} \cdot g_X^{-1}$) of 1.0 $g \cdot L^{-1}$ cultures, operated under continuous turbidostat cultivation, with different light intensities (80-1800 µmol photons·m$^{-2}$·s$^{-1}$ at 30 ˚C) and temperatures (20-40 ˚C at 300 µmol photons·m$^{-2}$·s$^{-1}$), respectively; **C & D)** Phycocyanin Productivity (■ $P_{PC}$, $mg_{PC} \cdot L^{-1} \cdot d^{-1}$) and Phycocyanin Extract Purity (■ *EP*) for different light intensities and temperatures, respectively. Data shown is the mean±stdev (n=6)

par with the average content of *A. platensis*, however there are reports of higher concentrations for *A. platensis* up to 184 $mg_{PC} \cdot g_X^{-1}$ [73,94]. At 45 ˚C, cell death occurred, which is concurrent with previous results obtained [77]. In terms of temperature effect, the number of studies on the effect of temperature of phycocyanin content and productivity are limited [21]. In temperature studies done on a number of different strains, such as *A. platensis, Anabena* sp. and *Lyngbya* sp., generally a peak in phycocyanin content is found for temperatures between 30-36 ˚C, with higher temperatures reducing the phycocyanin content [95-98]. For *A. platensis*, temperature optima for both biomass productivity and phycocyanin content are reported ranging from 27-

**Table 3.1** – Comparison of performance of *Leptolyngbya* sp. QUCCCM 56 and *A. platensis* from referenced studies, in terms of biomass productivity ($P_X$), phycocyanin content ($X_{PC}$), and phycocyanin productivity ($P_{PC}$) for various temperatures ($T$) and light intensities ($I$).

| Strain | Operational Conditions | | | Optima for $P_X$ | | | Optima for $X_{PC}$ | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Reactor Type | Cultivation Mode | Light:Dark | $T$ | $I$ | $P_X$ | $T$ | $I$ | $X_{PC}$ | $P_{PC}$ | |
| | | | $h$ | °C | $\mu mol \cdot m^{-2} \cdot s^{-1}$ | $g_X \cdot L^{-1} \cdot d^{-1}$ | °C | $\mu mol \cdot m^{-2} \cdot s^{-1}$ | $mg_{PC} \cdot g_X^{-1}$ | $mg_{PC} \cdot L^{-1} \cdot d^{-1}$ | |
| *A. platensis* | Glass Vessel (ø 9.5 cm) | Batch | 24:0 | 28 | 300[a] | 0.436 | 28 | 75[a] | 184 | 40.0 | [94] |
| | | Fed-batch | 24:0 | 28 | 300 | 0.588 | - | - | 161 | 94.8 | |
| *S. platensis* | Flat-PBR (depth n/a) | Batch | 24:0 | 30 | 700[a] | 0.75 | 30 | 100[a] | 140 | 110 | [99] |
| *S. platensis* | Flasks (500 mL) | Batch | 12:12 | 35[a] | ±27[a] | 0.023[b] | 35[a] | ±27[a] | 77 | 1.79[b] | [97] |
| *S. platensis* | PBR (ø 3.4 cm) | Batch | 14:10 | 30 | 200 | 0.39[b] | - | - | 168[b] | 66.1[b] | [100] |
| *A. platensis* | Flasks (1000 L) | Batch | 24:0 | 31 | 150[a] | 0.104 | 31 | 150[a] | 93 | 9.62[b] | [101] |
| *A. platensis* | U-shaped water basin | Batch | 16:8 | 27 | 800[a] | 0.110 | 27 | 70[a] | 130 | 5.4 | [102] |
| *A. platensis* | Vessel w. top lighting d: 9.8 cm | Chemostat | 12:12 | 30 | 403[a] | 0.30[b] | 30 | 124[a] | 92.3 | 8.3 | [103] |
| *Leptolyngbya* QUCCCM 56 | Flat-panel PBR (d: 14 mm) | Turbidostat | 12:12 | 40[a] | 300[a] | 1.09 | 40[a] | 80[a] | 86 | 78.8 | This study |

[a] Optimized temperature/light intensity for biomass productivity ($P_X$) or phycocyanin content ($X_{PC}$)
[b] Calculated based on referenced data

35 °C (Table 3.1). Our study shows a similar effect in terms of increasing temperatures leading to an increased phycocyanin content, however unlike the other studies, the optimum for both biomass productivity and phycocyanin content lies at a higher temperature (40 °C), and the maximum biomass productivity was 45% higher than reported biomass productivities for *A. platensis* (Table 3.1). This higher optimum temperature could give *Leptolyngbya* sp. QUCCCM 56 a competitive edge over other commonly cultivated strains for phycocyanin production, for cultivation both in desert climates, as well as in closed photobioreactors in temperate regions, where in summer cooling is generally required to reduce culture temperatures [39]. Furthermore,

the higher optimum temperature could also indicate that phycocyanin from *Leptolyngbya* sp. QUCCCM 56 could be more thermostable as compared to that isolated from other strains with lower temperature optima [72].

In regard to light intensity, the optimal for biomass productivity was found at 300 µmol photons·$m^{-2}$·$s^{-1}$. At higher light intensities, biomass productivities decreased slightly (up to 15%), however the biomass productivity of 0.72±0.01 $g_X$·$L^{-1}$·$d^{-1}$ even at 1800 µmol photons·$m^{-2}$·$s^{-1}$ is still on par with reported productivities of *A. platensis* under optimal conditions (Table 3.1). The results indicate that the strain is capable of maintaining high biomass productivities under a wide range of light intensities, even under dilute culture conditions (1.0 $g_X$·$L^{-1}$ and 14 mm culture depth). This could be very beneficial for cultivation in open raceway ponds in desert environments, where daily light intensities can fluctuate significantly. However, as has been reported for many other strains, including *A. platensis* [21], the optimal light intensity for phycocyanin content was found at low light intensities (80 µmol photons·$m^{-2}$·$s^{-1}$), and showed a significant decrease of 53.0% and 78.7% for increasing light intensities of 300 and 1800 µmol photons·$m^{-2}$·$s^{-1}$, respectively. Furthermore, even under the optimal conditions, the phycocyanin content of *Leptolyngbya* sp. QUCCCM 56 found was in the lower range (86.1±3.3 $mg_{PC}$·$g_X$) as compared to reported values for *A. platensis* (up to 184 $mg_{PC}$·$g_X$, Table 1). Nonetheless, overall phycocyanin productivities, which are a combination of both phycocyanin content and biomass productivity, were on par with that of *A. platensis*, and the maximum extract purity found for *Leptolyngbya* sp. (7.51±0.39) was considerably higher than generally reported for other strains, significantly increasing the value of the extract [104,105].

In this work, the biomass concentration, light:biomass ratio, as well as nitrogen availability, were kept constant for each condition studied, through applying a turbidostat cultivation regime with a fixed biomass concentration of 1.0 $g_X$·$L^{-1}$. A continuous culture permits the maintenance of cultures very close to the maximum growth rate, thereby increasing the biomass productivities, but also limiting the effects of nutritional limitations and changes in biomass concentration, allowing for the investigation into the effects of process parameters independently [76]. To the best of the authors' knowledge, there are no known studies in which the effect of light intensity and temperature on phycocyanin productivity have been investigated under such continuous culture regimes, and all referenced works researching these effects have been performed in (fed-) batch cultures. However, under batch cultivation conditions, the light:biomass ratio, as well as the availability of nitrogen and other nutrients, will change over the duration of the experiment. This causes the biomass productivity and phycocyanin content to be dependent not only on the process parameters under investigation, such as temperature and light intensity, but also on the cultivation stage, and related biomass and nitrogen concentrations. For example, both Chen *et al.* (2013) and

Xie *et al.* (2015) found that the maximum phycocyanin content during batch cultures coincided with nitrogen depletion and high biomass concentrations, and subsequent low light:biomass ratios [94,99]. The lower phycocyanin contents found in this work are therefore hypothesized to be due to the applied cultivation regime and set biomass density, in which neither (near) nitrogen depletion nor very high biomass concentrations occurred. Especially the latter would result in higher light:biomass ratios in continuous cultures as compared to batch cultures, which has a significant negative effect on the phycocyanin content. This was also suggested during the extraction assays, in which *Leptolyngbya* sp. QUCCCM 56 from batch cultures (flasks) was used. There, phycocyanin contents of 160 $mg_{PC} \cdot g_X$ were found, which were near double compared to the values found for the continuous cultures of the cultivation assays. More investigation is required to see how the phycocyanin content of the strain can be improved, for example by applying a higher biomass density, whilst maintaining the high biomass productivities of a continuous culture, thereby improving the overall phycocyanin productivity.



### 3.3.2 PHYCOCYANIN EXTRACTION OPTIMIZATION

The efficient extraction of phycocyanin from the biomass is essential to accurately determine the phycocyanin content and productivities. A number of different methods have been published, however they all differ considerably, mainly in terms of cellular disruption method, type of extraction buffer, biomass-buffer ratio, and extraction time [106-109]. Furthermore, the optimal extraction method can differ from strain to strain [107], and as this is the first known study of *Leptolyngbya* sp. for phycocyanin production, the most effective method of extraction was investigated and compared to *A. platensis*. Extraction yields and purities were analyzed for three different cell disruption methods (bead-beating, freeze-thawing and sonication), in combination with either phosphate buffer, milli-Q water or calcium chloride as extraction buffer. Furthermore, two biomass-buffer ratios were tested, and 4 incubation times, ranging from 0 - 96 h. The results for both *Leptolyngbya* sp. QUCCCM 56 and *A. platensis* UTEX 1940 for each treatment are shown in Figure 3.3.

The effect of extraction buffer and cell disruption method on the phycocyanin content and extract purity were found to be significant for all treatments tested for both strains ($p<0.05$). No significant effect of biomass concentration was found on the phycocyanin yield of *Leptolyngbya sp.* ($p=0.359$), nor on for the extract purity of *A. platensis* ($p=0.898$). Nonetheless, increasing biomass quantities from 5 to 10 mg were found to have a significant negative effect on the extracted phycocyanin content for *A. platensis*, and a significant positive effect on the extract purity for *Leptolygnbya* sp.. As the phycocyanin content found with 5 mg biomass for both *Leptolyngbya* sp. and *A. platensis* were either similar or higher as compared to higher biomass concentrations (10 mg), further statistical analyses were limited to the lower biomass concentration (5 mg).

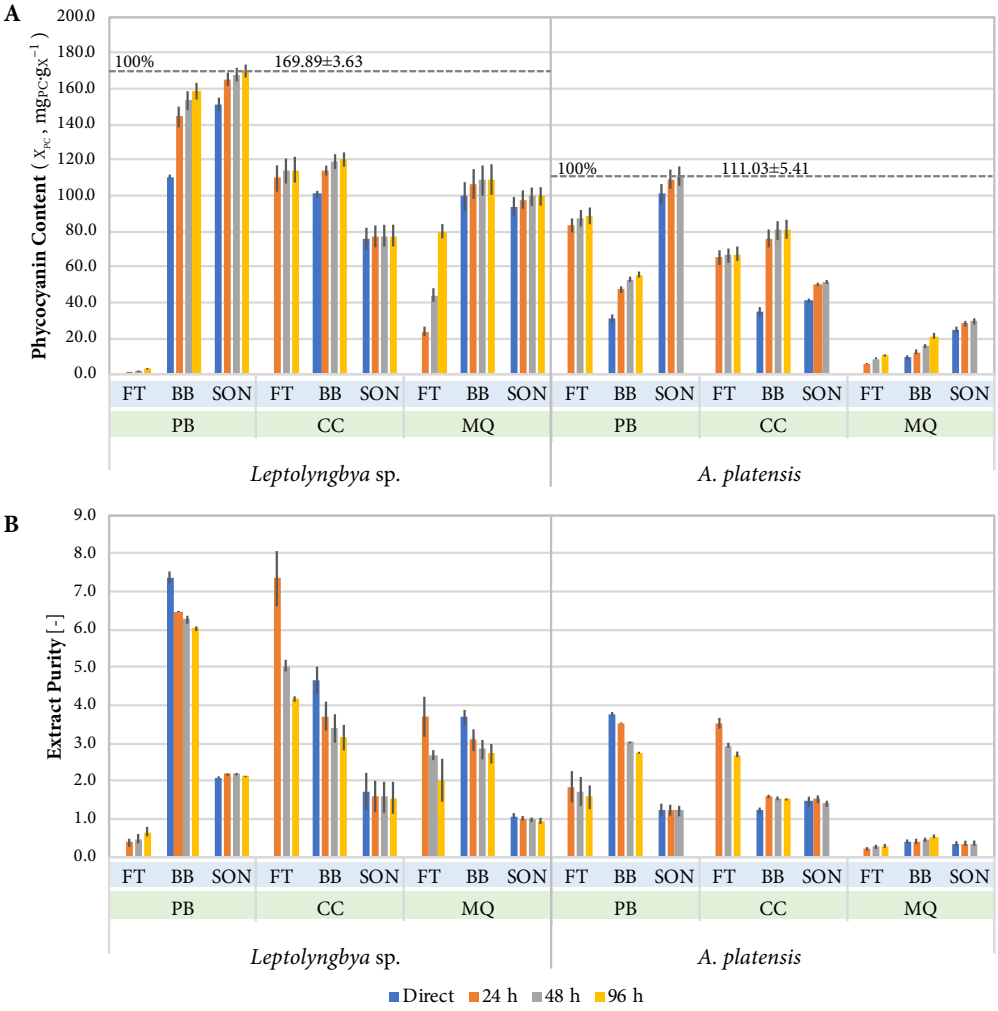


**Figure 3.3 – A)** Phycocyanin Content ($X_{PC}$ $mg_{PC} \cdot g_X^{-1}$) and **B)** Extract Purity (*EP*) from *Leptolyngbya* sp. QUCCCM 56 and *A. platensis* UTEX 1940 using different cell-disruption methods (FT: Freeze-Thawing, BB: Bead-Beating, SON: Sonication), extraction buffers (PB: Phosphate Buffer, CC: Calcium Chloride, MQ: Milli-Q Water), and incubation times, each sample containing 5 mg of biomass (10 mg data is provided in supplemental materials Table S.3.6 and Table S.3.8). Dashed line indicates the max. obtained phycocyanin assumed to be 100% extraction. Data shown is the mean±SD, n=3.

Sonication in phosphate buffer showed the highest extraction yields, with phycocyanin contents' of 169.89±3.63 and 111.03±5.41 $mg_{PC} \cdot g_X^{-1}$, after 96 h and 48 h incubation, for *Leptolyngbya* sp. and *A. platensis*, respectively. Freeze-thawing in phosphate buffer was the second-best extraction method for *A. platensis* (80% extraction as compared to sonication), however unexpectedly performed the least for *Leptolyngbya* sp., giving a phycocyanin content of only 2.73±0.10 $mg_{PC} \cdot g_X^{-1}$. Bead-beating with phosphate buffer performed very well for *Leptolyngbya* sp., with 93% extraction yields as compared to sonication. In terms of extract purity, for both strains, bead-beating in phosphate

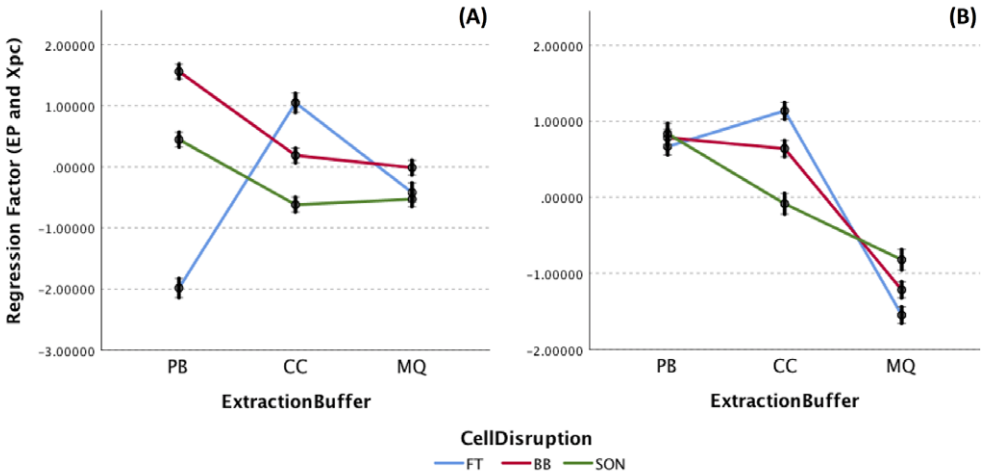


Figure 3.4 – Estimated means charts for significant ($p$<0.05) two-way effects of extraction buffer and cell disruption method on both extract purity (*EP*) and Phycocyanin Content ($X_{PC,}$ $mg_{PC}{\cdot}g_X^{-1}$) for (A) *Leptolyngbya* sp. QUCCCM 56 and (B) *A. platensis* UTEX 1940, represented by a combined regression factor (see text for more details). FT: Freeze-Thawing, BB: Bead-Beating, SON: Sonication, PB: Phosphate Buffer, CC: Calcium Chloride, MQ: Milli-Q Water.

buffer with direct measurement resulted in the highest purities, of 7.37±0.16 and 3.78±0.04 for *Leptolyngbya* sp. and *A. platensis*, respectively. Overall, a decrease of purity was found for increasing incubation times, whilst the extraction yield increased slightly.

In order to determine which treatment yielded both the highest extraction yield and purity, a regression factor was computed representing both values with equal weight for each strain. The estimated means on the regression factor for significant two-way effects (extraction buffer and cell disruption) were computed and are shown in Figure 3.4.

Figure 3.4 shows that when assessing the best method in terms of both extraction yield and purity, and considering interactions between the extraction buffer and the cell disruption method, bead-beating in phosphate buffer had the highest regression factor value for *Leptolyngbya* sp. For *A. platensis,* freeze-thawing with calcium chloride was the best performing, which was the second-best method for *Leptolyngbya* sp.. The differences between Figure 3.4 A and B clearly show how strain dependent the optimal phycocyanin extraction method is, as the results vary significantly between the two strains.

Cell disruption is one of the key factors for the extraction of phycocyanin with high yields and purities; inadequate disruption results in reduced extraction of phycocyanin, whilst excessive cell disruption can lead to release of other undesired biomolecules in addition to phycocyanin, thereby reducing the extract purity and possibly denaturing the phycocyanin [110]. Bead-beating,

a method commonly applied for cell disruption for lipid extraction [111,112], was not found to be described previously for phycobiliprotein extraction. In combination with phosphate buffer, however, the method had the highest combined extract yields and purities for *Leptolyngbya* sp., and the highest extract purity for *A. platensis*. As bead-beating allows for multiple variations from the currently applied protocol (number and duration of cycles, rpm, bead-size), it is hypothesized that this method could be optimized even further to increase the extract yield with direct measurement for both strains, without compromising the extract purity. Sonication resulted in the highest phycocyanin content values, which is supportive of results found by Lawrenz *et al.* (2011) [89]. However, the extract purity was low compared to other cell-disruption methods, suggesting that excessive cell disruption occurred, a factor which was not taken into account by Lawrenz *et al.*. The effectiveness of freeze-thawing for phycocyanin extraction from *Leptolyngbya* sp. QUCCCM 56 was found to be dependent on the extraction buffer used. With phosphate buffer, no significant phycocyanin amounts were extracted, which is surprising as it is one of the most commonly used methods for phycocyanin extraction from cyanobacteria [113,114], however with calcium chloride, the method performed well. Calcium chloride has been reported as an efficient salt for the extraction of cell wall proteins [115] and even though phycocyanin does not exist within the cell-wall membrane itself, it does forms clusters that adhere to the membrane. This could be a possible explanation as to why, when coupled to the least disruptive cell-disruption method (freeze-thawing), calcium chloride gives the highest extract yield and purity as compared to other extraction buffers combined with freeze-thawing.

The purity of the extracted phycocyanin significantly influences its commercial value, with analytical grade phycocyanin (purities of 4.0 and higher) having prices of 15 US$ per mg or more, compared to 0.13US$ and 1-5 US$ for food grade (0.7) and reagent grade (3.9), respectively [105]. Extract purities can differ from strain to strain, but are also dependent on the applied extraction methods, and additional purification steps are generally applied to increase the extracts' purity [107]. In this study, both strains showed the highest purities with bead-beating in phosphate buffer, indicating that this method of cell-disruption could be more effective for high-purity extraction as compared to other, more commonly applied methods. Furthermore, the highest extract purity found for *Leptolyngbya* sp. QUCCCM 56 (7.37±0.16) was significantly higher than obtained for *A. platensis* (3.78±0.04). Even phycocyanin extract purities reported from other studies for *A. platensis* were found ranging from 1.43 to maximum 6.69 after additional extract purification steps [73,104,107]. It is therefore hypothesized that not only the improved extraction method, but also the physiology of the strain, contributes to obtaining such high extract purities, and further investigation is recommended to study the scale-up of both *Leptolyngbya* sp. QUCCCM 56 and bead-beating for commercial scale high-grade phycocyanin production.

## 3.4 CONCLUSIONS

*Leptolyngbya* sp. showed to be able to grow well under elevated temperatures and light intensities, with an optimal biomass productivity found at 40 ˚C. Furthermore, bead beating was shown to be an effective and fast way to extract high-purity phycocyanin from both *Leptolyngbya* sp. and *A. platensis*. The obtained extract purities from *Leptolyngbya* sp. were higher than previously reported for any other strain. The improved productivities of both biomass and phycocyanin at higher temperatures, as well as the high purity of the obtained extract, suggest that the strain is an interesting candidate for commercial phycocyanin production in desert environments.

## 3.5 SUPPLEMENTAL MATERIALS

### SUPPLEMENT A STATISTICAL ANALYSIS DATA FOR LIGHT AND TEMPERATURE EXPERIMENTS

**Table S.3.1** – One-way ANOVA for temperature data

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| $P_x$ | **Between Groups** | 4.691 | 4 | 1.173 | 3010.844 | 0.000 |
| | **Within Groups** | 0.033 | 85 | 0.000 | | |
| | **Total** | 4.724 | 89 | | | |
| $X_{PC}$ | **Between Groups** | 32856.962 | 4 | 8214.240 | 1344.261 | 0.000 |
| | **Within Groups** | 519.401 | 85 | 6.111 | | |
| | **Total** | 33376.363 | 89 | | | |
| ***EP*** | **Between Groups** | 150.443 | 4 | 37.611 | 232.812 | 0.000 |
| | **Within Groups** | 13.732 | 85 | 0.162 | | |
| | **Total** | 164.174 | 89 | | | |

**Table S.3.2** – One-way ANOVA for light intensity data

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| $P_X$ | **Between Groups** | 2.113 | 5 | 0.423 | 868.416 | 0.000 |
| | **Within Groups** | 0.050 | 102 | 0.000 | | |
| | **Total** | 2.163 | 107 | | | |
| $X_{PC}$ | **Between Groups** | 56347.360 | 5 | 11269.472 | 2204.928 | 0.000 |
| | **Within Groups** | 521.326 | 102 | 5.111 | | |
| | **Total** | 56868.686 | 107 | | | |
| ***EP*** | **Between Groups** | 451.527 | 5 | 90.305 | 317.925 | 0.000 |
| | **Within Groups** | 28.973 | 102 | 0.284 | | |
| | **Total** | 480.500 | 107 | | | |

**Table S.3.3 –** Pearson Correlation for temperature data

| | | *Temp.* | $P_x$ | $X_{PC}$ | *EP* |
|---|---|---|---|---|---|
| ***Temp.*** | **Pearson Correlation** | **1** | 0.921** | 0.977** | 0.941** |
| | **Sig. (2-tailed)** | | **0.000** | **0.000** | **0.000** |
| $P_x$ | **Pearson Correlation** | 0.921** | **1** | 0.850** | 0.832** |
| | **Sig. (2-tailed)** | **0.000** | | **0.000** | **0.000** |
| $X_{PC}$ | **Pearson Correlation** | 0.977** | 0.850** | **1** | 0.940** |
| | **Sig. (2-tailed)** | **0.000** | **0.000** | | **0.000** |
| ***EP*** | **Pearson Correlation** | 0.941** | 0.832** | 0.940** | **1** |
| | **Sig. (2-tailed)** | **0.000** | **0.000** | **0.000** | |

** Correlation is significant at the 0.01 level (2-tailed).

**Table S.3.4** - Pearson Correlation for light data

| | | *Light Intensity* | $P_x$ | $X_{PC}$ | *EP* |
|---|---|---|---|---|---|
| ***Light Intensity*** | **Pearson Correlation** | **1** | **0.162** | -0.755** | -0.745** |
| | **Sig. (2-tailed)** | | **0.094** | **0.000** | **0.000** |
| $P_x$ | **Pearson Correlation** | **0.162** | **1** | -0.721** | -0.689** |
| | **Sig. (2-tailed)** | **0.094** | | **0.000** | **0.000** |
| $X_{PC}$ | **Pearson Correlation** | -0.755** | -0.721** | **1** | 0.971** |
| | **Sig. (2-tailed)** | **0.000** | **0.000** | | **0.000** |
| ***EP*** | **Pearson Correlation** | -0.745** | -0.689** | 0.971** | **1** |
| | **Sig. (2-tailed)** | **0.000** | **0.000** | **0.000** | |

** Correlation is significant at the 0.01 level (2-tailed).

## SUPPLEMENT B RESULTS OF PHYCOCYANIN EXTRACTION PROTOCOL DEVELOPMENT

**Table S.3.5** – Phycocanin content ($X_{PC}$) for different treatments and incubations times for 5 mg *Leptolyngbya* sp. QUCCCM 56 and *A. platensis* UTEX 1940

| Strain | Buffer | Cell Disruption | Direct | 24 h | 48 h | 96 h |
|---|---|---|---|---|---|---|
| *Leptolyngbya* QUCCCM 56 | Phosphate Buffer | Freeze-Thawing | | 0.80 ± 0.08 | 1.36 ± 0.08 | 2.73 ± 0.10 |
| | | Bead-Beating | 110.79 ± 1.07 | 144.14 ± 5.82 | 153.24 ± 5.32 | 158.62 ± 4.71 |
| | | Sonication | 151.24 ± 3.73 | 165.30 ± 3.74 | 167.90 ± 3.77 | 169.89 ± 3.63 |
| | Calcium Chloride | Freeze-Thawing | | 109.65 ± 7.33 | 113.87 ± 7.00 | 114.61 ± 7.24 |
| | | Bead-Beating | 100.75 ± 2.00 | 114.10 ± 2.78 | 119.11 ± 4.17 | 120.27 ± 4.02 |
| | | Sonication | 75.84 ± 6.22 | 77.17 ± 6.15 | 77.58 ± 6.10 | 77.69 ± 6.08 |
| | Milli-Q | Freeze-Thawing | | 23.97 ± 2.64 | 44.39 ± 3.81 | 80.07 ± 4.04 |
| | | Bead-Beating | 99.52 ± 8.07 | 106.55 ± 8.34 | 108.45 ± 8.52 | 109.09 ± 8.54 |
| | | Sonication | 94.01 ± 5.23 | 97.89 ± 5.12 | 99.44 ± 5.16 | 99.64 ± 5.19 |
| *A. platensis* | Phosphate Buffer | Freeze-Thawing | | 83.35 ± 3.93 | 87.38 ± 4.64 | 88.74 ± 4.67 |
| | | Bead-Beating | 31.09 ± 2.35 | 47.37 ± 1.86 | 53.09 ± 1.58 | 55.85 ± 1.65 |
| | | Sonication | 100.77 ± 5.75 | 109.31 ± 5.39 | 111.03 ± 5.41 | |
| | Calcium Chloride | Freeze-Thawing | | 65.33 ± 4.12 | 66.43 ± 3.99 | 67.50 ± 4.06 |
| | | Bead-Beating | 34.82 ± 2.78 | 76.08 ± 5.03 | 80.42 ± 5.30 | 81.20 ± 5.31 |
| | | Sonication | 41.18 ± 1.16 | 50.30 ± 0.98 | 51.69 ± 1.12 | |
| | Milli-Q | Freeze-Thawing | | 6.00 ± 0.22 | 8.82 ± 0.57 | 10.49 ± 0.59 |
| | | Bead-Beating | 9.31 ± 1.21 | 12.53 ± 1.30 | 15.71 ± 1.12 | 21.44 ± 1.67 |
| | | Sonication | 24.99 ± 1.65 | 28.18 ± 1.64 | 29.53 ± 1.76 | |

**Table S.3.6** – Phycocanin content ($X_{PC}$) for different treatments and incubations times for 10 mg *Leptolyngbya* sp. QUCCCM 56 and *A. platensis* UTEX 1940

| Strain | Buffer | Cell Disruption | Direct | 24 h | 48 h | 96 h |
|---|---|---|---|---|---|---|
| *Leptolyngbya* QUCCCM 56 | Phosphate Buffer | Freeze-Thawing | | 1.55 ± 0.66 | 2.20 ± 0.72 | 3.50 ± 0.79 |
| | | Bead-Beating | 90.21 ± 9.94 | 125.83 ± 11.19 | 138.12 ± 12.80 | 149.86 ± 12.25 |
| | | Sonication | 152.40 ± 6.35 | 165.92 ± 8.04 | 169.67 ± 8.30 | 172.13 ± 8.10 |
| | Calcium Chloride | Freeze-Thawing | | 84.35 ± 6.57 | 117.70 ± 7.22 | 117.98 ± 7.16 |
| | | Bead-Beating | 76.62 ± 3.02 | 86.85 ± 3.07 | 92.63 ± 2.60 | 93.03 ± 2.57 |
| | | Sonication | 79.66 ± 10.49 | 81.27 ± 10.09 | 81.57 ± 10.06 | 81.68 ± 10.04 |
| | Milli-Q | Freeze-Thawing | | 31.48 ± 3.08 | 62.41 ± 6.99 | 95.21 ± 3.76 |
| | | Bead-Beating | 58.54 ± 5.59 | 65.75 ± 6.38 | 67.94 ± 6.65 | 71.21 ± 6.29 |
| | | Sonication | 94.30 ± 5.39 | 98.18 ± 5.76 | 100.34 ± 6.01 | 101.77 ± 5.93 |
| *A. platensis* | Phosphate Buffer | Freeze-Thawing | | 56.34 ± 1.54 | 64.83 ± 2.45 | 67.46 ± 2.20 |
| | | Bead-Beating | 17.53 ± 1.03 | 27.94 ± 1.75 | 32.59 ± 2.18 | 35.46 ± 2.19 |
| | | Sonication | 64.96 ± 0.80 | 79.70 ± 1.90 | 82.30 ± 1.68 | |
| | Calcium Chloride | Freeze-Thawing | | 53.18 ± 1.34 | 53.98 ± 1.34 | 54.74 ± 1.26 |
| | | Bead-Beating | 23.69 ± 3.89 | 62.07 ± 3.47 | 68.58 ± 2.86 | 69.59 ± 2.86 |
| | | Sonication | 40.01 ± 1.76 | 53.60 ± 5.30 | 54.69 ± 5.36 | |
| | Milli-Q | Freeze-Thawing | | 6.57 ± 1.47 | 9.42 ± 2.39 | 11.34 ± 2.55 |
| | | Bead-Beating | 6.69 ± 0.80 | 12.06 ± 1.37 | 18.49 ± 0.72 | 21.52 ± 0.81 |
| | | Sonication | 23.43 ± 1.10 | 27.17 ± 1.05 | 28.76 ± 0.94 | |

**Table S.3.7** – Extract Purity (*EP*) for different treatments and incubations times for 5 mg *Leptolyngbya* sp. QUCCCM 56 and *A. platensis* UTEX 1940

| Strain | Buffer | Cell Disruption | Direct | 24 h | 48 h | 96 h |
|---|---|---|---|---|---|---|
| *Leptolyngbya* sp. QUCCCM 56 | Phosphate Buffer | Freeze-Thawing | | 0.38 ± 0.11 | 0.49 ± 0.12 | 0.67 ± 0.13 |
| | | Bead-Beating | 7.37 ± 0.16 | 6.48 ± 0.00 | 6.25 ± 0.11 | 6.02 ± 0.06 |
| | | Sonication | 2.10 ± 0.03 | 2.17 ± 0.03 | 2.19 ± 0.03 | 2.13 ± 0.02 |
| | Calcium Chloride | Freeze-Thawing | | 7.34 ± 0.73 | 5.05 ± 0.15 | 4.16 ± 0.07 |
| | | Bead-Beating | 4.66 ± 0.36 | 3.72 ± 0.38 | 3.39 ± 0.38 | 3.15 ± 0.34 |
| | | Sonication | 1.73 ± 0.49 | 1.60 ± 0.41 | 1.58 ± 0.41 | 1.57 ± 0.42 |
| | Milli-Q | Freeze-Thawing | | 3.70 ± 0.53 | 2.69 ± 0.13 | 2.03 ± 0.57 |
| | | Bead-Beating | 3.68 ± 0.20 | 3.08 ± 0.29 | 2.83 ± 0.26 | 2.72 ± 0.26 |
| | | Sonication | 1.09 ± 0.07 | 1.02 ± 0.06 | 0.98 ± 0.06 | 0.96 ± 0.08 |
| *A. platensis* | Phosphate Buffer | Freeze-Thawing | | 1.86 ± 0.41 | 1.73 ± 0.38 | 1.58 ± 0.31 |
| | | Bead-Beating | 3.78 ± 0.04 | 3.54 ± 0.01 | 3.02 ± 0.02 | 2.75 ± 0.02 |
| | | Sonication | 1.25 ± 0.16 | 1.24 ± 0.14 | 1.21 ± 0.14 | |
| | Calcium Chloride | Freeze-Thawing | | 3.53 ± 0.14 | 2.95 ± 0.06 | 2.71 ± 0.07 |
| | | Bead-Beating | 1.23 ± 0.08 | 1.60 ± 0.05 | 1.55 ± 0.05 | 1.52 ± 0.03 |
| | | Sonication | 1.46 ± 0.13 | 1.52 ± 0.11 | 1.41 ± 0.08 | |
| | Milli-Q | Freeze-Thawing | | 0.22 ± 0.04 | 0.27 ± 0.06 | 0.30 ± 0.05 |
| | | Bead-Beating | 0.41 ± 0.05 | 0.42 ± 0.07 | 0.46 ± 0.06 | 0.55 ± 0.05 |
| | | Sonication | 0.35 ± 0.07 | 0.36 ± 0.07 | 0.37 ± 0.07 | |

**Table S.3.8** – Extract Purity (*EP*) for different treatments and incubations times for 10 mg *Leptolyngbya* sp. QUCCCM 56 and *A. platensis* UTEX 1940

| Strain | Buffer | Cell Disruption | Direct | 24 h | 48 h | 96 h |
|---|---|---|---|---|---|---|
| *Leptolyngbya* sp. QUCCCM 56 | Phosphate Buffer | Freeze-Thawing | | 0.77 ± 0.26 | 0.74 ± 0.10 | 0.82 ± 0.09 |
| | | Bead-Beating | 7.63 ± 0.79 | 6.93 ± 0.89 | 6.59 ± 0.92 | 6.44 ± 0.76 |
| | | Sonication | 2.24 ± 0.48 | 2.33 ± 0.47 | 2.32 ± 0.44 | 2.30 ± 0.39 |
| | Calcium Chloride | Freeze-Thawing | | 7.91 ± 0.49 | 5.92 ± 0.71 | 5.20 ± 0.57 |
| | | Bead-Beating | 3.64 ± 0.49 | 3.15 ± 0.40 | 2.89 ± 0.33 | 2.74 ± 0.26 |
| | | Sonication | 1.89 ± 0.59 | 1.80 ± 0.54 | 1.76 ± 0.52 | 1.72 ± 0.52 |
| | Milli-Q | Freeze-Thawing | | 5.04 ± 0.36 | 5.11 ± 0.28 | 2.55 ± 0.08 |
| | | Bead-Beating | 4.63 ± 0.48 | 3.54 ± 0.33 | 3.24 ± 0.30 | 2.97 ± 0.28 |
| | | Sonication | 1.48 ± 0.60 | 1.31 ± 0.44 | 1.22 ± 0.36 | 1.16 ± 0.31 |
| *A. platensis* | Phosphate Buffer | Freeze-Thawing | | 1.54 ± 0.01 | 1.46 ± 0.03 | 1.39 ± 0.02 |
| | | Bead-Beating | 3.54 ± 0.26 | 3.10 ± 0.11 | 2.85 ± 0.09 | 2.54 ± 0.01 |
| | | Sonication | 1.15 ± 0.01 | 1.12 ± 0.02 | 1.10 ± 0.02 | |
| | Calcium Chloride | Freeze-Thawing | | 3.13 ± 0.24 | 2.77 ± 0.19 | 2.49 ± 0.13 |
| | | Bead-Beating | 1.61 ± 0.23 | 1.86 ± 0.31 | 1.85 ± 0.26 | 1.83 ± 0.25 |
| | | Sonication | 0.84 ± 0.04 | 0.95 ± 0.07 | 0.94 ± 0.07 | |
| | Milli-Q | Freeze-Thawing | | 0.29 ± 0.08 | 0.37 ± 0.09 | 0.40 ± 0.08 |
| | | Bead-Beating | 0.46 ± 0.11 | 0.55 ± 0.08 | 0.71 ± 0.06 | 0.72 ± 0.05 |
| | | Sonication | 1.79 ± 0.01 | 1.31 ± 0.02 | 1.18 ± 0.01 | |

# Chapter 4

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

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## ABSTRACT

The transition from small-scale laboratory experiments to large-scale outdoor cultivation is one of the most important steps in developing algae for commercial applications. There are however, multiple aspects which can cause discrepancies between the two scenarios, despite best efforts to recreate outdoor conditions in laboratory environments. Here, the effect of light intensity and inoculum volume on the occurrence of photooxidation for *Leptolyngbya* sp. QUCCCM 56 was investigated. Indoor, the strain was capable of growing at light intensities up to 5600 µmol photons·$m^{-2}$·$s^{-1}$, even at inoculation densities of as low as 0.1 g·$L^{-1}$ (10%). 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. Outdoor cultivation of the strain initially showed growth rates which were up to 15% higher as compared to indoor cultures, at 0.61±0.00 and 0.58±0.02 $d^{-1}$ for 10% and 20% inoculum volumes, respectively; however, a loss of chlorophyll, phycocyanin, and culture turbidity on day 3-4, were irrespective of inoculum volume, suggesting that the strain had difficulties adapting to the outdoor environment. Contrary to the initial hypothesis, the outdoor light intensity (1981±41 µmol photons·$m^{-2}$·$s^{-1}$) did not seem to be the main issue causing difficulties for the culture to grow. The culture did, however, recover, and clear morphological differences were observed, such as an increase in trichome length, as well as coiling of multiple trichomes to tightly packed strands. It was hypothesized that the morphological changes were induced by UV-radiation as an adaptation mechanism through increased self-shading. Furthermore, the presence of contaminating ciliates could have also affected the outdoor culture. Both UV and contaminants are, however, generally not simulated under laboratory environments, causing a mismatch between indoor optimizations and outdoor realizations.

## 4.1 INTRODUCTION

Successful outdoor cultivation of microalgae and cyanobacteria is the first step towards commercial application. However, this transition from indoor to outdoor is not always straightforward [116-118]. 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 [9,76,119].

Photoinhibition is a reversible phenomenon during which the photosynthetic capacity of cells is reduced, induced by overexposure 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 [120,121]. 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 [122]. 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. 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 photooxidative stress, ultimately, cell-death will occur [123]. 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 [76,124,125].

The sensitivity of cells to high irradiances is strain-dependent, with certain strains being more susceptible to photoinhibition and -oxidation than others [126]. The sensitivity can also be wavelength dependent, whereas ultraviolet radiation (UVR) can be the most inhibitory region of the spectrum [127]. Selection of strains, capable of withstanding high irradiances with limited photoinhibition and oxidation, is key for successful outdoor cultivation with high productivities [9]. 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 [128,129]. 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, however, also possible for cells to acclimatize to higher irradiances, known as photoadaptation, which decreases the effect of photoinhibition and -oxidation on the growth and survivability of the strain [130]. 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 [131-134].

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 (MENA) region [9]. 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 [77]. 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·$m^{-2}$·$s^{-1}$ [135]. This work, therefore, focusses 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.

## 4.2 MATERIALS & METHODS

### 4.2.1 STRAINS, MEDIA, AND BASIC CULTURE CONDITIONS

*Leptolyngbya* sp. QUCCCM 56 was obtained from the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM, Doha, Qatar). 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·$m^{-2}$·$s^{-1}$ and 12:12h light:dark cycle. Media for all experiments was prepared using locally sourced seawater, with a salinity of 40.0 ppt, filtered (VWR 0.45µm PES), autoclaved and supplemented with: $NaNO_3$, 4.71 mM; $KH_2PO_4$, 0.23 mM; $NaHCO_3$, 4.8 mM; $Na_2EDTA$, $2.56 \cdot 10^{-2}$ mM; $FeSO_4 \cdot 7H_2O$, $1.44 \cdot 10^{-3}$ mM; $MnCl_2 \cdot 4H_2O$, $1.41 \cdot 10^{-4}$ mM; $ZnSO_4 \cdot 7H_2O$, $3.06 \cdot 10^{-5}$ mM; $Co(NO_3)_2 \cdot 6H_2O$, $3.21 \cdot 10^{-6}$ mM; $CuSO_4 \cdot 5H_2O$, $1.28 \cdot 10^{-6}$ mM; and $Na_2MoO_4 \cdot 2H_2O$, $1.33 \cdot 10^{-5}$ mM.

### 4.2.2 INDOOR CULTIVATION CONDITIONS

The inoculum was cultivated in 2.0L Duran Bottles sparged with air, at a light intensity of 200 µmol photons ·$m^{-2}$·$s^{-1}$ (block, 12:12h 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 $g_X \cdot L^{-1}$ were reached, the biomass was used to inoculate a series of conical benchtop photobioreactors (PBR101, Phenometrics Inc., MI, USA), 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 [136]. The total illuminated surface area was 29 $cm^2$. The culture was sparged with air, and pH (non-controlled) 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% v/v, equivalent to initial biomass concentrations of 0.1, 0.2, and 0.5 $g_X \cdot L^{-1}$, respectively). Peak incident light intensities investigated were 2800, 4200 or 5600 µmol photons $\cdot m^{-2} \cdot s^{-1}$ (sinusoidal, 12:12h 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 [93]. Inoculation occurred at the same time for all conditions (10:00 AM), one hour prior to 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 4.1 [137].

$$I_z = I_0 \cdot e^{(-a_X \cdot C_X \cdot z)} \qquad \text{(eq. 4.1)}$$

In which $I_0$ and $I_z$ are the light intensities (µmol photons $\cdot m^{-2} \cdot s^{-1}$) at depth 0 and $z$ (m) of the reactor, $a_X$ is the the wavelength dependent dry weight specific absorption coefficient measured for low-light acclimatized cells of *Leptolyngbya* sp. QUCCCM 56, using the method described by Vejrazka *et al.* [138] in $m^2 \cdot kg^{-1}$, and $C_X$ is the biomass concentration in $g_X \cdot L^{-1}$.

### 4.2.3 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$\cdot m^{-2} \cdot s^{-1}$ (block, 12:12h light:dark), and cultures were maintained at 25 ˚C. Upon reaching a density of 1.0 $g \cdot L^{-1}$, the culture was used to inoculate outdoor 200 L raceway tanks, with a surface area of 1.0 $m^3$. Inoculation occurred at 4:00PM (afternoon), 2 h prior to sunset. The water level was maintained at 20 cm using fresh-water to maintain the salinity at 40 ppt. Agitation was achieved through a 4-blade paddle system rotating at 32 rpm, resulting in an average linear liquid velocity of 26.2 $cm \cdot s^{-1}$ [92]. Inoculation volumes of 10% and 20% (v/v) 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).

### 4.2.4 BIOMASS ANALYSIS

Biomass densities were monitored through optical density measurements at 680 and 750 nm, as well as through gravimetric analysis [139]. The growth rate was determined over the initial 72 h of cultivation, using equation 4.2.

$$\mu = \frac{\ln\left(C_{X,t} / C_{X,0}\right)}{t - t_0} \qquad \text{(eq. 4.2)}$$

In which μ is the growth rate ($d^{-1}$), and $C_{X,t}$ and $C_{X,0}$ are the biomass densities ($g \cdot L^{-1}$) at times $t$ and $t_0$ (d), respectively. For both the indoor and outdoor experiments, phycocyanin and chlorophyll contents in the biomass were monitored. 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. Samples were 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 absorbance of the extract solution was measured at 652 nm and 665 nm using a quartz cuvette and a DR3900 VIS-Spectrophotometer (Hach-Lange, CO, USA). Arnon's equation was used to determine the chlorophyll concentration as per Lichtenhaler (1987) [140], see equations 4.3, 4.4, and 4.5 for chlorophyll *a*, *b*, and total chlorophyll, respectively:

$$C_{Chl_a} = (16.72 \cdot Abs_{665} - 9.16 \cdot Abs_{652}) \cdot \frac{V_{buffer}}{V_{sample}} \qquad \text{(eq. 4.3)}$$

$$C_{Chl_b} = (34.09 \cdot Abs_{652} - 15.28 \cdot Abs_{665}) \cdot \frac{V_{buffer}}{V_{sample}} \qquad \text{(eq. 4.4)}$$

$$C_{Chl_{tot}} = Chl_a + Chl_b \qquad \text{(eq. 4.5)}$$

In which $Chl_a$, $Chl_b$ and $C_{Chl_{tot}}$ are the concentrations of chlorophyll *a*, chlorophyll *b,* and total chlorophyll, respectively, in $mg_{Chl} \cdot L^{-1}$. $Abs_{665}$, and $Abs_{652}$ are the absorptions measured at 665 nm and 652 nm, respectively, $V_{buffer}$ and $V_{sample}$ are the methanol and sample volumes, respectively. Chlorophyll content ($X_{Chl}$, $mg_{Chl} \cdot g_X^{-1}$) was determined by dividing the chlorophyll concentration with the biomass concentration ($C_X$, $g_X \cdot L^{-1}$). 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.* [135].

### 4.2.5 STATISTICAL ANALYSIS

The reported values are the means of individual samples, whilst the error bars represent the range. One-way ANOVA 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, Chicago, IL, USA).

## 4.3 RESULTS & DISCUSSION

### 4.3.1 INITIAL OUTDOOR SCALE-UP CULTIVATION TRIALS

*Leptolygnbya* sp. QUCCCM 56, a cyanobacteria isolated from the Qatari desert [77] was previously studied under simulated desert conditions of high temperatures and light intensities of up to 40 °C and 1800 µmol photons·m$^{-2}$·s$^{-1}$, respectively [135]. Due to its promising growth rate (>1 g·L$^{-1}$·d$^{-1}$), 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, 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 (supplemental materials Figure S.4.1). Average peak light-intensities during these months, as calculated from onsite measurements, were 1812±111, 2278±236, 1757±98 µmol photons·m$^{-2}$·s$^{-1}$ for October, April and September, respectively. Peaks of up to 2871 µmol photons·m$^{-2}$·s$^{-1}$ were found to occur (Figure 4.1). Overall, the highest average peak light intensities observed were around 2250 µmol photons·m$^{-2}$·s$^{-1}$ for 4 months of the year, with lowest averages of approx. 1500 µmol photons·m$^{-2}$·s$^{-1}$ 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.

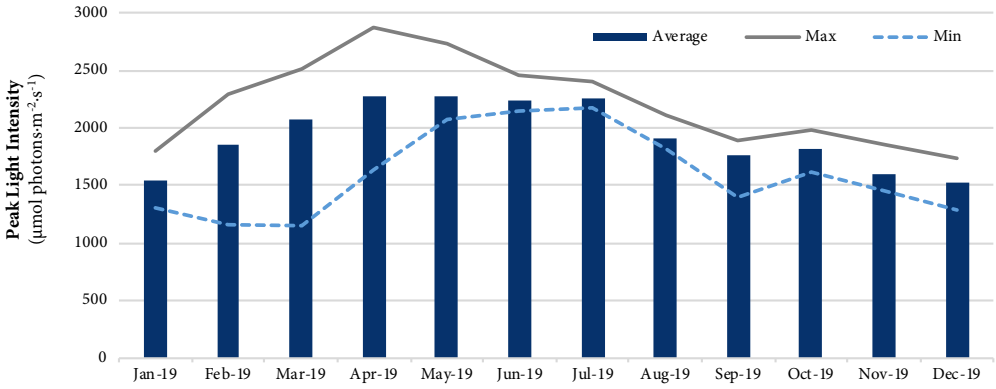



Figure 4.1 – Monthly average, absolute maximum, and absolute minimum peak light intensity (µmol photons·m$^{-2}$·s$^{-1}$) as recorded in Qatar (25°48'06.4"N 51°21'06.4"E) over the course of 2019.

Similar results have been found for certain strains of *Arthrospira,* which is why it is recommend to scale up with a factor 5 (20% inoculum volume) rather than the factor 10 (10% inoculum volume) industry standard [76,119,129]. The occurrence of photooxidation during the outdoor trials, however, 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·$m^{-2}$·$s^{-1}$ in flat panel photobioreactors (14 mm light path) [135]. 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.

### 4.3.2 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 10-50% (v/v), 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 4.2 A.

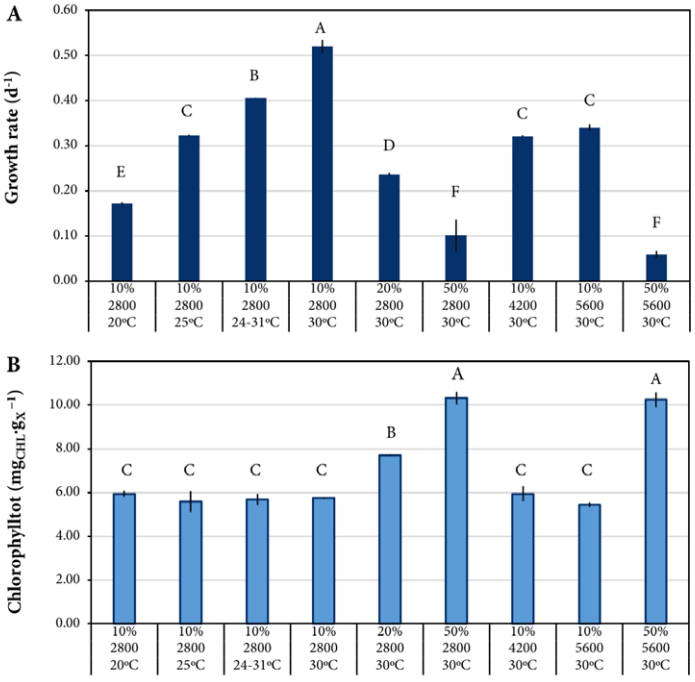



Figure 4.2 – A) Growth rate, µ ($h^{-1}$) calculated over day 0-2; and B) Chlorophyll content ($mg_{CHL}$·$g_X^{-1}$) after 24 h of cultivation, of indoor cultivated *Leptolyngbya* sp. QUCCCM 56 under different inoculum volumes (10, 20 and 50% v/v), light intensities (2800, 4200 and 5600 µmol photons·$m^{-2}$·$s^{-1}$) and temperatures (20, 25, and 30°C and 24-31 °C night:day cycle). Values are mean±range n=2, different letters indicating significant differences ($p < 0.05$) between the individual means.

Contrary to the preliminary outdoor experiments, *Leptolyngbya* sp. grew well under all conditions tested. Even at the highest light intensities and lowest dilution rates, growth rates were positive, and no visible photooxidation was observed. The maximum growth rate of 0.519±0.015 $d^{-1}$ was obtained when the culture was inoculated at the lowest biomass concentration (10% inoculum volume, equivalent to 0.11±0.02 $g_X \cdot L^{-1}$), with a light intensity of 2800 µmol photons$\cdot m^{-2} \cdot s^{-1}$ and 30 °C. At higher light intensities (4200 and 5600 µmol photons$\cdot m^{-2} \cdot s^{-1}$) and similar inoculum volumes (10%), the growth rates were up to 35% lower as compared to 2800 µmol photons$\cdot m^{-2} \cdot s^{-1}$, despite the increased light availability. This slight reduction in growth rate would suggest that photoinhibition occurred to some degree, however not sufficient to cause a culture crash. This, as well as the decrease in growth rate with decreasing temperatures, is concurrent with previous results [135].

Besides temperature, increasing inoculum volumes also caused a decrease in growth rates. The latter is easily understood by analyzing the overall light availability over the reactor depth, which decreases with increasing biomass densities, as is shown in Figure 4.3. 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$\cdot m^{-2} \cdot s^{-1}$, and 50% inoculum volume with 5600 µmol photons$\cdot m^{-2} \cdot s^{-1}$ however, 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.

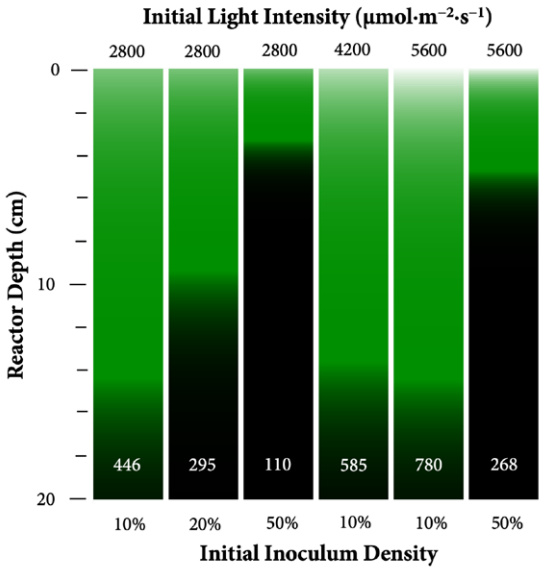



Figure 4.3 - Illustration of theoretical light intensities over the reactor depth (cm) calculated using eq. 4.1, for the various inoculum volumes and light intensities tested. White represents the highest light intensity and black the lowest (5600 and 0 µmol photons$\cdot m^{-2} \cdot s^{-1}$, respectively), and green 50$^{th}$ percentile; scale is uniform across all conditions. Values in white represent the average light intensity over the reactor depth in µmol photons$\cdot m^{-2} \cdot s^{-1}$

### 4.3.3 PIGMENTS AS INDICATORS OF PHOTOINHIBITION AND -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 to similar ($p$>0.05) values for all conditions within 72 h (Figure 4.4 A). When analyzing the chlorophyll content after 24 h (Figure 4.2 B) 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 $mg_{Chl} \cdot g_X^{-1}$, regardless of temperature and light intensity. Increasing inoculum volumes showed a significant increase in chlorophyll content, which was 10.28±0.27 $mg_{Chl} \cdot g_X^{-1}$ for the highest inoculum volumes. 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 4.4 B). The recovery, however, occurred in a very different pattern, with higher phycocyanin values for low light intensity cultures (2800 µmol photons·$m^{-2}$·$s^{-1}$) as compared to the phycocyanin levels found for higher light intensities (4200 and 5600 µmol photons·$m^{-2}$·$s^{-1}$). 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 [21,135]. Furthermore, high-inoculum volume cultures had a higher final phycocyanin content, as

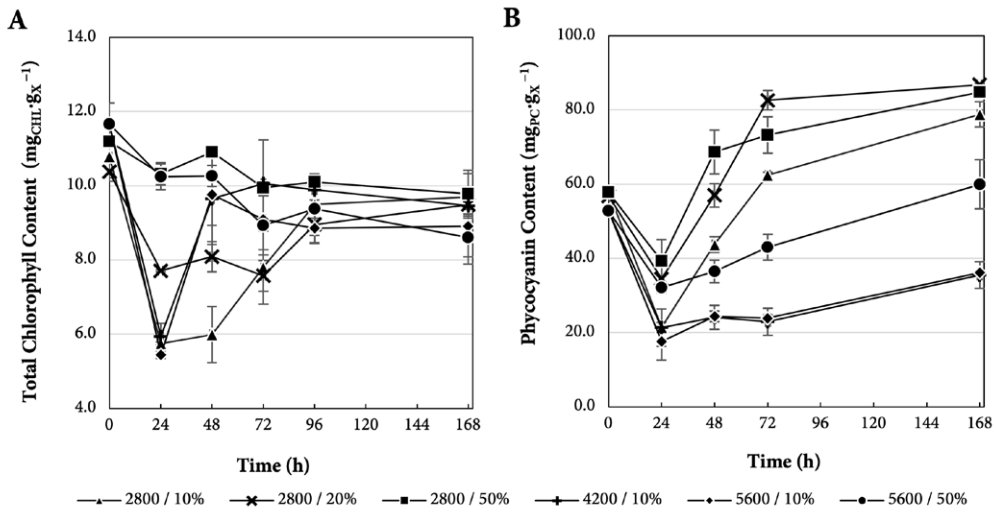



Figure 4.4 – A) Total Chlorophyll content ($mg_{Chl} \cdot g_X^{-1}$) and B) Phycocyanin Content ($mg_{PC} \cdot g_X^{-1}$) over time for *Leptolyngbya* sp. QUCCCM 56 cultivated with different inoculum dilutions (10,20 and 50%), and light intensities (2800, 4200 and 5600 µmol photons·$m^{-2}$·$s^{-1}$) at 30 °C. Values are mean±range n=2.

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,* however 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 [141]. This phenomenon would explain the different ratios between chlorophyll and phycocyanin found for the different light intensities and biomass densities.



Even though no extreme photooxidation 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. However, at low inoculum volumes, increasing 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 photooxidation, regardless of light intensity. It is however unclear whether this is only the case at high light intensities as applied (≥ 2800 µmol photons·$m^{-2}$·$s^{-1}$), which could have already been over-saturating, 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 sub-saturation levels. All in all, the results would suggest that the culture crash outdoor is more likely due to the sub-optimal inoculum volumes, rather than high light intensities, and higher inoculum volumes could prove beneficial.

### 4.3.4 OUTDOOR CULTIVATION WITH VARYING INOCULUM VOLUMES

In order 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% (v/v). Cultivation occurred in August-2020, and average peak light intensities of 1981±41 µmol photons·$m^{-2}$·$s^{-1}$ 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 4.5 B). Initially, the cultures grew,

with biomass densities increasing, and no significant difference in growth rate was observed for the two inoculum volumes (0.61±0.00 and 0.58±0.02 $d^{-1}$ for 10% and 20%, respectively). At the time of inoculation, there was still light penetration over the entire culture depth, thus no light limitation occurred for either culture, which would explain the similar growth rates for both inoculum volumes. The phycocyanin content of the strain did drop significantly between the inoculum (32.2±1.2 $mg \cdot g_X^{-1}$), and the outdoor cultures within 24 h of inoculation, at 2.7±0.4

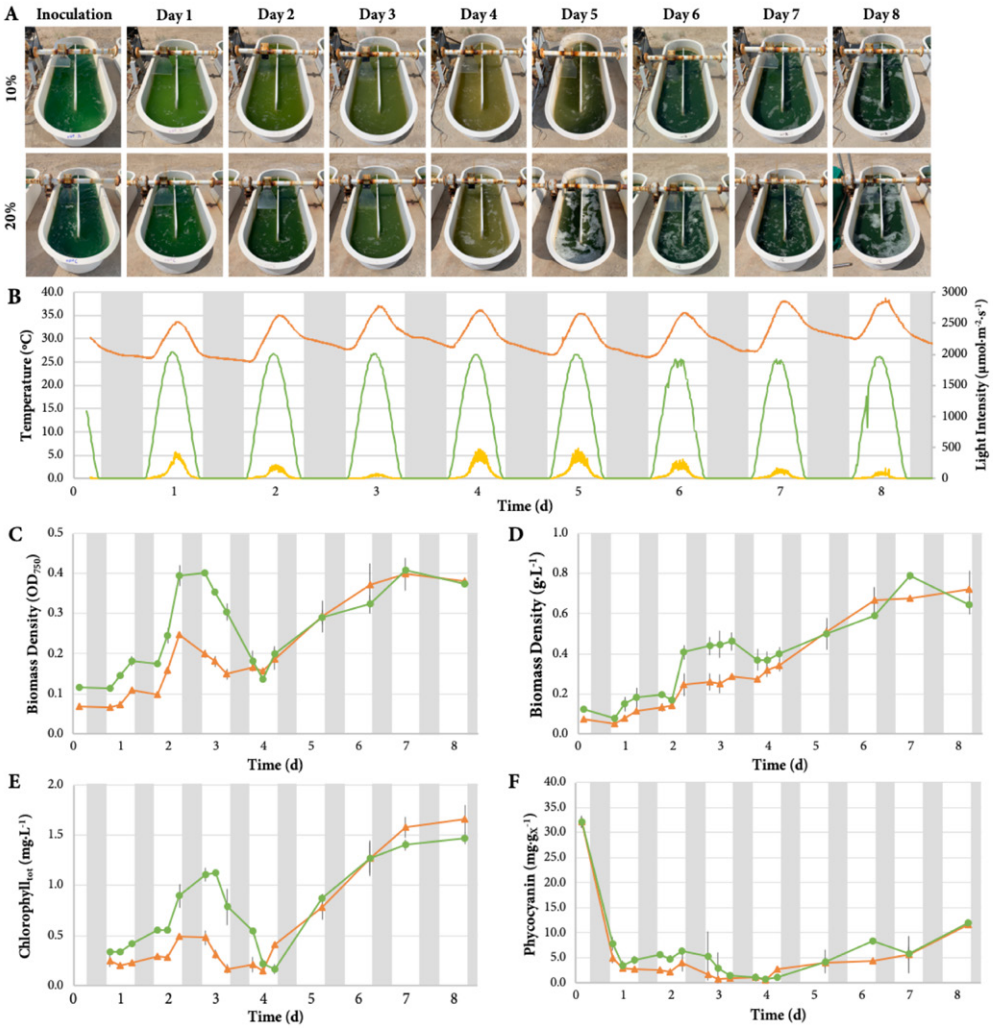



Figure 4.5 - Outdoor growth trials of *Leptolyngbya* sp. QUCCCM 56 performed in August 2020, inoculated at 10% and 20% (v/v) inoculum volumes. A) Daily photos of 10% (top) and 20% (bottom) 200L raceway tanks; B) water temperature (orange line, °C), PAR light intensity at water surface (green line, µmol photon·$m^{-2}$·$s^{-1}$), and PAR light intensity at 20 cm culture depth (yellow line, µmol photons·$m^{-2}$·$s^{-1}$); C-F) Culture Turbidity ($OD_{750}$), Biomass Density ($g \cdot L^{-1}$), $Chlorophyll_{tot}$ concentration ($mg \cdot L^{-1}$) and Phycocyanin content ($mg \cdot g_X^{-1}$), respectively, with orange line representing 10% inoculum volumes, and the green line 20% inoculum volumes. Data shown in the mean±range (n=2).

and 4.5±0.8 $mg \cdot g_X^{-1}$ for 10% and 20% inoculum volumes, respectively (Figure 4.5 F). This was to be expected seeing the drastic shift between dense indoor cultures at low light intensity, to low-density cultures at high light intensities. A slight recovery, to 4.0±1.7 and 6.3±0.7 $mg_{PC} \cdot g_X^{-1}$ 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 culture turbidity ($OD_{750}$), chlorophyll concentration and phycocyanin content was observed, and visual observations showed a change in culture color (Figure 4.5 A,C and E). There was no significant difference in response between the two inoculum volumes. Microscopic observations on day 4, when culture turbidity and 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 4.6 B), in addition to some contamination from other species, most obvious being a fast moving ciliated protozoa of approximately 16-19 µm in length (Figure 4.6 C).



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

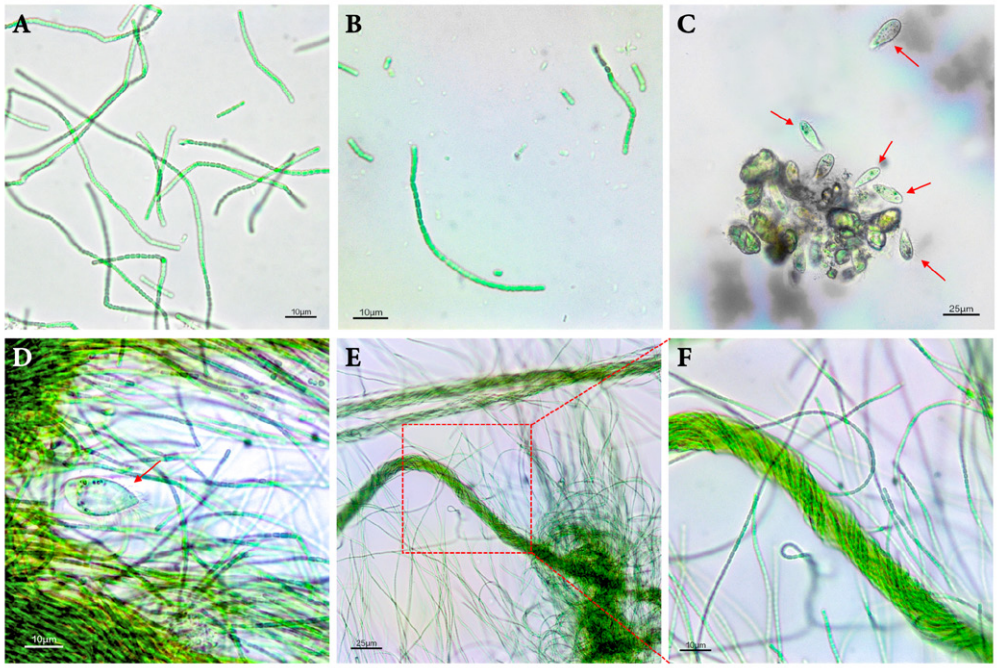



Figure 4.6 – Light microscope images of *Leptolyngbya* sp. QUCCCM 56 during indoor and outdoor cultivation. A) indoor cultivation in photobioreactors; B-C) Outdoor culture on day 4, D-F) culture on day 6. Red arrows indicating ciliates; scale bar representing 10 µm for A, B, D, and F, and 25 µm for C and E.

day 6 showed an increase in trichomes, both in number and in length, and very few contaminating species (Figure 4.6 D-F). The trichomes were longer as compared to those found during cultivation in aerated photobioreactors (Figure 4.6 A). Furthermore, the coiling of multiple trichomes, a phenomenon not observed before during laboratory experiments either, was also observed (Figure 4.6 E-F).

### 4.3.5 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·$m^{-2}$·$s^{-1}$. This would suggest that the cultures indoor were more photoinhibited, as compared to the outdoor experiments, where light intensities peaked at 2034 μmol·$m^{-2}$·$s^{-1}$. Both the higher growth rate, as well as the insignificant differences in response between the two inoculum volumes during the outdoor experiments, would however suggest that the culture crash on day 3-4 is not related to the culture's response to (PAR) light intensity.

The initial response of the culture strongly resembles the description of culture crash occurrence found by Troschl *et al.* in cultures of cyanobacteria *Synechocystis* sp., which was caused by contamination by the ciliate *Colpoda steinii* [142]. Ciliates are protozoa which feed on smaller organisms, such as bacteria and algae, and can rapidly wipe out algae cultures. However, 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 4.6 D), 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, however, adaptation of the strain, seen through an increase in trichome length and trichome coiling, aided in preventing full culture crash. Furthermore, removal of other smaller contaminant algae and bacteria by the ciliate could have perhaps even benefited the survival 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 [142]. Furthermore, the strategy of selectively applying ciliates for beneficial biological-contaminant control in microalgal cultures was also shown to be effective by

Cho *et al.* [143], and could be further investigated as method for successful outdoor cultivation of *Leptolyngbya* sp. QUCCCM 56.

Another possible reason for the experienced culture crash could be the presence of damaging UV-radiation (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 $W \cdot m^{-2}$, for UVA and UVB respectively, have been found [144]. 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 [127]. 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 ($20.2 \pm 0.4$ $mg_{PC} \cdot g_X^{-1}$) 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 [145]. In the case of morphology, it has been found that larger cells are less susceptible to physical damage caused by ionizing radiation [146]. 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 [147]. Similarly, *Leptolyngbya* sp. QUCCCM 56 showed an initial decrease in trichome length during outdoor cultivation, however 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$\cdot m^{-2} \cdot s^{-1}$), 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 [148]. 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.

All in all, the response of the outdoor culture, which was irrespective of inoculum volume, was considerably different as compared to that which was observed for the indoor experiments. Discrepancies between laboratory and outdoor cultivation are unfortunately very common, as the full spectrum of outdoor conditions, not only light and temperature variations, but also other factors such as contaminants, and full light-spectra, are exceedingly difficult to replicate under laboratory settings. It is clear that the strain required an adaptation period in order to allow for stable cultivation outdoors, and the change in morphology was one of the obvious adaptations, however 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 although 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.

## 4.4 CONCLUSIONS

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, with limited photooxidation occurring at low inoculum volumes. Outdoor however, no difference in response was found for varying inoculum volumes, and an adaptation period of multiple days was required, inducing changes in cell morphology, before stable growth was observed. 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.

## 4.5 SUPPLEMENTAL MATERIALS

### SUPPLEMENT A: IMAGES OF INITIAL OUTDOOR TRIALS

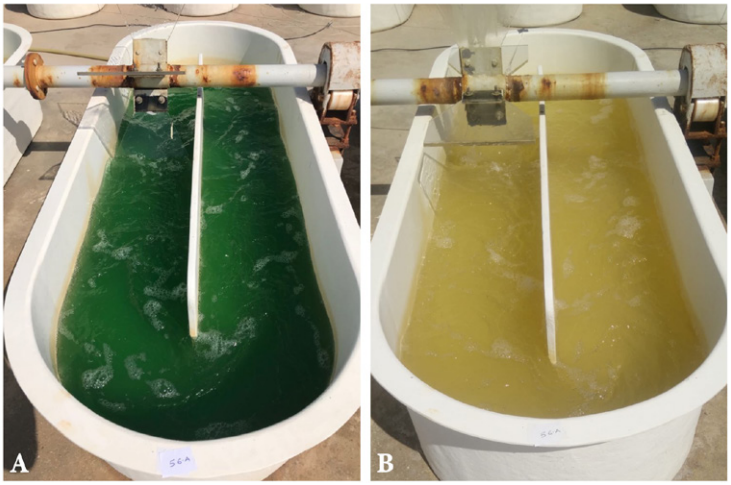
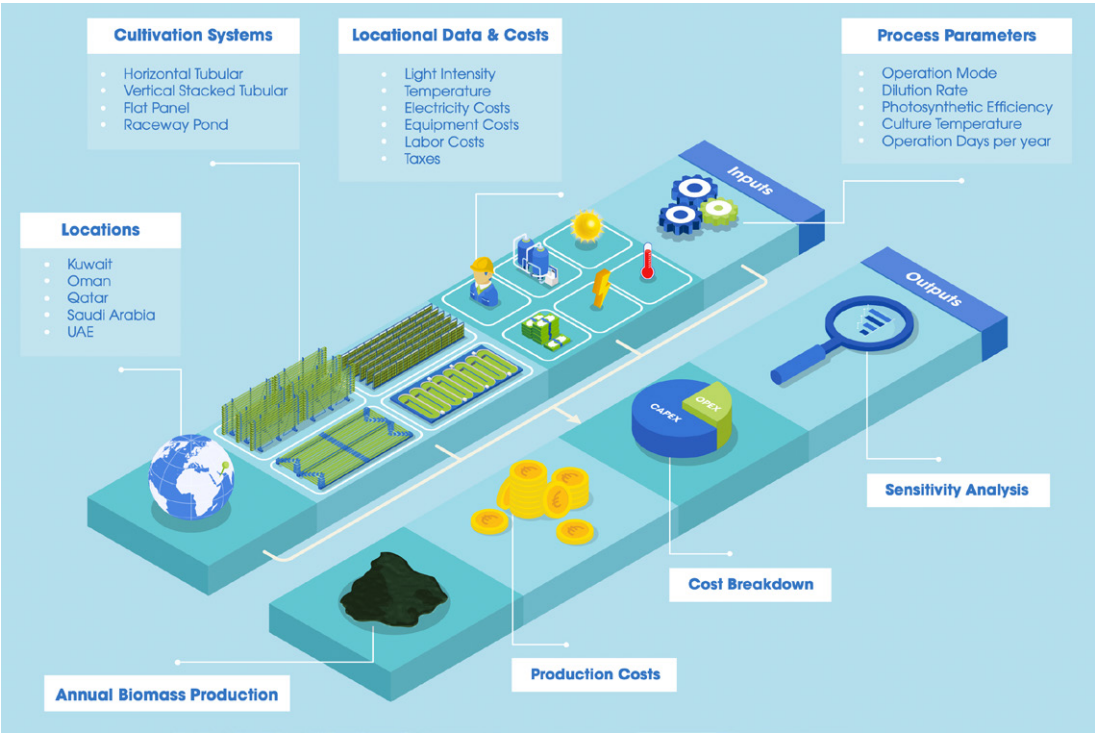



**Figure S.4.1** - Cultivation of *Leptolyngbya* sp. QUCCCM 56 at 200 L scale under outdoor climate conditions (September 2018), A) at time of inoculation and B) after 48 h


Cultivation Systems
Horizontal Tubular
Vertical Stacked Tubular
Flat Panel
Raceway Pond
Locational Data & Costs
Light Intensity
Temperature
Electricity Costs
Equipment Costs
Labor Costs
Taxes
Process Parameters
Operation Mode
Dilution Rate
Photosynthetic Efficiency
Culture Temperature
Operation Days per year
Locations
Kuwait
Oman
Qatar
Saudi Arabia
UAE
Inputs
Outputs
CAPEX
OPEX
Sensitivity Analysis
Cost Breakdown
Production Costs
Annual Biomass Production

# Chapter 5

# Techno-economics of algae production in the Arabian Peninsula

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## ABSTRACT

The Arabian Peninsula's advantageous climate, availability of non-arable land, and easy access to seawater and $CO_2$-rich flue gas point sources, make it an interesting location for large-scale microalgae biomass production. This study aimed at analyzing the techno-economic feasibility of different cultivation systems and production locations in the Arabian Peninsula, in order to assess the commercial potential for algae biomass production in the region. Compared to tubular horizontal and vertical stacked tubular photobioreactors, flat panel and raceway pond cultivation systems had the lowest projected biomass production costs, at 3.0 and 2.9 €·kg$^{-1}$, respectively, for production at 100 ha scale. Locational differences in production costs throughout the region were minimal. In scaling up from 1 ha to 100 ha production facility, the largest reductions in production costs were made within the first 10 ha (67%). Further scale-up to 100 ha, resulted in a mere 13% additional cost-savings. Potential further cost reductions of up to 42.5% and 25% could be accomplished in flat panel bioreactors, with improvements in photosynthetic efficiencies (5.4% compared to 2.7%) and increased temperature optima (60 °C compared to 40 °C), respectively. Successful implementation both improvements could result in a biomass production cost of 1.46 €·kg$^{-1}$, with further cost reduction opportunities through the use of recovered $CO_2$ (-10.7%), increased operational days (-7.5%) and alternative harvesting methods (-4.9%). Efforts to source local thermo- and photo- tolerant strains could be the key to unlock the potential of the region for algae commercialization.

## 5.1 INTRODUCTION

The drive towards more sustainable feedstocks for fuels, feed, food and chemicals has been ever increasing over the past decades. Microalgae, microscopic plant-like organisms which perform photosynthesis and produce a plethora of different commercially interesting metabolites, have caught the interest of many researchers, both in academic and industrial contexts, as such a sustainable feedstock [149]. Despite the increasing interest into algae commercialization over the last decades, the largest part of algae developments remain at research scale [118,129]. This is largely attributed to the limited knowledge on the associated costs with large-scale algae production, as well as to the requirements for ongoing improvements in strain selection, cultivation systems, and harvesting mechanisms [150]. Overall, in order to establish a successful global algae-based industry, more insight is needed into the overall economics of algae production, and more specifically, which processes most significantly impact cost reductions. This will allow researchers, developers and investors to make the right decisions regarding algae R&D, scale-up and commercialization.

Biomass productivity, and related photosynthetic efficiencies, are one of the areas in which significant advances can be made to decrease the production costs [9,151]. As algae utilize light and prefer moderate temperatures, climate and light availability play a major role in maximizing productivities. Large regions in the Middle East and North Africa have been identified to be able to support the highest theoretical biomass productivities of up to 200-240 $t \cdot ha^{-1} \cdot y^{-1}$, based on their climates and light availability, however such productivities have not yet been obtained for long-term cultivation under outdoor conditions [9]. Besides climate, cultivation systems also have a significant impact on biomass productivities, and associated production costs [152-154]. Closed systems, such as flat panels and tubular reactors, have shown to have higher photosynthetic efficiencies and subsequent areal productivities compared to open raceway ponds [152,154]. These higher productivities however do come at a cost, as construction and operation of closed systems is more expensive compared to open systems [154,155]. Furthermore, the benefits of closed systems are not equal for all locations, and especially in locations with hot desert and tropical climates, the increased construction and operation costs related to requirements for cooling systems, do not always weigh up to the increased productivities [156,157].

The Arabian Peninsula, more specifically the Gulf Cooperation Council (GCC) countries, present great opportunity for large-scale algae production. The region offers an advantageous climate allowing for year-round production, large availability of non-arable land, easy access to seawater, and high number of $CO_2$-rich flue gas point sources. Despite these promising aspects, the GCC region has seen very few investigations into algae commercialization, and studies are

generally limited to strain identification and isolation, and small-scale investigation into high-value secondary metabolites or biofuels [15-18]. Additionally, some studies have investigated the cultivation of halotolerant species [92,158-160], and a limited number of strains have been cultivated in semi-large-scale outdoor cultivation systems [27,28,92]. All in all, the current state of research and development of algal technologies in the GCC region has unfulfilled potential. This study therefore aims to investigate and compare the economics of algae production in various locations across the GCC (Kuwait, Oman, Qatar, Saudi Arabia and the United Arab Emirates (UAE)). The impact of reactor systems (raceway ponds, horizontal tubular, vertical stacked tubular and flat panel), and different process choices on production costs are investigated in order to provide a tool for strategic planning and evaluating the economic viability of a GCC-based algae production industry.

## 5.2 MATERIALS & METHODS

The applied techno-economic model for biomass production has been developed and described in detail by Ruiz *et al.* (2016) [39]. The model is based on available empirical information and literature, and allows for projections of different scenarios for algae production in various locations, based on inputs such as climate data, productivities, equipment and consumable costs, as well as location dependent social and utilities related costs such as labor, taxes, electricity. Alterations to the original model are described here.

### 5.2.1 LOCATIONS AND CLIMATOLOGY

Seven locations across the GCC were included in the study (Figure 5.1). The locations were selected based on their proximity to the coast for sea-water access, as well as to be spatially distributed and to cover all GCC countries, with the exception of Bahrain, in order to provide an accurate representation of the differences which can be expected across the Arabian Peninsula. Bahrain was excluded due to its close proximity to Qatar, and minimal differences in climate. Location-dependent parameters which have been included in the study are: climate data and seawater temperatures (Table 5.1), as well as salaries (Table 5.3), and energy costs (Table 5.4).

### 5.2.2 PRODUCTION PROCESS CONSIDERATIONS

The model assumes the algae biomass production process, starting from nutrient enrichment of seawater and sterilization, to algae cultivation and biomass harvesting. Harvesting through centrifugation is assumed (base-case), with a 15% (w/w) algal slurry as the end-product of the simulated production process. Furthermore, different scales of cultivation were simulated, ranging from 1 to 100 hectares, and 10% of the cultivation area was assumed to be required to produce

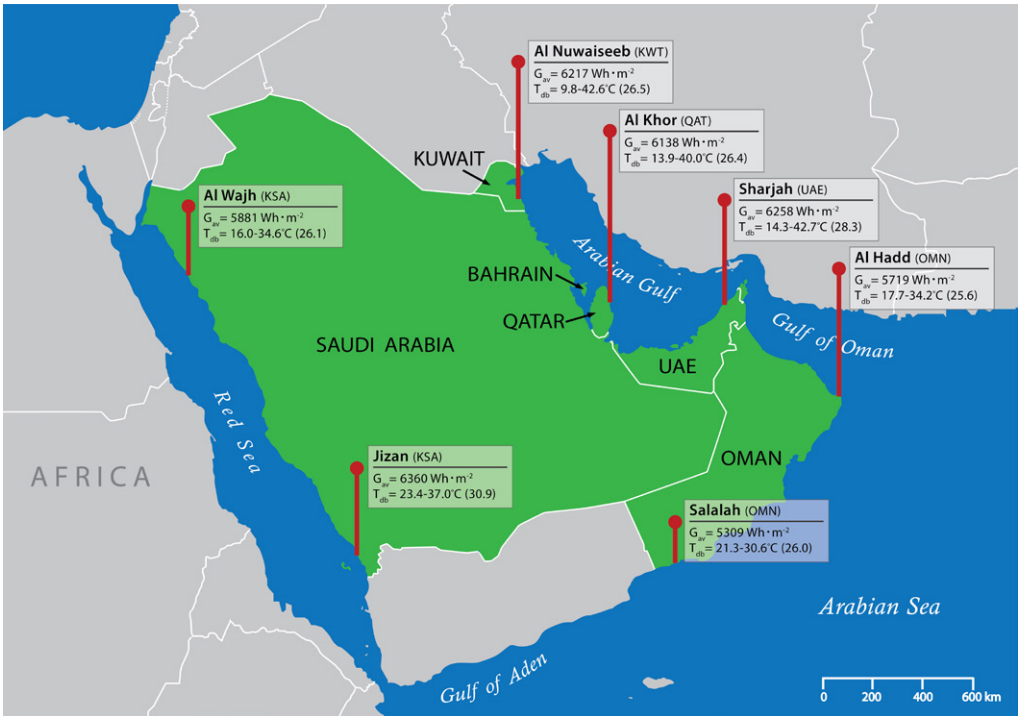



**Figure 5.1** – Selected locations across the GCC were assessed in the techno-economic model for algae biomass production. $G_{av}$ is the annual global average solar radiation (Wh·m$^{-2}$), and $T_{db}$ is the annual dry bulb temperature range (°C) (annual average between brackets).



**Table 5.1** – Climate data inputs used for the study, data is average±stdev of annual data.

| Parameter | Unit | Kuwait | Oman | | Qatar | Saudi Arabia | | UAE | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | *Nuwaiseeb* | *Al Hadd* | *Salalah* | *Al Khor* | *Al Wajh* | *Jizan* | *Sharjah* | |
| Global solar radiation[a] | Wh·m$^{-2}$ | 6217 | 5719 | 5309 | 6138 | 5881 | 6360 | 6258 | [26] |
| Dry Bulb Temperature[a] | °C | 26.5±9.3 | 25.6±3.1 | 26±2.1 | 26.4±7.0 | 26.1±4.6 | 30.9±3.5 | 28.3±7.3 | [26] |
| Relative Humidity[a] | % | 36±14.7 | 72.5±11.4 | 73.8±14.2 | 58.3±15.7 | 64.9±9.7 | 65.9±7.3 | 55.3±15.6 | [26] |
| Dew Point Temperature[a] | °C | 7.2±3.9 | 19.8±3.7 | 20.4±4.3 | 16.1±4.5 | 18.5±5.8 | 23.6±1.8 | 17.1±4.1 | [26] |
| Wind Speed[a] | m·s$^{-1}$ | 3.6±0.9 | 5.2±1.5 | 2.2±0.7 | 4.1±1.3 | 4.0±1.9 | 3.1±1.8 | 3.1±1.3 | [26] |
| Seawater Temperature[b] | °C | 24.9±6.0 | 27.2±2.1 | 26.5±1.6 | 26.9±5.3 | 26.8±2.4 | 30.2±1.9 | 28.0±4.3 | [27] |

[a] Annual average of hourly data over 2004-2018
[b] Annual average of monthly temperatures over 2009-2019

inoculum (non-productive area). The total facility size was assumed to be 120% of the cultivation area, with an additional 20% of land necessary for auxiliary processes, such as (office) buildings, roads, and major equipment. It was assumed that the land costs were 1200 €·ha$^{-1}$·yr$^{-1}$ (rented basis).

## *PRODUCTION SYSTEMS*

Four cultivation systems were taken into consideration, the designs of which are based on the AlgaePARC pilot facility (Wageningen, the Netherlands), and have been described in detail by Ruiz *et al.* (2016) [39]. A brief description of each system is provided:

- **Horizontal tubular photobioreactor (HT)**; closed system consisting transparent low-density polyethylene tubes (Ø 0.057 m), placed on the ground at 0.05 m distance, connected by loops at the end of each tube, with a volume:ground ratio of 23.8 L·m$^{-2}$. Culture is circulated through the tubes at a liquid velocity of 0.45 m·s$^{-1}$, and passed through a degasser (separate vessel) for the removal of excess oxygen. Tube length is dependent on oxygen buildup, with the maximum dissolved oxygen content prior to the degasser set at 300% of oxygen saturation.
- **Vertical stacked horizontal tubular photobioreactor (VT)**; closed system consisting of transparent borosilicate glass tubes (Ø 0.065 m), stacked parallel to the ground in a vertical structure (0.95 m high). Each unit contains loops of 8 vertically stacked tubes, and a distance of 0.50 m between each unit is assumed. The configuration results in a volume:ground ratio of 47 L·m$^{-2}$. The circulation liquid velocity of the culture is equivalent to the horizontal tubular system, and the length of the tubes was determined based on oxygen buildup, taking into consideration the same process constraints as for the horizontal tubular photobioreactor system.
- **Flat panel photobioreactor (FP)**; closed system consisting of transparent polyethylene 'bags', supported by a steel mesh casing, with a height of 0.50 m, and a light path of 0.02 m, each panel placed 0.25 m apart (volume:ground ratio 37 L·m$^{-2}$). Culture mixing is provided through air sparging from the bottom, which also prevents the buildup of excess oxygen, at a flow of 0.32 vvm. The entire front surface area is assumed to be exposed to direct radiation, and diffuse and reflected light can reach the back surface.
- **Raceway pond (RW)**; open system consisting of a shallow pond (0.20 m depth) with a single recirculation loop, and a total volume of 2856 m$^3$ (width: 28 m, length: 510 m), and volume:ground ratio of 200 L·m$^{-2}$. A single paddlewheel provides mixing and culture circulation at a liquid velocity of 0.25 m·s$^{-1}$. A carbonation sump (1.0 m deep and 0.65m long) across the width of one channel is assumed, to promote carbon transfer to the liquid of pH-dosed $CO_2$.

## BIOMASS PRODUCTIVITY & OPERATING CONDITIONS

The model utilizes photosynthetic efficiencies as the main determining factor for biomass productivities. In Ruiz *et al.* (2016), these productivities are based on empirical data from AlgaePARC (Wageningen University, the Netherlands), where different reactor types were evaluated side-by-side, and assuming that the same photosynthetic efficiencies applied for the same reactor types in different locations [39]. To improve the accuracy of the model, however, local productivities should be applied. Empirical data of (semi)large-scale outdoor cultivation in the GCC region is limited, especially in closed photobioreactors. There are, however, a number of cultivation studies in open raceway ponds, located in Qatar. The photosynthetic efficiency of these different experiments was calculated using equation 5.1, and can be found in Table 5.2:

$$PE = \frac{P_{X,areal} \cdot \Delta H_C^0 \cdot 10^{-3}}{\left(\frac{I_{day}/E_{PAR}}{0.43}\right)} \qquad \text{(eq. 5.1)}$$

In which *PE* is the photosynthetic efficiency (% sunlight), $P_{X,areal}$ is the average areal productivity in $g_X \cdot m^{-2} \cdot d^{-1}$, $\Delta H_C^0$ is the enthalpy of biomass combustions (22.5 $KJ \cdot g^{-1}$), $I_{day}$ is the average areal daily photon flux density (mol photons$\cdot m^{-2} \cdot d^{-1}$), $E_{PAR}$ is the energetic content of the PAR fraction of sunlight (4.76 $mol \cdot J^{-1}$), and 0.43 the conversion factor from sunlight to PAR light ($J \cdot J^{-1}$) [154].

The average photosynthetic efficiency obtained for the local studies in open raceway ponds, consisting of cultivation of a number of different strains in different seasons, was 1.92% (Table 5.2). In the model, this value was assumed for the photosynthetic efficiency of cultivation in open raceway ponds for the different locations. For horizontal tubular, vertical stacked horizontal tubular and flat panel photobioreactors, as no empirical regional data was available, photosynthetic

**Table 5.2** – Experimental data used in the study; obtained from outdoor cultivation trials in raceways ponds at Qatar University's Algal Technologies Program

| Strain | Period | Duration | Volume | Cultivation Mode | Dilution | Average Prod.[a] | Light Intensity | PE | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | | *d* | $m^3$ | | $d^{-1}$ | $g \cdot m^{-2} \cdot d^{-1}$ | $mol \cdot m^{-2} \cdot d^{-1}$ | % | |
| *Tetraselmis* sp. | May-2018 | 30 | 0.2 | R Batch[b] | 0.12-0.25 | 20.77 | 55.70 | 1.72% | [92] |
| | Jan-2018 | 31 | 0.2 | R Batch[b] | 0.12-0.25 | 12.97 | 31.01 | 1.93% | [92] |
| *Picochlorum* sp. | Oct-2018 | 8 | 25 | Batch | - | 17.25 | 41.52 | 1.91% | [161] |
| *Chrococcidiopsis* sp. | Oct-2016 | 60 | 0.2 | R Batch[b] | 0.25 | 16.08 | 33.28 | 2.23% | [162] |
| *Geitlerinema* sp. | May-2019 | 7 | 0.2 | Batch | - | 23.62 | 56.63 | 1.92% | [unpub.] |
| *Leptolyngbya* sp. | Aug-2020 | 7 | 0.2 | Batch | - | 20.56 | 52.92 | 1.79% | [*Chapter 4*] |
| Average | | | | | | | | 1.92% | |

[a] Average areal biomass productivity over the duration of the cultivation trial
[b] Repeated Batch

efficiencies of 1.5%, 2.4% and 2.7% were applied, respectively, as per de Vree *et al.* (2015) [154]. Chemostat operation of the cultivation systems was assumed, with 0.16, 0.25, 0.27 and 0.27 $d^{-1}$ dilution rates set for the raceway pond, horizontal tubular, vertical stacked tubular and flat panel photobioreactors, respectively. Furthermore, in the base case, it was assumed that the facility would be operational for 300 $d \cdot yr^{-1}$.

### *NUTRIENTS*

Urea and triple superphosphate were assumed as nitrogen and phosphate sources, respectively. The average price from August 2015-2020 was used for both urea and triple superphosphate, at 205 $€ \cdot ton^{-1}$ and 272 $€ \cdot ton^{-1}$, to minimize the effect of price fluctuations. For $CO_2$, commercial grade was assumed (base-case), at a price of 184 $€ \cdot ton^{-1}$ [163].

### *TEMPERATURE CONTROL*

The culture temperature was simulated, considering factors of irradiance, radiation and convection. For the raceway pond, the effect of evaporation and condensation was also determined, and no temperature control was assumed. For the closed cultivation systems, the maximum culture temperature was set to 40 °C, above which seawater cooling was applied. As the Arabian Gulf is not very deep, and ambient air temperatures fluctuate significantly over the course of a year, the seawater temperatures fluctuate strongly as well [164]. Thus, contrary to Ruiz *et al.* (2016) who assumed fixed water temperatures, a monthly fluctuating seawater temperature was assumed for each location (Table 5.1). The simulation assumed that cooling occurred though culture submerged heat-exchangers with an efficiency of 75%. The costs of the heat exchangers were based on the same considerations as Ruiz *et al.* (2016).

### *MAJOR EQUIPMENT*

Units of various capacities were added to the model for most major equipment (pumps, centrifuge, tanks etc.). These, as well as the costs and power requirements, are provided in the supplemental data (Table S.5.1). The ultimate capacity of each unit used for the cost-projection for the different facility sizes and production systems, was based on the calculated maximum capacity requirement during the highest irradiation (thus highest productivity). Furthermore, for each unit, a maximum of 90% load was assumed, and where required, multiple smaller units were assumed until the cost of a larger unit was equal or less in comparison. Prices of major equipment were corrected for inflation where necessary, and a 5% purchase tax was assumed [32].

## 5.2.3 LABOR COSTS

The total labor costs were based on number of personnel, salary, and employer's contribution. The required number of personnel of a 1-hectare facility formed the base-case, considering a

total of 10 employees (1 plant manager, 1 supervisor, and 8 operators of different skill levels). For the scale-up, a non-linear relation between labor requirements and size was assumed, according to the 0.25 power of the capacity ratio [165]. Salaries were based on average salaries for each country for Operations Manager, Supervisor, and Process Operator in the engineering sector [166], and employer's contributions were added to the labor costs to cover for liability of work-related accidents and occupations illness [167] (Table 5.3).

### 5.2.4 ELECTRICITY COSTS

All GCC countries provide subsidized electricity costs for agricultural activities – these subsidized rates were used for each location, and can be found in Table 5.4.

### 5.2.5 CURRENCY

Fluctuations in currency conversion rates were taken into account; all prices were added in their original currency, and conversion to the desired end-currency (EUR) considered the average

**Table 5.3 –** Labor cost considerations for different facility sizes and locations

| Facility Size | Plant Manager<br>*FTE* | Supervisor<br>*FTE* | Operator<br>*FTE* | Total<br>*FTE* |
|---|---|---|---|---|
| 1 ha | 1 | 1 | 8 | 10 |
| 10 ha | 1 | 2 | 15 | 18 |
| 100 ha | 1 | 3 | 28 | 32 |

| Location | Plant Manager<br>*Cost·month$^{-1}$* | Supervisor<br>*Cost·month$^{-1}$* | Operator<br>*Cost·month$^{-1}$* | Employer's contribution |
|---|---|---|---|---|
| Kuwait | KWD 2,070 | KWD 1,200 | KWD 680 | 21.0% |
| Oman | OMR 2,990 | OMR 1,920 | OMR 870 | 18.0% |
| Qatar | QAR 25,600 | QAR 14,400 | QAR 7,720 | 17.0% |
| Saudi Arabia | SAR 26,900 | SAR 14,400 | SAR 8,520 | 20.5% |
| UAE | AED 32,800 | AED 17,600 | AED 10,500 | 22.0% |

**Table 5.4 –** Subsidized electricity tariffs for agricultural consumers, for each location.

| Location | Electricity<br>*Cost·kWh$^{-1}$* [i] | | Ref |
|---|---|---|---|
| Kuwait (Nuwaiseeb) | KWD 0.010 | € 0.029 | [168] |
| Oman (Hadd) | OMR 0.010 | € 0.023 | [169] |
| Oman (Salalah) | OMR 0.020 | € 0.046 | [169] |
| Qatar (Al Khor) | QAR 0.070 | € 0.017 | [170] |
| Saudi Arabia (all locations) | SAR 0.160 | € 0.038 | [171] |
| UAE (Sharjah) | AED 0.075 | € 0.018 | [172] |

[i] Conversion to EURO using average conversion rate over 2019

conversion rates over the year of the quotation (in the case of CAPEX). For OPEX, such as labor and electricity costs, the average conversion rates over 2019 were used [173].

### 5.2.6 OPTIMIZATION: SENSITIVITY ANALSYSIS

A sensitivity analysis was performed to determine the effects of different parameters on the predicted biomass production costs. Seven different aspects were analyzed, more specifically: 1) increased photosynthetic efficiency, 2) increased temperature optima, 3) alternative harvesting methods, 4) increased operational days, 5) use of waste urea, 6) use of recovered $CO_2$, and 7) reduced wastewater treatment costs. The aspect of production scale was discussed separately. Each parameter was assessed individually for operation of the different reactor types in Qatar (Al Khor). An overview of the optimized scenarios vs. the reference scenarios is shown in Table 5.5.

The photosynthetic efficiencies were doubled for the different reactor types, whereas the temperature optima was chosen as such to eliminate the need for cooling in all cultivation systems. The alternative harvesting methodology (tilted screen and vacuum filter belt) were selected as they are common method for harvesting of larger (filamentous) cyanobacteria such as *Arthrosprira* sp. and *Leptolyngbya* sp. [120], which could be suitable candidates for production in the region [135] [*Chapter 4*]. The increase in operational days assumed chemical engineering industry standard operating days (8000 $h \cdot y^{-1}$) [174], the costs for waste urea and flue-gas $CO_2$ were estimates of transport costs only, assuming no purchase costs [39], and wastewater treatment costs were optimized through a 50% reduction.

**Table 5.5 –** Comparison of reference and improvement scenarios, applied to 100 ha cultivation scenario located in Qatar (Al Khor).

| Parameter | Reactor System | Reference Scenario | Optimized Scenario |
|---|---|---|---|
| ***Photosynthetic efficiency*** | RW | 1.92% | 3.84% |
| | HT | 1.50% | 3.00% |
| | VT | 2.40% | 4.80% |
| | FP | 2.70% | 5.40% |
| ***Temperature Optima*** | RW,HT,VT,FP | 40 °C | 60 °C |
| ***Harvesting*** | RW,HT,VT,FP | Centrifugation | Vibrating screen & filter press |
| ***Operational Days*** | RW,HT,VT,FP | 300 d | 333 d |
| ***Alternative Urea Source*** | RW,HT,VT,FP | 205 €·$t^{-1}$ | 50 €·$t^{-1}$ |
| ***Recovered $CO_2$*** | RW,HT,VT,FP | 0.184 €·$kg^{-1}$ | 0.029 €·$kg^{-1}$ |
| ***Wastewater treatment costs*** | RW,HT,VT,FP | 0.43 €·$m^{-3}$ | 0.215 €·$m^{-3}$ |

## 5.3 RESULTS & DISCUSSION

### 5.3.1 PRODUCTIVITIES

Algae biomass productivity predictions were made for various cultivation systems and locations across the GCC, (Figure 5.2). The different locations had only a minor impact on the projected productivities, attributed to similar climates across the region, with larger differences predicted for the different cultivation systems. Flat panel photobioreactors had the highest potential productivities, ranging from 62-74 $t \cdot ha^{-1} \cdot yr^{-1}$ across the different locations, followed by vertical stacked tubular reactors and raceway ponds. Lowest productivities (34-41 $t \cdot ha^{-1} \cdot yr^{-1}$) were predicted for the horizontal tubular system. The predicted productivities are quite promising compared to other global productivity predictions made, such as by Tredici *et al.* (2016), who estimated an annual biomass productivity of 36 and 54 $t \cdot ha^{-1} \cdot yr^{-1}$ for flat panel photobioreactors operated in Italy and Tunisia, respectively [175].

The productivities were strongly dependent on cultivation system and the associated productivity parameters, which form the basis of the model, most specifically the photosynthetic efficiency. The photosynthetic efficiency is dependent on many factors, such as strain, cultivation system, and cultivation conditions (light, temperature etc.), and can also be reduced by sub-optimal cultivation conditions [157]. Theoretical maximum photosynthetic efficiency values for outdoor cultivation range from 8 to 12%, however, such efficiencies have yet to be obtained for long-term outdoor cultivation [9,157]. In order to make robust predictions about productivities and associated production costs, outdoor data for selected strains, locations, and cultivation systems is essential. For the cultivation in raceway ponds, this data is available for the region (Table 5.2). For other



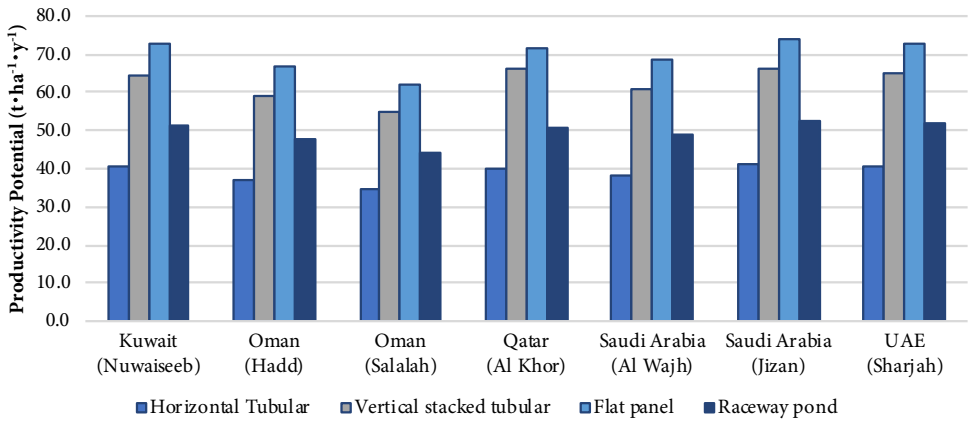



Figure 5.2 – Biomass productivity potential for various locations in the GCC and different cultivation systems.

cultivation systems however, the values applied are estimations based on results from AlgaePARC in the Netherlands, with *Nannochloropsis* sp. [39]. Nonetheless, the photosynthetic efficiencies found for cultivation of various strains over multiple seasons in raceway ponds in Qatar are higher (1.92%) as compared to those reported for *Nannochloropsis* sp. in the Netherlands (1.2%), as well as the average value of 1.5% generally applied [176] – if this trend can be extrapolated to the other cultivation systems as well, the current predictions could underestimate the biomass production potential of the region.

In terms of predicted biomass production costs (Figure 5.3), repeatedly, reactor type is the main determining factor, with minimal variations between the different locations. Both tubular systems had the highest overall cost (4.3-4.9 and 4.1-4.6 €·kg$^{-1}$ for horizontal tubular and vertical stacked tubular respectively), as compared to the flat panel reactor and raceway pond, at 3.0-3.2 and 2.9-3.5 €·kg$^{-1}$, respectively.

When focusing on Qatar (Al Khor), the lowest biomass production costs of 2.9 €·kg$^{-1}$ is predicted for raceway pond cultivation, followed by 3.1 €·kg$^{-1}$ for a flat panel photobioreactor. These costs are lower than recorded previously by Ruiz *et al.* (2016) for production in Saudi Arabia (4.0 and 3.2 €·kg$^{-1}$ for raceway ponds and flat panel reactors, respectively), and the projections for production in flat panels by Tredici *et al.* (2016) at 3.2 €·kg$^{-1}$ [39,175]. The lower costs compared to Ruiz *et al.* are most significant for raceway ponds, which can be related back to the higher photosynthetic efficiency assumed (1.92% as compared to 1.2%), based on empirical data from the region.

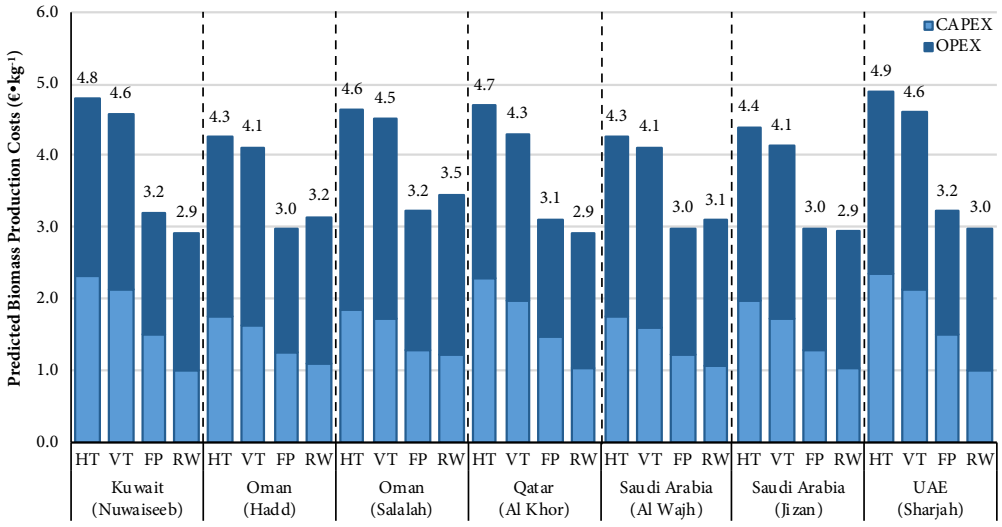



**Figure 5.3 –** Projected biomass production costs for both CAPEX (■) and OPEX (■) in €·kg$^{-1}$, for 100 ha facility in different GCC locations, and for bioreactor types (HT: Horizontal tubular, VT: Vertical stacked tubular, FP: Flat panel, RW: Raceway pond).

### 5.3.2 FACILTIY SCALE

he scale of a facility has a significant impact on the cost of production, with smaller scales having higher costs per unit of biomass produced. The above predictions were all based on a 100 hectare facility, however reaching such production capacities takes time. Nonetheless, cost predictions showed that the biomass production cost could already be reduced with up to 67% through an increase of scale from 1 to 10 hectares (Figure 5.4). Scaling up further to 100 hectares is projected to reduce the production costs with a mere extra 13%.

Largest gains are made in the operation costs (OPEX); at 1 hectare, up to 46.7% of the total biomass production costs are for labor (raceway ponds), whilst at 10 hectare this is only 15-24% depending on the reactor type, and at 100 hectare scale, labor costs can even go down to as low as 3.4% for vertical stacked tubular photobioreactors (Figure 5.5 and Table S.5.2). Ruiz *et al.* (2016) also showed that a scale increase from 1 hectares to 100 reduced the production costs [39], but

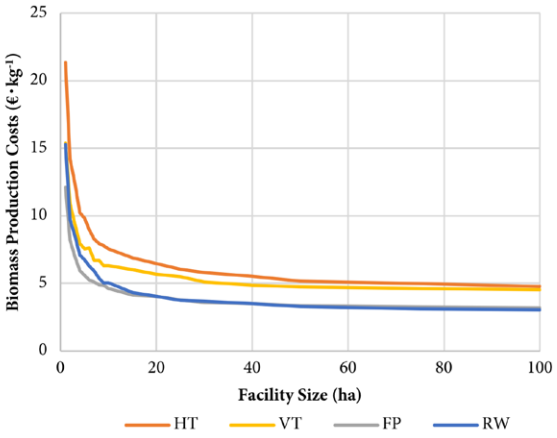
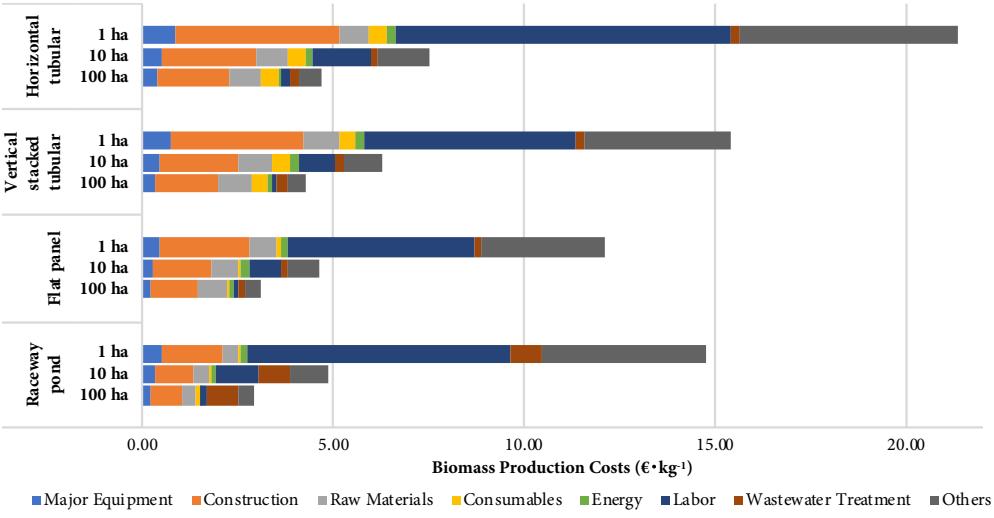



**Figure 5.4** (right) – Projected biomass production costs for raceway ponds (RW), horizontal tubular (HT), flat panel (FP) and vertical stacked tubular (VT) photobioreactors, as a function facility size (ha). Location: Qatar (Al Khor)

**Figure 5.5** (down) – Cost breakdown of projected biomass production costs in €·kg$^{-1}$, for a 1, 10 and 100 ha cultivation facility located in Qatar (Al Khor), and different bioreactor types (HT: Horizontal tubular, VT: Vertical stacked tubular, FP: Flat panel, RW: Raceway pond).



here we show that most of the gains can be made within the first 10 hectare scale-up alone. This would indicate that it could be feasible to have multiple smaller production facilities spread out, without significant impacts on production costs.

### 5.3.3 TEMPERATURE

Generally speaking, higher solar irradiances are paired with higher ambient temperatures. Especially in closed cultivation systems, the combination of these two factors can lead to very high culture temperatures if no external cooling is applied [152]. Considering the effects of irradiance, radiation, convection, evaporation and condensation on the heat fluxes of each cultivation system, maximum culture temperatures were estimated for each location by running the model without temperature control (Figure 5.6 A). Not surprisingly, due to the lack of evaporative cooling, and with no external cooling applied, the culture temperature profiles in the closed systems

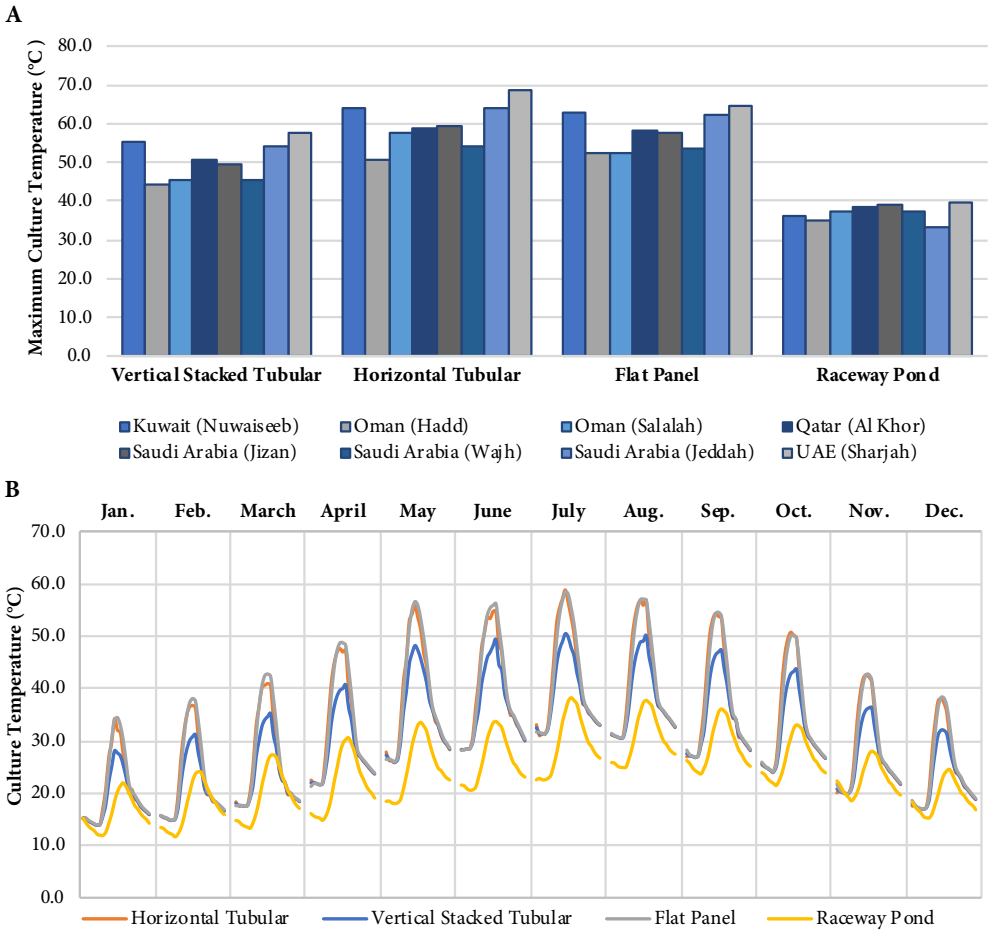



**Figure 5.6 – A)** Maximum culture temperatures simulated in photobioreactors without cooling for the different GCC locations and **B)** Average diurnal (00:00–24:00 h) temperature profiles simulated for each month, for the different cultivation systems without cooling, for cultivation in Qatar (Al Khor).

showed higher maxima as compared to the raceway ponds. Furthermore, locational differences in temperature maxima were significant, with differences of Δ 17.7 °C for horizontal tubular photobioreactors, with UAE (Sharjah) having the highest peak temperatures, of up to 68.6 °C for horizontal tubular reactors, and 39.7 °C for raceway ponds.

The monthly average diurnal culture temperatures were also modeled for Qatar (Al Khor) (Figure 5.6 B). Maximum daily fluctuation predicted were Δ 30.6 °C, for flat panel photobioreactors without cooling. Such extreme culture temperatures and fluctuations can significantly impact the productivity of a strain, with temperatures above optima rapidly leading to cell death [177], which is why temperature control through external cooling is necessary. The maximum culture temperature and fluctuations in the open raceway pond were considerably smaller, with a maximum of 39.7 °C, and maximum diurnal fluctuation of Δ 15.9 °C. In this scenario, where the optimum culture temperature is 40 °C, cooling is not required in the raceway ponds, however, as the lower temperature is mainly due to evaporative heat losses, this in turn will be paired with concerns of increasing salinities, and requirement for non-saline make-up water. Furthermore, the biological effects of diurnal variations will also need to be taken into consideration, as this could significantly reduce productivities depending on the strain [178].



### 5.3.4 SENSITIVITY ANALYSIS

In order to optimize the production process, and bring costs down, research and development is needed. A sensitivity analysis can guide the research focus areas, in order to obtain the largest gains. Here, a sensitivity analysis was performed, investigating the influence of various parameters on the overall biomass production costs, for production in the various reactor types simulated in Qatar (Al Khor) (Figure 5.7).

Regardless of reactor type, the foremost influencer of biomass production costs is the photosynthetic efficiency, with a doubling in PE resulting in cost reductions of 32.7-42.5% as compared to the base case. Second most influential parameter, specifically for the closed systems, was the temperature optima of the strain. Increasing this parameter to 60 °C, thereby eliminating the need for temperature control – maximum predicted culture temperature in Qatar was 58.8 °C in horizontal tubular reactors – showed to reduce the projected production costs with up to 25%. In raceway ponds there was no benefit for increased temperature optima, as the maximum culture temperature was lower compared to the optimal temperature set in the base case (40 °C).

In multiple studies, the improvement of photosynthetic efficiencies has been recognized as a major player in reducing production costs [39,163]. In order to come close to the optimized scenario, especially for the Arabian Peninsula where light is readily available, strains which maintain high

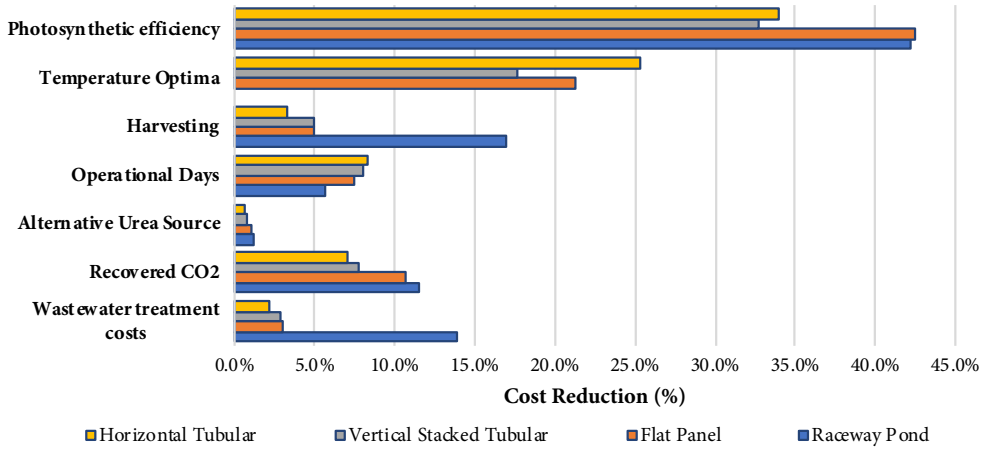



**Figure 5.7** - Sensitivity analysis on biomass production cost for production in Qatar. Reference and improvement scenario parameters can be found in Table 5.5. Effect of individual parameters on cost is shown in horizontal axis. Detailed values can be found in Table S.5.3).

photosynthetic efficiencies under high light intensities will be essential [9]. Different approaches have been investigated to optimize photosynthetic efficiencies, both biological (strain selection, strain adaptation) and non-biological (bioreactor design, process optimization) in nature [157]. High light intensities are a common climatic conditions across the Arabian Peninsula, and as of such, local strains have the potential of being well adapted to thrive under such conditions. Increasing the bioprospecting efforts in the region could potentially result in strains which are optimally adapted to maintain high photosynthetic efficiencies even under high light intensities, and high temperatures as well. All in all, establishing long-term outdoor cultivation with high photosynthetic efficiencies will require an interplay between strain selection, bioreactor design, and climate conditions (light and temperature), in order to obtain the envisioned improvements.

The effect of other improvements on predicted biomass production costs had limited overall impacts on cost reductions, with the exception of harvesting and wastewater treatment for raceway pond cultivation. Not surprisingly, as cultivation in raceway ponds requires handling of larger culture volumes due to more dilute cultures, improvements in liquid handling, such as harvesting and wastewater treatment costs, can improve the raceway pond production costs with 16.9% and 13.8%, respectively. The impact of the two parameters on closed systems is less, ranging from 3.2-5.0% for harvesting and 2.2-3.0% for wastewater treatment. The integration with industrial waste sources, such as flue gas $CO_2$ and waste urea, would have a slight impact on the production costs as well (reductions of up to 11.5% for $CO_2$ and 1.2% for urea). The use of waste urea for algae biomass cultivation has been demonstrated as a feasible alternative by Al Jabri *et al.* (2020), and although the cost benefit is minimal, other secondary benefits, such as reduction of industrial waste, should also be taken into consideration [179].

## 5.4 CONCLUSIONS & RECOMMENDATIONS

The aim developing the presented model was to enable generation of biomass production costs projections in multiple cultivation systems and locations in the GCC region, as well as identifying key focus areas for possible cost-reductions. The model outcome indicates that raceway ponds and flat panel photobioreactors represent the most credible options for large-scale production in the region, resulting in the lowest calculated biomass production costs. These projected production costs levels were found to be competitive with similar global studies, confirming the region's potential as an economically attractive location for algae production, linking into feed, food and nutraceutical industries.

The photosynthetic efficiency was found to be the most significant variable influencing the production costs, yet therefore also introduced the most uncertainty into the model. For raceway pond base-cases, the photosynthetic efficiency used for the predictions was based on empirical data from the region. Such regional data is however not (yet) available for other cultivation systems. Improvements in photosynthetic efficiency, as well as temperature optima of the strain, will make the most gains towards significant reductions in the production costs. This is a clear indicator that bioprospecting efforts in the region for photo- and thermo- tolerant strains could be the differentiating factor the algae industry needs for competitive and commercially viable establishment.



A recommended forward route towards validation of these model outcomes would be to conduct regional pilot-scale studies of different reactor types side-by-side, most specifically flat panel photobioreactors and open raceway ponds. Besides productivity data, which can be used to support the assumptions made within the techno-economic analysis, other practical aspects of scale-up can and should be investigated concurrently. For example:

- Impact of ultraviolet radiation levels on long-term integrity of (plastic) bioreactors and associated outdoor facility equipment;
- The effect of sand and dust ingress (which is a prevalent constant in the region's atmosphere) on open cultivation systems and downstream-processing;
- Industrial integration for process inputs, such as carbon dioxide
- Water management strategies to deal with high levels of evaporative losses during cultivation in open systems

Studying such aspects at pilot scale will provide the key-insights needed to create a clear road-map towards commercialization of an algae-based industry within the GCC region.

# SUPPLEMENTAL MATERIALS

## SUPPLEMENT A: COST OVERVIEW OF MAJOR EQUIPMENT

**Table S.5.1** - Major Equipment Capacities and Costs (corrected for inflation)

| Details | Capacity | | Costs·unit$^{-1}$ (€) | Power Req. (kWh) | Efficiency |
|---|---|---|---|---|---|
| ***Pumps*** | | | | | |
| Pump (seawater) | 2 | $m^3 \cdot h^{-1}$ | € 480 | 0.18 | |
| | 4 | $m^3 \cdot h^{-1}$ | € 1,091 | 0.4 | |
| | 20 | $m^3 \cdot h^{-1}$ | € 3,163 | 12 | |
| | 200 | $m^3 \cdot h^{-1}$ | € 14,292 | 5.9 | |
| Culture circulation pump | 700 | $m^3 \cdot h^{-1}$ | € 29,628 | | 0.75 |
| | 7000 | $m^3 \cdot h^{-1}$ | € 206,623 | | 0.75 |
| | 28000 | $m^3 \cdot h^{-1}$ | € 628,510 | | 0.75 |
| ***Tanks*** | | | | | |
| Liquid Holding Tank | 10 | $m^3$ | € 2,556 | | |
| | 20 | $m^3$ | € 4,711 | | |
| | 50 | $m^3$ | € 7,337 | | |
| | 400 | $m^3$ | € 48,493 | | |
| | 4000 | $m^3$ | € 501,539 | | |
| ***Sterilization Process*** | | | | | |
| Medium Filter Unit | 5.99 | $m^3 \cdot h^{-1}$ | € 16,010 | | |
| | 59.9 | $m^3 \cdot h^{-1}$ | € 113,343 | | |
| ***Mixing Unit*** | | | | | |
| Media Mixing Unit | 0.1 | $m^3$ | € 14,759 | 0.05184 | |
| | 4 | $m^3$ | € 231,924 | 2.0736 | |
| | 8 | $m^3$ | € 256,171 | 4.1472 | |
| | 25 | $m^3$ | € 306,772 | 12.96 | |
| ***$CO_2$ Supply*** | | | | | |
| $CO_2$ Supply Unit | 1 | $ha^{-1}$ | € 4,978 | | |
| ***Aeration (for degasser and flat panel photobioreactors)*** | | | | | |
| Air Blower | 200 | $m^3 \cdot h^{-1}$ | € 3,191 | 0.99 | |
| | 1000 | $m^3 \cdot h^{-1}$ | € 5,959 | 3.96 | |
| | 2499 | $m^3 \cdot h^{-1}$ | € 11,788 | 11.15 | |
| ***Degasser*** | | | | | |
| Degasser | 60 | $m^3 \cdot h^{-1}$ | € 1,280 | | |
| | 600 | $m^3 \cdot h^{-1}$ | € 2,641 | | |
| ***Harvesting*** | | | | | |
| Centrifuge | 0.13 | $m^3 \cdot h^{-1}$ | € 28,463 | 1.1 | |
| | 2.1 | $m^3 \cdot h^{-1}$ | € 53,764 | 4 | |
| | 30 | $m^3 \cdot h^{-1}$ | € 180,000 | 18 | |
| | 70 | $m^3 \cdot h^{-1}$ | € 360,000 | 28 | |
| Tilted Screen (120$m^2$) | 200 | $m^3 \cdot h^{-1}$ | € 7,768 | 0.2 | |
| Vacuum Suction filter belt | 2 | $m^3 \cdot h^{-1}$ | € 93,211 | 0.45 | |
| ***Reactor Frames & Support*** | | | | | |
| Steel Mesh Casing (Flat Panel) | 0.875 | $kg \cdot m^{-2}$ | € 650 ·$ton^{-1}$ | | |
| Metal Poles (Vertical Tubular) | 3.8 | $kg \cdot m^{-1}$ | € 720 ·$ton^{-1}$ | | |

**Table S.5.2 –** Biomass production capacity, and breakdown of projected biomass production costs, in capital expenses (CAPEX) and operational expenses (OPEX) for 1, 10 and 100 ha production facility sizes located in Qatar (Al Khor).

| | | Biomass Capacity | Biomass Costs | CAPEX | OPEX | Initial Investment | Major Equipment | Construction | Raw Materials | Consumables | Energy | Labor | Wastewater Treatment | Others |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $t \cdot y^{-1}$ | $€ \cdot kg^{-1}$ | $€ \cdot kg^{-1}$ | $€ \cdot kg^{-1}$ | M€ | $€ \cdot kg^{-1}$ | $€ \cdot kg^{-1}$ | $€ \cdot kg^{-1}$ | $€ \cdot kg^{-1}$ | $€ \cdot kg^{-1}$ | $€ \cdot kg^{-1}$ | $€ \cdot kg^{-1}$ | $€ \cdot kg^{-1}$ |
| **Raceway pond** | **1 ha** | 51 | 14.7 | 2.1 | 12.6 | 1.3 | 0.5 | 1.6 | 0.4 | 0.1 | 0.1 | 6.9 | 0.8 | 4.3 |
| | **10 ha** | 509 | 4.9 | 1.4 | 3.5 | 8.2 | 0.3 | 1.0 | 0.4 | 0.1 | 0.1 | 1.2 | 0.8 | 1.0 |
| | **100 ha** | 5091 | 2.9 | 1.0 | 1.9 | 61.7 | 0.2 | 0.8 | 0.4 | 0.1 | 0.0 | 0.2 | 0.8 | 0.4 |
| **Horizontal tubular** | **1 ha** | 40 | 21.4 | 5.1 | 16.2 | 2.6 | 0.9 | 4.3 | 0.8 | 0.5 | 0.2 | 8.8 | 0.2 | 5.7 |
| | **10 ha** | 398 | 7.5 | 3.0 | 4.5 | 15.4 | 0.5 | 2.5 | 0.8 | 0.5 | 0.2 | 1.5 | 0.2 | 1.4 |
| | **100 ha** | 3977 | 4.7 | 2.3 | 2.4 | 116.8 | 0.4 | 1.9 | 0.8 | 0.5 | 0.1 | 0.2 | 0.2 | 0.6 |
| **Flat panel** | **1 ha** | 72 | 12.1 | 2.8 | 9.3 | 2.6 | 0.5 | 2.3 | 0.7 | 0.1 | 0.2 | 4.9 | 0.2 | 3.2 |
| | **10 ha** | 716 | 4.6 | 1.8 | 2.8 | 16.5 | 0.3 | 1.5 | 0.7 | 0.1 | 0.2 | 0.8 | 0.2 | 0.8 |
| | **100 ha** | 7159 | 3.1 | 1.5 | 1.6 | 136.4 | 0.3 | 1.2 | 0.7 | 0.1 | 0.1 | 0.1 | 0.2 | 0.4 |
| **Vertical stacked tubular** | **1 ha** | 64 | 15.4 | 4.3 | 11.1 | 3.5 | 0.7 | 3.5 | 0.9 | 0.5 | 0.2 | 5.5 | 0.3 | 3.8 |
| | **10 ha** | 636 | 6.3 | 2.5 | 3.8 | 20.7 | 0.4 | 2.1 | 0.9 | 0.5 | 0.2 | 0.9 | 0.3 | 1.0 |
| | **100 ha** | 6629 | 4.3 | 2.0 | 2.3 | 168.9 | 0.3 | 1.6 | 0.9 | 0.4 | 0.1 | 0.1 | 0.2 | 0.5 |

## SUPPLEMENT C: SENSITIVITY ANALYSIS

**Table S.5.3 –** Biomass production cost reductions for the different optimization parameters compared to the base-case, assessed for each cultivation system at 100 ha scale in Qatar (Al Khor).

| Reactor System | Parameter | Reference Costs | Optimized Costs | Cost Reduction) |
|---|---|---|---|---|
| | | €·kg$^{-1}$ | €·kg$^{-1}$ | % |
| **Horizontal Tubular** | *Photosynthetic efficiency* | 4.78 | 3.18 | -33.4% |
| | *Temperature Optima* | | 3.58 | -25.0% |
| | *Harvesting* | | 4.62 | -3.3% |
| | *Operational Days* | | 4.42 | -7.5% |
| | *Alternative Urea Source* | | 4.74 | -0.8% |
| | *Recovered $CO_2$* | | 4.44 | -7.0% |
| | *Wastewater treatment costs* | | 4.68 | -2.0% |
| | *Electricity costs* | | 4.71 | -1.4% |
| **Vertical Stacked Tubular** | *Photosynthetic efficiency* | 4.53 | 3.04 | -32.9% |
| | *Temperature Optima* | | 3.80 | -16.1% |
| | *Harvesting* | | 4.31 | -4.9% |
| | *Operational Days* | | 4.20 | -7.3% |
| | *Alternative Urea Source* | | 4.50 | -0.7% |
| | *Recovered $CO_2$* | | 4.20 | -7.3% |
| | *Wastewater treatment costs* | | 4.40 | -2.9% |
| | *Electricity costs* | | 4.47 | -1.4% |
| **Flat Panel** | *Photosynthetic efficiency* | 3.19 | 1.83 | -42.7% |
| | *Temperature Optima* | | 2.53 | -20.7% |
| | *Harvesting* | | 3.04 | -4.8% |
| | *Operational Days* | | 2.98 | -6.6% |
| | *Alternative Urea Source* | | 3.16 | -1.0% |
| | *Recovered $CO_2$* | | 2.86 | -10.4% |
| | *Wastewater treatment costs* | | 3.10 | -2.9% |
| | *Electricity costs* | | 3.13 | -1.9% |
| **Raceway Pond** | *Photosynthetic efficiency* | 2.95 | 1.70 | -42.3% |
| | *Temperature Optima* | | 2.95 | 0.0% |
| | *Harvesting* | | 2.45 | -16.9% |
| | *Operational Days* | | 2.80 | -5.0% |
| | *Alternative Urea Source* | | 2.91 | -1.3% |
| | *Recovered $CO_2$* | | 2.61 | -11.5% |
| | *Wastewater treatment costs* | | 2.54 | -13.8% |
| | *Electricity costs* | | 2.93 | -0.6% |

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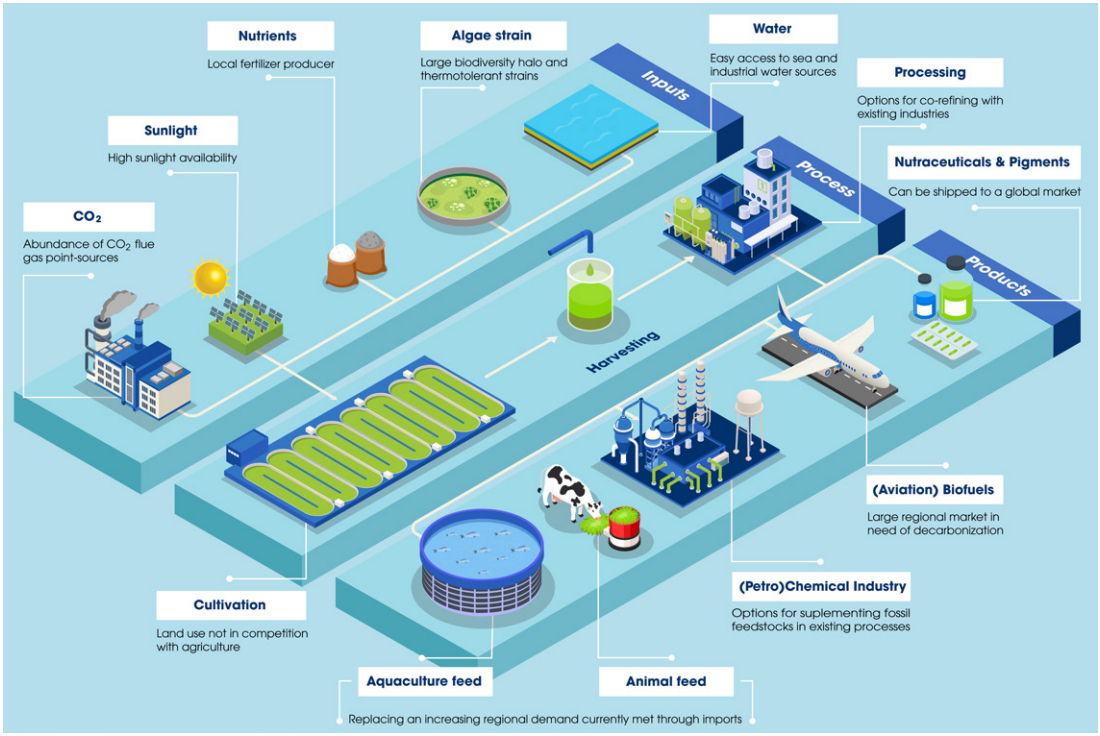
Nutrients
Local fertilizer producer
Algae strain
Large biodiversity halo and thermotolerant strains
Water
Easy access to sea and industrial water sources
Processing
Options for co-refining with existing industries
Inputs
Sunlight
High sunlight availability
Process
Nutraceuticals & Pigments
Can be shipped to a global market
$CO_2$
Abundance of $CO_2$ flue gas point-sources
Products
Harvesting
(Aviation) Biofuels
Large regional market in need of decarbonization
Cultivation
Land use not in competition with agriculture
(Petro)Chemical Industry
Options for suplementing fossil feedstocks in existing processes
Aquaculture feed
Animal feed
Replacing an increasing regional demand currently met through imports

# Chapter 6

## General Discussion:

## Algae as a sustainable feedstock for the Arabian Peninsula

## ABSTRACT

Algae have promising potential as a sustainable feedstock for the production of feed, fuels, chemicals, materials, and nutraceuticals. In particular in regions such as the Arabian Peninsula and the Gulf Cooperation Council (GCC), algae could play a significant role in enhancing local economic diversification, as well as in reducing dependence on fossil fuels and ecological footprint. The strengths of commercial algae production within a GCC context were studied, looking into opportunities the region offers, as well as challenges which could be encountered when establishing a regional algae industry. Favorable climate conditions, as well as the availability of non-arable land, seawater and flue-gas $CO_2$, were some of the major identified benefits that could contribute towards an economically feasible production process. Furthermore, the large indigenous biodiversity of algal and cyanobacterial strains with thermo-, photo-, and halo-tolerant properties could provide a valuable cornerstone for viable outdoor production processes. Regional challenges, however, include climate change, securing human resources, and the vital transitioning from research- to commercial scales. With sufficient regional ambition, however, as well as proactive and balanced management of the foreseen risks, there is significant potential for commercially viable algae-based value chains in the GCC, spanning product ranges which encompass aquaculture and livestock feeds, fuels, and high-value metabolites.

## 6.1 INTRODUCTION

Global climate change, increasing global population and associated demand for food, as well as the rapidly accelerating energy transition, have led to an increased global interest in the development of sustainable resources. This is certainly prevalent in the GCC region (Box 1), where economic activity sustainability levels are currently limited, as the GCC countries' GDPs are based mainly on fossil fuel exports, and arid climates cause food security to rely heavily on imports. While the fossil fuel-rich economies of the Gulf exhibit some of the highest ecological footprints in the world, this same fact also generates the drive and the resources to develop sustainable local solutions [180,181].

One of the proposed local solutions with a high regional potential is the production of microalgae. Microalgae are considered as one of the most promising renewable feedstocks for sustainable production of food, feed, chemicals, materials and fuels [32]. Contrary to traditional crops, algae have higher aerial productivities, do not require arable land or fresh water for cultivation, and can be harvested year-round [9,182]. There is an immense variety in types of algae strains, which can vary in optimal cultivation conditions, biomass productivity and composition. Selecting the right algae strain for a specific location and product, is key in achieving a successful production process.

Despite the mentioned benefits, the production costs for algae-based products are high relative to the conventional products they intend to replace [39]. Global R&D efforts are therefore focused on optimizing algal productivities and related technologies, with the aim of reducing its cost footprint, and enabling these to become cost-competitive and commercially viable. Here we discuss the potential of algae as a sustainable feedstock for the GCC region, exploring regional factors such as climate and strains, local resources and end-users, as well as implementation challenges.



**BOX 1:**
GULF COOPERATION COUNCIL

The Gulf Cooperation Council (GCC) is a regional intergovernmental political and economic union of six countries on the Arabian Peninsula: Bahrain, Kuwait, Oman, Qatar, Saudi Arabia (KSA) and the United Arab Emirates (UAE).

## 6.2 INPUTS FOR SUCCESSFUL ALGAE PRODUCTION

### 6.2.1 LOCATION: MAXIMIZING POTENTIAL

The economic feasibility of algae cultivation is largely dependent on the localization of production, especially when considering outdoor cultivation. More often than not, location is the driving factor behind choices in strain, cultivation systems, and products [39,183,184]. As photosynthetic algae cultivation is dependent on light, maximum theoretical biomass productivities are obtained in locations where solar irradiances are highest (Figure 6.1). Furthermore, most cultivated algae thrive in moderate to warm temperatures, ranging from 20 °C to 30 °C [185], with sub-optimal temperatures significantly reducing biomass productivities [177]. Seasonal variability in climatic conditions, as well as the occurrence of other adverse climate conditions - such as significant precipitation and storms - are equally important factors, as they could prevent year-round production, thereby reducing the overall annual yields which could be obtained. For the GCC region, the balance of such climatic aspects is favorable: high solar irradiances, moderate to warm temperatures, and low annual precipitation have been shown to be able to support high biomass productivities all year round [92]. Furthermore, the region holds further advantages for algae production such as nutrient, non-arable land, and sea-water availability, as well as an rich environment with a high degree of algal biodiversity.

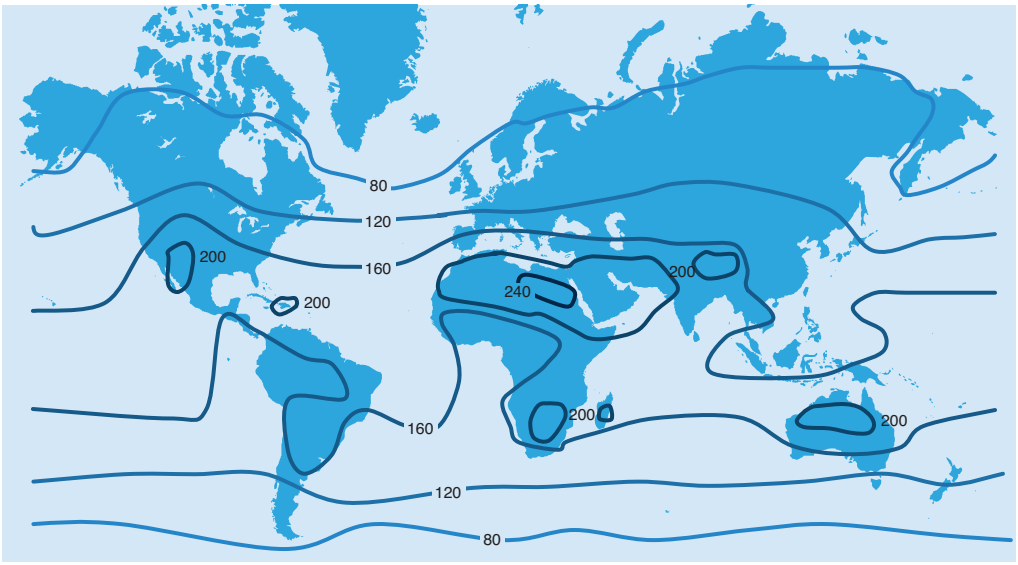


**Figure 6.1** – Global hypothetical algae biomass productivities ($t \cdot ha^{-1} \cdot y^{-1}$) which can be obtained assuming 5% photosynthetic efficiency and 20 $MJ \cdot kg^{-1}$ dry biomass From Tredici (2010) **[9]**

### 6.2.2 'SUPER-STRAINS'

The potential productivity of algae is not only dependent on local conditions, but also largely on strain. A large number of algae-related research revolves around the isolation of new strains, and investigation of their cultivation potential [*Chapter 2*]. The large diversity of algae, characterized by very different physiological attributes, implies that wherever algae cultivation is located, local algal strains isolated from natural habitats will be the best adapted to those specific conditions, and would therefore likely serve best as candidates for large-scale cultivation [186,187]. For example, high temperatures and high irradiances can cause sub-optimal biomass productivities and low photosynthetic efficiencies, as strains can be photo inhibited, or heat stress can occur. The light intensities and temperatures at which this occurs however are highly strain dependent. As local species have long been adapted to the prevailing regional abiotic and biotic factors, they are evolutionarily primed to thrive under such local conditions [*Chapter 3*]. For that reason, location is a key determining factor for the selection of microalgae for cultivation, and an 'ideal' strain will likely be different for each location, especially if outdoor cultivation is envisioned.

Despite the high potential of the GCC region for algae cultivation, efforts into the investigation of local strains for commercial production purposes are thus far limited. In Saudi Arabia, studies into the lipid productivity potential of various isolates have been performed [40,188], and several research groups in the UAE, Saudi Arabia, and Qatar have been investigating local halotolerant species [10,27,28,92]. The most comprehensive resource for regional strains (to the best of the author's knowledge) is the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM) (Figure 6.2) [10]. Established by the Algal Technologies Program, the QUCCCM houses over 200 strains isolated from the Qatar environment, a number of which have been investigated for the production of biofuels, fish feed, biofertilizer, and phycocyanin, as well as their potential for (large-scale) outdoor production [10,27,28,77,162,189-191][*Chapters 2 & 3*].



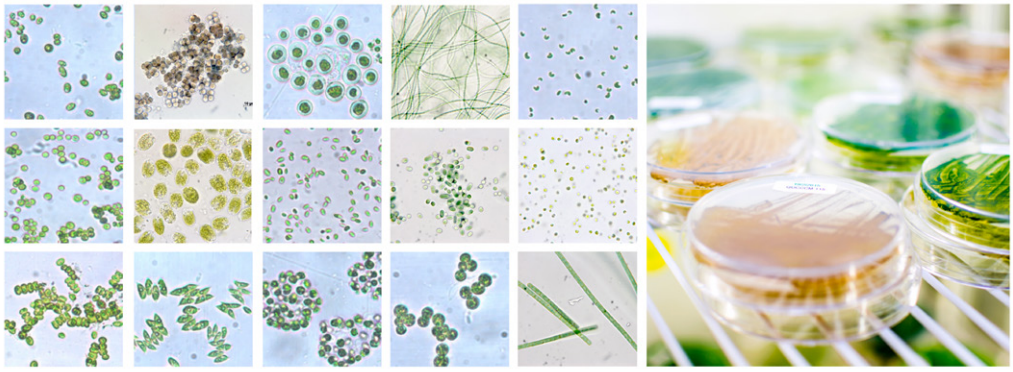

**Figure 6.2** – Microscope images of some examples of strains isolated from the Qatar environment, housed in the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM)

### 6.2.3 NATURAL RESOURCES & UTILITIES

The decision of where to locate an algae production facility is based on many more aspects than just climatic conditions. Additional requirements include the availability of natural resources and utilities, such as water, land and power. When assessing the potential for sustainable production of algae biomass, the use of such natural resources requires specific consideration, as utilization thereof should not be in competition with food-production, nor deplete already limited non-renewable reserves.

The GCC countries are scarce in natural fresh-water, and existing agricultural activities deplete these resources faster than they can be replenished. This causes salt-water intrusion and quality deterioration of ground water reservoirs. It is therefore not surprising that the GCC are the most water stressed countries globally, with Kuwait, UAE and Saudi Arabia holding global ranks 1-3 (Table 6.1). Over 60% of the GCC's potable water is sourced from desalination plants (in Qatar even 99%) – however this production method brings with it significant sustainability concerns, both in terms of energy requirements and environmental impact [192,193]. As water is one of the largest requirements for algae production, sustainable production will require the use of sustainable water sources such as seawater, and selected marine microalgae strains. With over 8,000 km of coastline however, seawater is easily accessible in the GCC. Furthermore, the availability of brackish ground-water unsuitable for agriculture, or high quality treated wastewater, create opportunities for use as evaporation-loss compensation, or cultivation of strains with lower salt tolerances [5].

The GCC covers a total land area of over 2.5 million km$^2$, of which over 98% is non-arable [181]. Some of the GCC nations have undertaken extensive efforts to increase the amount of land on which agriculture is viable, however this is at a great expense of both water stress and fertilizer use

**Table 6.1** – Overview of land availability and use, water stress, fertilizer consumption, and other geographical characteristics

| Country | Total Area | Arable land | Agricultural land[a] | Fertilizer Consumption[b] | Water Stress[c] | Coast Line | Land area below 5 m |
|---|---|---|---|---|---|---|---|
| | *km$^2$* | *km$^2$* | *km$^2$* | *kg·ha$^{-1}$* | - | *km* | *km$^2$* |
| Bahrain | 778 | 16 (2.1%) | 86 (11.1%) | 1,319 (0.7%) | 206 | 161 | 265 (34.0%) |
| Kuwait | 17,820 | 80 (0.4%) | 1,500 (8.4%) | 751 (1.3%) | 2,603 | 499 | 1,461 (8.2%) |
| Oman | 309,500 | 560 (0.2%) | 14,361 (4.6%) | 468 (275.4%) | 106 | 3,165 | 2,476 (0.8%) |
| Qatar | 11,610 | 143 (1.2%) | 670 (5.8%) | 6,755 (3.3%) | 473 | 563 | 1,405 (12.1%) |
| Saudi Arabia | 2,149,690 | 34,760 (1.6%) | 1,736,197 (80.8%) | 177 (13.3%) | 1,243 | 2,640 | 8,599 (0.4%) |
| UAE | 71,020 | 445 (0.6%) | 3,888 (5.5%) | 715 (3.4%) | 2,346 | 1,318 | 3,409 (4.8%) |

Data source: The World Bank https://data.worldbank.org/ - accessed June 23rd 2020

[a] Agricultural land refers to the share of land area that is under permanent crops, and under permanent pastures

[b] Fertilizer consumption as kg per hectare of arable land, and fertilizer consumption percentage of local production

[c] Water stress refers to freshwater withdrawal as a proportion of available freshwater resources

**Figure 6.3** – Typical topography of a Sabkha (Dukhan Sabkha, Qatar)

(Table 6.1). At the same time however, the abundance of non-arable land poses a great opportunity for sustainable algae cultivation. Large areas of this non-arable land can be found in the coastal regions of the Arabian Peninsula; salt intrusion has increased the salinity of the soil, making the land unsuitable for traditional agricultural. Furthermore, coastal saline mudflats, also known as Sabkha (سبخة Figure 6.3), are especially interesting due to their close proximity to the sea, easy access to shallow saline groundwater, and often sub-sea-level elevations. These characteristics would provide minimal pumping requirements for seawater, as well as offering the opportunity for groundwater cooling. Sabkhas, of which 6% of the GCC's land area consists, are generally considered as unproductive, with little to no economic value [194]. Conversely and remarkably, they actually represent a great sustainable option for algae cultivation.

Finally, the sunlight availability in the region does not only make it an attractive location for algae production, but also for solar power. With kWh-prices for solar power stations dropping rapidly, a number of countries in the GCC have joined the race for constructing the world's cheapest solar photovoltaic (PV) energy plants. On a costs-per-kWh capacity basis, such PV power plants are more economically attractive than their natural gas- or nuclear power- equivalents [195]. Numerous PV power plants are being built or planned in the region, driven by the ambition to replace significant parts of fossil-fuel power requirements in the coming years. Sourcing the power requirements to operate an algae production facility from solar energy in the GCC will not only be cheaper, but also far more sustainable.



## 6.3 INTEGRATION OPPORTUNITIES FOR MICROALGAE IN GCC'S ECONOMY

The GCC's member state economies, although largely fossil-fuel driven, provides many opportunities for integration with microalgae production. These can range from integration with industrial (waste) streams for process inputs such as nutrients, water, $CO_2$, or residual heat, up to spanning beyond the production process, to availability of investment potential, and local end-users.

### 6.3.1 SUSTAINABLE PROCESS INPUTS & INDUSTRIAL INTEGRATION

It has been well established that algae-based processes can be used for integration with industrial $CO_2$ point sources, wastewater treatment, and other industry streams [196,197]. Various studies have shown that microalgae are capable of growing on $CO_2$-rich flue gas from point sources such as in cement factories and power plants [198-200]. Although challenges exist – the effect of flue-gas composition, incl. $CO_2$ concentration and the presence of $NO_x$, $SO_x$ and heavy metals, on the algae cultivation process as well as the product quality for example. These could be overcome however with adequate strain selection, implementation of gas purification technologies, and other technological advances [*Chapter 2*] [200,201]. The integration of flue-gas $CO_2$ with algae cultivation has been estimated to lead to a reduction in biomass production costs of 11.5 %, as compared to when commercial $CO_2$ is used [*Chapter 5*]. Within the GCC there are numerous large-scale sources of high concentration $CO_2$, such as natural gas processing and gas-to-liquid (GTL) plants, as well as the cement and steel industry. All in all, over 74% of the GCC's $CO_2$ emissions originate from such point sources from the energy, and construction industries, totaling over 750 Mt $CO_2$ annually in 2016 (Figure 6.4), offering enticing opportunities for potential monetization thereof through linking into algae-derived product value chains.

The same regional industries which might provide $CO_2$ feedstocks, also hold potential to supply even further requirements of algae production. Water is an essential part of many industrial processes, and with 'zero liquid discharge' policies for industrially used and produced water spreading across the region, the demand for onshore water recycling options is increasing. Although the main (sustainable) source of water for algae cultivation in the region is seawater, the requirement of non-saline water as make-up for evaporation and other process steps will

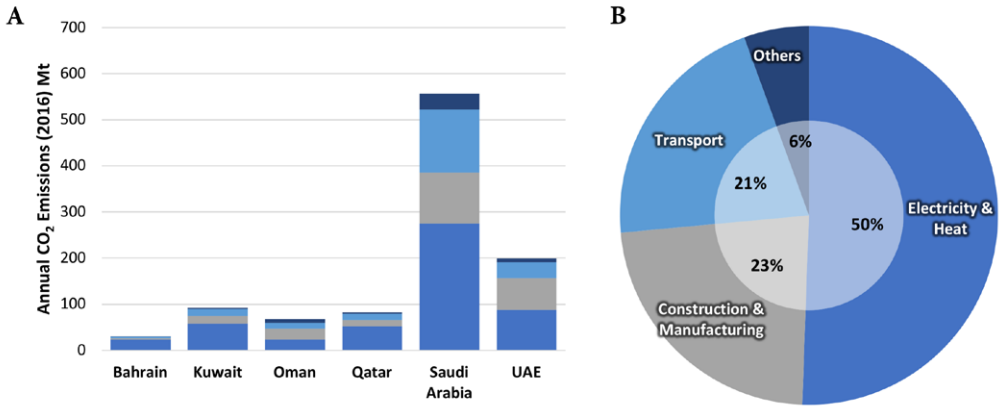


**Figure 6.4 –** Annual Carbon Dioxide emissions (2016, in million tonnes) in the GCC (A) per country and (B) per sector (adapted from Climate Watch 2018, data source: CAIT 2020).

remain – a demand which could be easily met through the use of industrial water or treated sewage effluent [5].

Besides the integration with algae cultivation requirements, existing industrial infrastructure in the region could also support in the downstream processing of algal-products. For example, bio-crude oil produced through the hydrothermal liquefaction of microalgal biomass can be processed in existing oil-refineries without modification, either as sole process-feed, or blended with petroleum feedstocks [202]. This could open the door to a gradual transition to renewable fuels which are compatible with the existing petroleum infrastructure.

### 6.3.2 PRODUCTS & LOCAL END-USERS

The algae product portfolio is large and diverse, ranging from biofuels, to food and feed, and pharmaceuticals. Although the 'high-value low volume' specialty-products such as pigments and pharmaceuticals could easily be shipped to a global market, bulk products, such as feed or fuels, are more credible when fulfilling a local demand. As most of the region's current demand for feed and food is met by imports, development of sustainable local food resources is a priority [14,180]. One such development has been seen in the growth of the aquaculture industry in the GCC countries, with a projected increase of 7.2% over 2020-2025 [203]. Saudi Arabia, the largest regional aquaculture producer, at over 60.000 $t \cdot y^{-1}$ (2016), aims to increase its production ten-fold over the coming 10 years. A multitube of other projects are under development in Oman, UAE and Qatar [204,205].



Although promising, the development of the aquaculture industry in the region also has challenges; the requirement for imported fish-feed to support the growing business only replaces the dependency on imports from one commodity to another. The same is applicable to the growing livestock industry, where the dependence on imported animal feed, such as wheat or soy bean meal, still poses a food-security risk. Algae biomass however can be used as a (partial) replacement of animal and aquaculture feed, and can be produced sustainably under the conditions prevalent in the GCC. The comparison of algae-based feed with other existing (imported) feeds (Table 6.2), only further substantiates the potential that microalgae pose for the regional market.

Additionally, the GCC is home to a number of large airlines, such as Etihad, Emirates, and Qatar Airways – potential end-users for algae-based aviation fuels. Starting 2021, these airlines will be subject to the Carbon Offsetting and Reduction Scheme for International Aviation (CORSIA) adopted by the International Civil Aviation Organization (ICAO), which aims at stabilizing net $CO_2$ emissions from international civil aviation, and further reducing them to 50% of 2005 levels by 2050 [208]. Recognizing the potential that microalgae pose, Qatar Airways co-invested in a

**Table 6.2 –** Overview of different animal feed products, and their yields, water requirement, harvesting frequency and competition with human food.

| | Existing Feed Products (non-algae) | | | | Algae Feed Products | |
|---|---|---|---|---|---|---|
| | Soy Bean Meal | Wheat | Fishmeal | Poultry Meal | Algae (imported) | Algae (local) |
| **Yield ($t \cdot ha^{-1} \cdot yr^{-1}$)** | 3[206] | 8 [207] | n/a | n/a | 30-50 | 50-70 [*Chapter 5*] |
| **Water Requirement** | Fresh Water | Fresh Water | n/a | n/a | Fresh/ Seawater | Seawater |
| **Harvesting Frequency** | Seasonal | Seasonal | n/a | n/a | Seasonal[i] | Year-round |
| **Transport Requirements** | High | High | Medium | Medium | Medium/ High | Very Low |
| **Competition with Food** | High | High | High | Medium/ High | Low | Very Low |

[i] Depending on cultivation location

| Data not available | Very Poor | Poor | Neutral | Good | Very Good |
|---|---|---|---|---|---|

research project for the development of algae aviation fuels in 2010 [209]. Although the techno economical potential and of biofuels as aviation fuels stills needs to be demonstrated, it could be a potential co-product in a downstream biorefinery scenario [39]. Similar to the aviation industry, a large part of the revenue would come from the 'business class' low-volume, high-value specialty products such as pigments and pharmaceuticals, with the remaining biomass components and products, such as biofuels, supplementing the revenue streams as the 'economy class' products.

### 6.3.3 INVESTMENT POTENTIAL

In recognition of the requirement to reduce their dependence on a fossil-fuel based economy, governments in the GCC region are actively investing in economic diversification as a driver to increase economic, environmental as well as social sustainability. This has resulted in strategic 'National Vision' documents (Box 2), in which current significant revenues from oil and gas, are recognized as essential means for achieving these ambitions.

Under these Visions, the GCC member states' needs for expansion into new markets and sectors is led by a search for alternative revenue streams. This search is driven from the ground up, starting with investments in academia, R&D, and innovation, as well as fostering an ecosystem for entrepreneurship [210,211]. Increasing spending on research over the last decade has led to the establishment of multiple national research funding agencies, financially supporting local researchers in 'Vision'-aligned research. Furthermore, the application and commercialization

## **BOX 2:** GCC FUTURE DEVELOPMENT: NATIONAL VISIONS

The GCC countries have found themselves in a challenging position, with supplying the global energy demand, but at the same time realizing that climate change and a global shift towards a low-carbon economy will leave its economies and societies vulnerable. Adding to this conundrum is the knowledge that improving environmental quality through conventional means would reduce economic growth, with no industries to fall back on other than petroleum driven [181].

In recognition of this, the governments in all GCC countries have established ambitious future development plans in so-called "Vision" documents. The Sultanate of Oman was the first to release "Oman Vision 2020" in 1995 (with a 2040 update in 2019), followed by Bahrain (The Economic Vision 2030) and Qatar (Qatar National Vision 2030) in 2008, Kuwait (Kuwait Vision 2035) and the UAE (UAE Vision 2021) in 2010, and finally Saudi Arabia with its Vision 2030 strategy issued in 2016.

These strategic frameworks form the basis for future economic, social, human, and environmental development in the region, in recognition that increasing non-fossil fuel-based revenues (economic diversification) will be critical to reduce the economic impact of oil-price volatility on GDP as well as address environmental sustainability.

of research outputs is promoted through access to equity finance, establishment of business incubators, and driving collaborations through establishment of science parks [212,213]. Such initiatives will be essential in forming the basis of a critical mass of new ventures and fostering entrepreneurship in SMEs needed to drive sustainable economic change in the GCC.

With this as underlying concept, now is the perfect time for development of algae-based technologies for implementation in the region, as it represents both a sustainable feedstock for a large variety of products, as well as supports the aim of moving away from fossil-based feedstocks. For some of the regional research-funding organizations, such as the Qatar National Research Fund, Kuwait Foundation for the Advancement of Science, and Oman Scientific Research Council, algae have already been on the agenda, with a number of projects funded through various research funding programs [214,215]. With further funding of scale-up projects to apply fundamental research outcomes, the GCC could become a major player in the development of an algae-based industry as 'kickstart' investments in both R&D and start-ups could be the driving factor needed for the industry to succeed globally.

## 6.4 CHALLENGES FOR REALIZATION

Although opportunities are numerous, there are of course also challenges when it comes to the realization of an algae-based value-chains in the GCC countries. These challenges will need to be addressed in order to realize the full potential algae can provide towards the economic development of the region.

### 6.4.1 FROM STRAIN TO PROCESS

Contrary to traditional agricultural crops, targeted selection and domestication of microalgae is still underdeveloped. Although over 35.000 strains have been described to date, only a few are produced at industrial scale [216]. Global culture collections providing algae and cyanobacterial strains are an important resource to accelerate research into novel strains for large-scale production. Only a relatively small proportion of the total number of naturally occurring microalgae species, however, can be found in such culture collections. Species from unusual habitats tend to be heavily underrepresented compared to those isolated from fresh water ponds, soils, and coastal marine environments [217]. As such, it is of vital importance for efforts into the isolation of strains to continue and to expand, especially in locations where cultivation is most likely to occur. Furthermore, an important factor in the domestication of microalgae which can't be overlooked is a strain's ability to be cultivated under large-scale and industrially relevant conditions. Experience has shown that not all isolated strains are capable of growing in mid- to large-scale outdoor conditions, despite rigorous testing and optimization. Although many reviews and papers recognize the importance of selection of strains capable of highly productive outdoor cultivation, there are limited studies which address prevalent scale-up issues, or suitability for large-scale year-round cultivation [129]. One of the main issues encountered is the limited capability of simulated lab-scale environments to represent the full spectrum of environmental conditions which the strain is subjected to when grown outdoor [*Chapter 4*]. For this reason, it is crucial that newly isolated high-potential strains are tested under mid-scale outdoor conditions as soon as possible, in order to identify whether there will be any hurdles when scaling up for large scale production.

### 6.4.2 PROCESS INTEGRATION

When looking further down the line of development, despite the many opportunities for industrial integration, the currently still limited scale of microalgae production limits the integration opportunities with well-established large-scale industries due to a mis-match in supply and demand. At this stage this therefore leads to stressed value propositions for established industries. Considering a carbon capture utilization and storage (CCUS) example, for instance: a GTL plant

producing a nominal 34.300 bbl·day$^{-1}$ in liquid products, is estimated to produce 1.6 Mt of $CO_2$ per year [218]. Even if maximum theoretical biomass productivities of 200 tonnes·ha$^{-1}$·yr$^{-1}$ are reached [9], a 100 hectare algae production facility would require only 2.3% of the total $CO_2$ emissions of one such production plant. Furthermore, the scale-up process towards a 100-hectare facility will require multiple phases, with less $CO_2$ demand, and due to these differences in scale it is often challenging to study the commercial potential of microalgae production taking into account the possibilities of industrial integration. Algae are however not the only CCUS option on the market, and as the drive towards carbon capture becomes larger in the GCC, so do the opportunities for its use. For example, Qatar has announced the commissioning of a 5 Mt $CO_2$·yr$^{-1}$ carbon capture facility, the largest of its kind in the region. This, together with the construction of $CO_2$ pipelines across the country, will allow for the delivery of $CO_2$ to various industries, mainly for enhanced oil recovery (EOR) [219]. With this infrastructure in place, it is not unrealistic to envision the coupling of an algae industry to such a $CO_2$ network.

### 6.4.3 CLIMATE CHANGE

Climate change, although a strong driver of economic change and subsequent opportunities for development of the GCC's algae industry, also poses a threat. Rising sea levels will affect the GCC's coastlines, with Qatar being particularly prone to loss of land area (up to 13% with 1 m sea-level rise), impacting up to 10% of the population as well as GDP [220]. More frequent weather extremes such as heat waves and sand storms are also expected to be further consequence of climate change in the region [180]. As regional temperatures rise, so will the demand for fresh water, which is supplied by desalination. Both factors will inevitably lead to an increase in salinity in the Arabian Gulf [221], which in turn can impact any sea-water based process. In order to ensure long-term viability of an algae-based industry in the GCC, special attention will be required to source and develop strains and technologies which can cope with the changing environmental conditions, such as thermo- and halo-tolerant strains. This aspect however also underlines the urgency to deploy such bio-based economies as soon as possible, to support in potentially (partially) circumventing the effects of climate change.

### 6.4.4 HUMAN CAPITAL

Human capital has been recognized as the main driver and necessity for regional efforts on economic diversification and long-term sustainable growth. The GCC member states have therefor sought to increase local capacities through education, nationalization of human resources and promotion of entrepreneurship ideals among the local population [222]. Despite these efforts, there remains a large dependence on foreign labor, especially in the private sector. This is mainly attributed to a non-alignment between educational outcomes and labor-market requirements,

**Table 6.3** – SWOT analysis targetting the development of an algae-based industry in the GCC region

| Strengths | Weaknesses |
|---|---|
| - Algae can be grown without using scarce freshwater or agricultural land resources<br>- Strong alignment with regional 'Visions', playing into needs for sustainable economic diversification<br>- Application of a unique regional biodiversity with thermo- and halo-tolerant strains<br>- Possibility of production of multiple products accessing different markets with one production facility | - Limited local human capital in relevant fields<br>- Lack of awareness of the certification and oversight mechanisms associated with regard to various products (e.g. ISO certification, EU Emission Trading Schemes)<br>- Decreased photosynthetic efficiencies with elevated light intensities could inhibit the envisioned productivity gains<br>- Still commercially immature technology – not many large-scale companies in production<br>- Large-scale production could present unforeseen drawbacks compared to those found in laboratory experiments |
| **Opportunities** | **Threats** |
| - Favourable climate, land and seawater availability, $CO_2$ and nutrient resources, all in close proximity<br>- Access to research-funds and investments for start-ups<br>- Addresses environmental issues in the region, such as $CO_2$ emissions<br>- Building national human resource expertise in the emerging science<br>- Playing into growing local markets needs for local sustainable feedstocks<br>- Downstream integration possibilities with existing industries can support a gradual decarbonization<br>- Showcasing "Green" bio-based industry to enhance region's global reputation | - Loss of foreign expertise to other countries due to regional job-insecurities and public policies<br>- Climate change could threaten long-term regional process feasibility<br>- Limited availability of local talent due to mismatch between educational curricula and business needs<br>- Studies into industrial integration challenging due to mismatch in scale of research requirements and industrial supply<br>- Long-term commitment needed from industry to enable industrial integration for $CO_2$, waste water and nutrients |

a focus on credentials rather than skill, as well as a prevalent preference for public-sector employment. Human capital development and labor market reforms will therefore be critical in addressing the availability of local talent needed to improve productivity and competitiveness, in order to achieve sustainable knowledge-based and private sector-driven economies [223].

The human capital needed for an algae-based industry will come in many forms: from researchers, to engineers, operators and management. Whilst the educational efforts of the GCC countries have improved drastically over the past decades, some fields remain underrepresented. As the biotechnology and bioengineering curricula at leading regional universities are relatively new and still developing, this results in a very small, mainly foreign-educated, local talent pool to draw upon. On the short term, there will therefore be a need for foreign expertise. This however poses a risk on the long term, as expatriate workers have limited opportunities for permanent residence in the GCC countries. Focus should therefore lie on improving relevant regional educational curricula, from which prospecting young nationals can be recruited, as well as knowledge transfer and continuing education through international partnerships to enhance regional capacity building.

## 6.5 CONCLUSIONS

Algae are a promising feedstock for sustainable economic diversification in the GCC countries and offer an exciting prospect for the establishment of novel value chains. Favorable climatic conditions, potential of local strains, and possibilities for integration with existing industries are just a few of the of the strengths and opportunities which the region possesses. There are however also risks to the establishment of an algae-based industry in the region, such as the limited availability of human capital, uncertainties in process scale-up and integration, and the effects of climate change. The SWOT analysis provided in Table 6.3 serves to summarize the various social, technical and economical dimensions which should be considered when progressing the development of such a novel industry. Nonetheless, the intrinsic positioning of the GCC region for commercial algae production is favorable from a global perspective, and the foreseen risks can be averted with focused and proactive management, thereby helping to open the doors towards a (blue-)green GCC.

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# Summaries

# SUMMARY

The Arabian Peninsula, due to its climate, availability of non-arable land, seawater, and carbon dioxide, is one of the best global locations for commercial cultivation of algae and cyanobacteria. This work focused on the screening of multiple locally isolated strains for their capability to thrive under industrially relevant conditions, such as high temperatures and carbon dioxide levels. One identified cyanobacteria was further investigated for its potential to produce phycocyanin, a nutraceutical with high commercial value, under desert climate conditions. The indoor to outdoor transition of the strain was also studied to further asses its potential as a commercially interesting strain for production in Qatar, with specific focus on the effect of high light intensities on the occurrence of photooxidation. A techno-economic analysis was then applied to determine the biomass production costs for various cultivation systems and facility sizes. Finally, a sensitivity analysis indicated which improvements would have the largest impact on the overall costs of the process, as a recommendation for future research and development.

## CHAPTER 2: ISOLATION OF NOVEL STRAINS AND SCREENING FOR CARBON CAPTURE AND PRODUCT POTENTIAL

The isolation and identification of novel strains is necessary to funnel into the algae-value chain development process. The best strain for a process will differ based on cultivation locations as well as on desired end-products. In **Chapter 2**, novel microalgae and cyanobacteria strains isolated from the Arabian Gulf were screened and characterized for their carbon capture and product potential under industrially relevant conditions of elevated temperatures (up to 40 ˚C) and carbon dioxide levels (up to 30%). Two strains, *Leptolyngbya* sp. and *Picochlorum* sp., grew well at temperatures of up to 40 ˚C, and also showed a tolerance towards elevated $CO_2$ concentrations. Both microalgae isolated, *T. subcordiformis* and *Picochlorum* sp., presented significant amounts of lipids, including high-value omega-3 fatty acids EPA and DHA. On the other hand, both cyanobacteria, *Leptolyngbya* sp. and *Chroococcidiopsis* sp., presented levels of phycobiliproteins, and the latter strain also showed indications of possible nitrogen fixation characteristics. The isolates, all very diverse in response and products, showed promising characteristics, making them valuable strains for further investigation towards commercial applications and $CO_2$ capture.

## CHAPTER 3: GROWTH AND PRODUCT OPTIMIZATION UNDER SIMULATED DESERT CLIMATE CONDITIONS

In **Chapter 3**, one of the isolates described in **Chapter 2** was further investigated for its potential for the production of phycocyanin-rich biomass under desert climate conditions. Under elevated temperatures and light intensities, of up to 40 ˚C and 1800 µmol photons·$m^{-2}$·$s^{-1}$, *Leptolyngbya*

sp. biomass productivity was up to 45% higher as compared *A. platensis*, the commercially most commonly produced strain for phycocyanin. High temperatures were found to improve both the biomass productivity and phycocyanin content, with maxima of 1.09±0.03 $g_X \cdot L^{-1} \cdot d^{-1}$ and 72.12±3.52 $mg_{PC} \cdot g_X^{-1}$, respectively. At the highest light intensities (1000-1800 µmol photons$\cdot m^{-2} \cdot s^{-1}$), biomass productivities were slightly photo inhibited, with a limited reduction of up to 15%. Furthermore, various cell disruption methods and buffers were tested for the efficient extraction of high-purity phycocyanin. The best results were found through bead-beating in phosphate buffer, which showed the highest combined phycocyanin yield (169.9±3.6 $mg_{PC} \cdot g_X$) and purity (7.37±0.16). The extract purities obtained for *Leptolyngbya* sp. are considerably higher than other reported phycocyanin purities. This, together with the strains capability to maintain relatively high biomass productivities compared to *A. platensis*, even under such high light intensities, make it a feasible candidate for high-value phycocyanin production in desert environments.

## CHAPTER 4: OUTDOOR SCALE UP: EFFECTS OF OUTDOOR CONDITIONS ON SUCCESFULL CULTIVATION

The transition from small-scale laboratory experiments to large-scale outdoor cultivation is one of the most important steps in developing algae for commercial applications. In **Chapter 4**, this transition for *Leptolyngbya* sp. is studied, with a focus on the effect of inoculum volume (and subsequent culture densities) on the occurrence of photooxidation. Indoor, the strain was capable of growing at light intensities up to 5600 µmol photons$\cdot m^{-2} \cdot s^{-1}$, even at inoculation densities of as low as 0.1 $g \cdot L^{-1}$ (10%). 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. Outdoor cultivation of the strain however showed a different response as compared to indoor experiments; within days of inoculation a loss of chlorophyll, phycocyanin, and culture turbidity was observed, irrespective of inoculum volume, suggesting that the strain had difficulties adapting to the outdoor environment. The culture did, however, recover, and clear morphological differences were observed, such as an increase in trichome length, as well as coiling of multiple trichomes to tightly packed strands. It was hypothesized that the morphological changes were induced by UV-radiation as an adaptation mechanism through increased self-shading. Furthermore, the presence of contaminating ciliates could have also affected the outdoor culture. Both UV and contaminants are, however, generally not simulated under laboratory environments, causing a mismatch between indoor optimizations and outdoor realizations.

## CHAPTER 5: TECHNO-ECONOMIC POTENTIAL OF ALGAE PRODUCTION ON THE ARABIAN PENINSULA

Using the outdoor production data from **Chapter 4**, together with previously obtained results for other strains, the techno-economic potential of algae production in the Arabian Peninsula was studied (**Chapter 5**). Different cultivation systems, production locations and facility scales were assessed in terms of their effect on biomass production costs. Flat panel and raceway pond cultivation systems had the lowest projected biomass production costs, at 3.0 and 2.9 €·kg$^{-1}$, respectively, at 100 ha scale. Biomass production costs in tubular systems, both horizontal and vertically stacked, were up to 1.5 times more expensive. Locational differences in production costs throughout the region were minimal. In scaling up from 1 ha to 100 ha production facility, the largest reductions in production costs were made within the first 10 ha (67%), with further scale-up resulting in a mere 13% additional cost-savings. Optimization of process parameters, such as a doubling in photosynthetic efficiency, increased temperature optima to circumvent the needs for cooling, alternative harvesting methods, and the use of recovered $CO_2$, were also projected to reduce production costs, with a lowest projected biomass production cost for raceway ponds and flat panel reactors at 0.82 and 0.89 €·kg$^{-1}$, respectively. Increased photosynthetic efficiencies and temperature optima had the largest impact on projected costs, which is why efforts to source local thermo- and photo- tolerant strains, such as *Leptolyngbya* sp., could be the key to unlock the potential of the region for algae commercialization.

## CHAPTER 6: GENERAL DISCUSSION: ALGAE AS A SUSTAINABLE FEEDSTOCK FOR THE ARABIAN PENINSULA

In **Chapter 6**, the results from the previous chapter are used as a basis to discuss the potential of algae as a sustainable feedstock for the Arabian Peninsula countries, including Qatar, from scientific, social, and economic perspectives. Regional aspects, such as beneficial climate and high local biodiversity were evaluated as promising contributors towards the establishment of a technically and economically feasible algae production process. This was further contributed to by the availability of non-arable land, seawater, and industrial $CO_2$ point sources. Furthermore, the regional requirements for locally produced sustainable feed and food sources, and the ambition for economic diversification transitioning away from fossil-fuel based industries, could be the drivers the algae industry needs to grow into a commercially successful value-chain within the region. Risks, such as uncertainties in process scale-up and integration, and limited regional availability of human capital, can be averted with proactive management, there opening doors towards a (blue-)green Qatar.

# SAMENVATTING

Het Arabisch Schiereiland is vanwege het klimaat, de beschikbaarheid van niet-agrarisch land, zeewater en koolstofdioxide, één van de beste locaties ter wereld voor de commerciële productie van algen en cyanobacteriën. In dit proefschrift zijn meerdere lokaal geïsoleerde stammen gescreend op hun vermogen om te gedijen onder industrieel relevante omstandigheden, zoals verhoogde temperaturen en $CO_2$ niveaus. Eén zo'n geïdentificeerde cyanobacterie is verder bestudeerd op zijn potentieel om phycocyanine te produceren, een neutraceutisch middel met een hoge commerciële waarde. De transitie van laboratorium-cultivatie naar cultivatie onder het Qatarese klimaat is onderzocht, om de commerciële potentie van de stam verder te bepalen. Er was hierbij een specifieke focus op het effect van hoge licht intensiteit en het optreden van mogelijke foto-oxidatie. Een techno-economische analyse is vervolgens toegepast om de productiekosten van biomassa te bepalen voor verschillende cultivatie-systemen en faciliteitengroottes. Ten slotte gaf een gevoeligheidsanalyse aan welke verbeteringen de grootste invloed zouden hebben op de totale kosten van het proces, als aanbeveling voor toekomstig onderzoek en ontwikkeling.

## HOOFDSTUK 2: ISOLATIE VAN NIEUWE STAMMEN EN SCREENING VOOR KOOFSTOF-AFVANG EN PRODUCT-POTENTIEEL

De isolatie en identificatie van nieuwe stammen is noodzakelijk voor de commerciële ontwikkeling van een algenwaardeketen. De beste stam voor een proces zal verschillen op basis van locatie van cultivatie en gewenste eindproducten. In **Hoofdstuk 2** zijn een aantal nieuwe microalgen en cyanobacteriën stammen geïsoleerd uit de Arabische Golf, en gescreend op hun potentie voor $CO_2$ afvang en productpotentieel, onder industrieel relevante omstandigheden van verhoogde temperaturen (tot 40 ˚C) en kooldioxide (tot 30%). Twee stammen, *Leptolyngbya* sp. en *Picochlorum* sp., groeiden goed bij temperaturen tot 40 ˚C en vertoonden ook tolerantie voor verhoogde $CO_2$-concentraties. Beide geïsoleerde microalgen, *T. subcordiformis* en *Picochlorum* sp., vertoonden aanzienlijke hoeveelheden lipiden, waaronder hoogwaardige omega-3-vetzuren EPA en DHA. Aan de andere kant presenteerde beide cyanobacteriën, *Leptolyngbya* sp. en *Chroococcidiopsis* sp., significante hoeveelheden phycobiliproteïnen, en de laatste stam vertoonde ook indicaties van mogelijke stikstofbindingskarakteristieken. De stammen vertoonden ieder veelbelovende eigenschappen, waardoor ze waardevol zijn voor verder onderzoek naar commerciële toepassingen en $CO_2$-afvang.

## HOOFDSTUK 3: GROEI EN PRODUCTOPTIMALISATIE ONDER GESIMULEERDE WOESTIJNKLIMAATOMSTANDIGHEDEN

Eén van de stammen welke in **Hoofdstuk 2** was omschreven, is verder onderzocht in **Hoofdstuk 3** voor zijn potentie voor het produceren van phycocyanine onder woestijnklimaatomstandigheden. Onder verhoogde temperaturen en lichtintensiteiten, tot wel 40 ˚C en 1800 µmol fotonen·$m^{-2}$·$s^{-1}$, was de biomassaproductiviteit van *Leptolyngbya* sp. tot wel 45% hoger in vergelijking met *A. platensis*, de meest geproduceerde commerciële stam voor phycocyanine. Hoge temperaturen bleken zowel de biomassaproductiviteit als het phycocyaninegehalte te verbeteren, met maxima van respectievelijk 1.09±0.03 $g_X$·$L^{-1}$·$d^{-1}$ en 72.12±3.52 $mg_{PC}$·$g_X^{-1}$. Bij de hoogste lichtintensiteiten (1000-1800 µmol fotonen·$m^{-2}$·$s^{-1}$), was de biomassaproductiviteit tot 15% gereduceerd in vergelijking met lagere lichtintensiteiten. Verder zijn verschillende extractiemethoden (buffers en cel lyseringsmethodes) voor het efficiënt extraheren van hoog-zuiver phycocyanine onderzocht. De beste resultaten werden gevonden met bead-beating in fosfaatbuffer, resulterend in de hoogst gecombineerde phycocyanine-opbrengst (169.9±3.6 $mg_{PC}$·$g_X$) en zuiverheid (7.37±0.16). De extractzuiverheden verkregen van *Leptolyngbya* sp. zijn aanzienlijk hoger dan andere gerapporteerde phycocyanine-zuiverheden. Dit, samen met het vermogen van de stam om een relatief hoge biomassaproductiviteit te behouden in vergelijking met *A. platensis*, zelfs bij hoge temperaturen en lichtintensiteiten, maakt het een potentiele kandidaat voor hoogwaardige phycocyanineproductie in woestijnomgevingen.

## HOOFDSTUK 4: OPSCHALING: EFFECTEN VAN BUITENOMSTANDIGHEDEN OP SUCCESVOLLE CULTIVATIE

De overgang van kleinschalige laboratoriumexperimenten naar grootschalige buitenteelt is een van de belangrijkste stappen bij het ontwikkelen van algen voor commerciële toepassingen. In **Hoofdstuk 4** is deze overgang voor *Leptolyngbya* sp. bestudeerd, met de nadruk op het effect van inoculatievolume (en resulterende inoculatiedichtheden) op het optreden van foto-oxidatie. Binnen was de stam in staat om te groeien onder extreme lichtintensiteiten tot wel 5600 µmol fotonen·$m^{-2}$·$s^{-1}$, zelfs bij inoculatiedichtheden van slechts 0.1 g·$L^{-1}$ (10%). Niveaus van chlorofyl en phycocyanine vertoonden een significante afname binnen de eerste 24 uur van cultivatie, wat wijst op een optreden van een zekere mate van foto-oxidatie, maar beide waren in staat om te herstellen. Buitencultivatie van de stam liet echter een andere respons zien dan bij de laboratorium experimenten; binnen enkele dagen na inoculatie werd een verlies aan chlorofyl, phycocyanine en dichtheid in de cultuur waargenomen, ongeacht het inoculatievolume, wat suggereert dat de soort moeite had zich aan te passen aan de buitenomgeving. De cultuur herstelde zich echter, en er werden duidelijke morfologische verschillen waargenomen, zoals een toename van de lengte van de trichomen, evenals het oprollen van meerdere trichomen tot dicht opeengepakte

strengen. De hypothese is dat de morfologische veranderingen werden geïnduceerd door UV-straling als een aanpassingsmechanisme. Verder had de aanwezigheid van ongewenste ciliaten ook een mogelijke invloed op de buitencultuur. Zowel UV als contaminerende organismen worden echter doorgaans niet nagebootst onder laboratoriumomgevingen, waardoor een afwijking ontstaat tussen laboratorium optimalisaties en buiten realisaties.

## HOOFDSTUK 5: TECHNO-ECONOMISCH POTENTIEEL VAN ALGENPRODUCTIE OP HET ARABISCHE SCHIEREILAND

Met behulp van de buitenproductiegegevens uit **Hoofdstuk 4**, samen met eerder verkregen resultaten voor andere stammen, is het techno-economische potentieel van algenproductie op het Arabische Schiereiland bestudeerd (**Hoofdstuk 5**). Verschillende cultivatiesystemen, productielocaties en faciliteit schalen zijn beoordeeld op hun effect op de productiekosten van algen biomassa. Vlakke panelen en kanaalvijver cultivatie systemen hadden de laagste verwachte biomassaproductiekosten, van respectievelijk 3.0 en 2.9 €·kg$^{-1}$, bij een productie schaal van 100 ha. De productiekosten van biomassa in tubulaire cultivatie systemen, zowel horizontaal als verticaal gestapeld, waren tot 1.5 keer duurder. Locatieverschillen in productiekosten in de regio waren minimaal. De grootste verlaging van productie kosten kon worden behaald bij het opschalen van 1 ha naar 10 ha productiefaciliteit (67%), waarbij verdere opschaling naar 100 ha slechts 13% extra kostenbesparing opleverde. Optimalisatie van procesparameters, zoals een verdubbeling van de fotosynthetische efficiëntie, verhoogde temperatuuroptima om de behoefte aan koeling te omzeilen, alternatieve oogstmethoden, en het gebruik van afgevangen $CO_2$, zouden ook de productiekosten kunnen verlagen, met de laagste verwachte biomassaproductiekosten voor kanaalvijver en vlakke panelen reactoren van respectievelijk 0.82 en 0.89 €·kg$^{-1}$. Verhoogde fotosynthetische efficiëntie en temperatuuroptimalisatie hadden de grootste impact op de verwachte kosten, en daarom zouden inspanningen om lokale thermo- en fototolerante stammen te vinden, zoals *Leptolyngbya* sp., de sleutel kunnen zijn om het commercialisatiepotentieel van algen in de regio te realiseren.

## HOOFDSTUK 6: ALGEMENE DISCUSSIE: ALGEN ALS EEN DUURZAME GRONDSTOF VOOR HET ARABISHE SCHIEREILAND

In **Hoofdstuk 6** worden de resultaten uit de vorige hoofdstukken gebruikt als basis om het potentieel van algen te bespreken, als duurzame grondstof voor de landen van het Arabische Schiereiland, waaronder Qatar, vanuit wetenschappelijk, sociaal en economisch perspectief. Regionale aspecten, zoals een gunstig klimaat, hoge lokale biodiversiteit, beschikbaarheid van grond niet geschikt voor agrarische doeleinden, makkelijk toegankelijk zeewater en industriële $CO_2$-bronnen, worden aangemerkt als veelbelovende bijdragen aan de totstandbrenging van

een technisch en economisch haalbaar algenproductieproces. De regionale drijfveer voor lokaal geproduceerde duurzame voedselbronnen, en de ambitie voor economische diversificatie (weg van op fossiele brandstoffen) zouden hierbij de drijfveren kunnen zijn die de algenindustrie nodig heeft om uit te groeien tot een commercieel succesvolle waardeketen binnen de regio. Risico's, zoals onzekerheden in procesopbouw en integratie, en beperkte regionale beschikbaarheid van menselijk kapitaal, kunnen worden vermeden met proactief beheer, en de deuren openen naar een (blauw) groen Qatar.

## الفصل الثالث: تحسين عمليات النمو و المنتج المحتمل في ظل محاكاة الظروف المناخية الصحراوية

في الفصل الثالث، خضعت إحدى السلالات المعزولة والموضحة في الفصل الثاني لمزيد من التحقيق لمعرفة قدرتها على إنتاج الكتلة الحيوية الغنية بمادة الفيكوسيانين تحت ظروف المناخ الصحراوي. تحت درجات حرارة مرتفعة تصل إلى 40 درجة مئوية وشدة ضوئية تصل و1800 فوتون ضوئي، أظهرت سلالة *Leptolyngbya* sp. أعلى إنتاجية للكتلة الحيوية بنسبة 45٪ بالمقارنة مع سلالة *A. platensis*، وهي السلالة التجارية الأكثر شيوعًا لإنتاج الفيكوسيانين. كشفت الدراسة على أن درجات الحرارة العالية تساعد على تحسين كل من إنتاجية الكتلة الحيوية ومحتوى الفيكوسيانين، وبحد أقصى (1.09 ± 0.03 جم/لتر/ اليوم) و ( 72.12 ± 3.52 مجم/جم ) على التوالي. في أعلى شدة ضوء (1000-1800 فوتون ضوئي)، تم تثبيط إنتاجية الكتلة الحيوية بشكل طفيف، مع انخفاض محدود يصل إلى 15٪. علاوة على ذلك، تم إجراء العديد من الاختبارات عن طرق تعطيل الخلايا ومحاليل الاستخراج الفعال للفيكوسيانين عالي النقاء. تم العثور على أفضل النتائج من خلال تقنية تكسير الخلايا ومحلول الفوسفات، والذي أظهر أعلى إنتاجية من تركيب الفيكوسيانين (169.9 ± 3.6 مجم /جم) ونقاوة (7.37 ± 0.16). تم الحصول على المستخلص من الفايكوسيانين من سلالة *Leptolyngbya* sp. بدرجة أعلى بكثير من نقاوة الفيكوسيانين الأخرى المذكورة في المراجع والابحاث المنشورة. هذا، جنبًا إلى جنب مع قدرة السلالات على الحفاظ على إنتاجية عالية نسبيًا للكتلة الحيوية مقارنة بـ سلالة *A. platensis* ، حتى في ظل الظروف القاسية مثل كثافات الإضاءة ودرجات الحرارة العالية ، مما تجعلها مرشحًا ممكنًا لإنتاج الفيكوسيانين عالي القيمة في البيئات الصحراوية.

## الفصل الرابع: توسيع نطاق الزراعة الخارجية: آثار الظروف الخارجية على الزراعة الناجحة

يعد الانتقال من التجارب المعملية إلى الزراعة الخارجية على نطاق واسع أحد أهم الخطوات في تطوير تطبيقات الطحالب على المستوي التجاري. هذا الانتقال لـ *Leptolyngbya* sp تمت دراسته في الفصل الرابع مع التركيز على تأثير حجم التلقيح الأولي للاستزراع (وكثافة الاستزراع) على حدوث الأكسدة الضوئية. في المختبر، كانت السلالة قادرة على النمو بكثافة ضوئية تصل إلى 5600 فوتون ضوئي، حتى عند كثافة تلقيح منخفضة تصل إلى 0.1 جم / لتر (10٪). أظهرت مستويات الكلوروفيل والفيكوسيانين انخفاضًا كبيرًا خلال الـ 24 ساعة الأولى، مما يشير إلى مستوى معين من الأكسدة الضوئية، ومع ذلك، كلاهما عاد إلى المستوى الطبيعي. رغم ذلك، أظهرت الزراعة الخارجية للسلالة استجابة مختلفة مقارنة بالتجارب الداخلية. في غضون أيام من التلقيح، لوحظ فقدان الكلوروفيل والفيكوسيانين مع وجود تعكر في الوسط الزراعي، بغض النظر عن حجم اللقاح، مما يشير إلى أن السلالة واجهت صعوبات في التكيف مع البيئة الخارجية. ومع ذلك، فقد عاد الوسط الزراعي إلى طبيعته، ولوحظت اختلافات شكلية في طول الخيوط والتفافها بشدة. تم الافتراض بأن التغيرات الشكلية للسلالة كانت ناجمة عن الأشعة فوق البنفسجية كآلية للتكيف من خلال زيادة التظليل الذاتي. علاوة على ذلك، يمكن أن يكون وجود الملوثات قد أثر أيضًا على الوسط الزراعي. ومع ذلك، لم يتم محاكاة كل من الأشعة فوق البنفسجية والملوثات بشكل عام في المختبر، مما يتسبب في عدم التوافق بين عمليات التحسين في الأماكن المغلقة وضبط العمليات في الهواء الطلق.

# ملخص

تعد شبه الجزيرة العربية، نظرًا لمناخها وتوافر الأراضي غير الصالحة للزراعة ومياه البحر وثاني أكسيد الكربون، واحدة من أفضل المواقع لزراعة الطحالب والبكتيريا الزرقاء في العالم على المستوى التجاري. ركز هذا العمل على دراسة العديد من السلالات المعزولة محليًا ومدى قدرتها على النمو في أنظمة الاستزراع تحت الظروف الصناعية، مثل درجات الحرارة المرتفعة ومستويات ثاني أكسيد الكربون العالية. كما تم إجراء مزيد من التحقيق في إحدى سلالات البكتيريا الزرقاء التي تم اختيارها لإمكانية إنتاج مادة الفيكوسيانين، وهي مادة مغذية ذات قيمة تجارية عالية، في ظل الظروف المناخية الصحراوية. تمت أيضا دراسة الاستزراع للسلالة تحت الظروف المناخية الطبيعية بعد دراستها في المختبرات من أجل تقييم قدرتها على النمو بشكل أكبر باعتبارها سلالة مثيرة للاهتمام تجاريًا للإنتاج في قطر، مع التركيز بشكل خاص على تأثير شدة الإضاءة العالية على حدوث الأكسدة الضوئية. ثم تم تطبيق التحليل الاقتصادي التقني لتحديد تكاليف إنتاج الكتلة الحيوية لأنظمة الزراعة المختلفة ومساحات الإنتاج. أخيرًا، أشارت الدراسات التحليلية إلى التحسينات التي سيكون لها أكبر تأثير على التكاليف الإجمالية للعملية، كتوصية للبحث والتطوير في المستقبل.

## الفصل الثاني: عزل السلالات الجديدة وفحص قدرات احتجاز الكربون و المنتج المحتمل

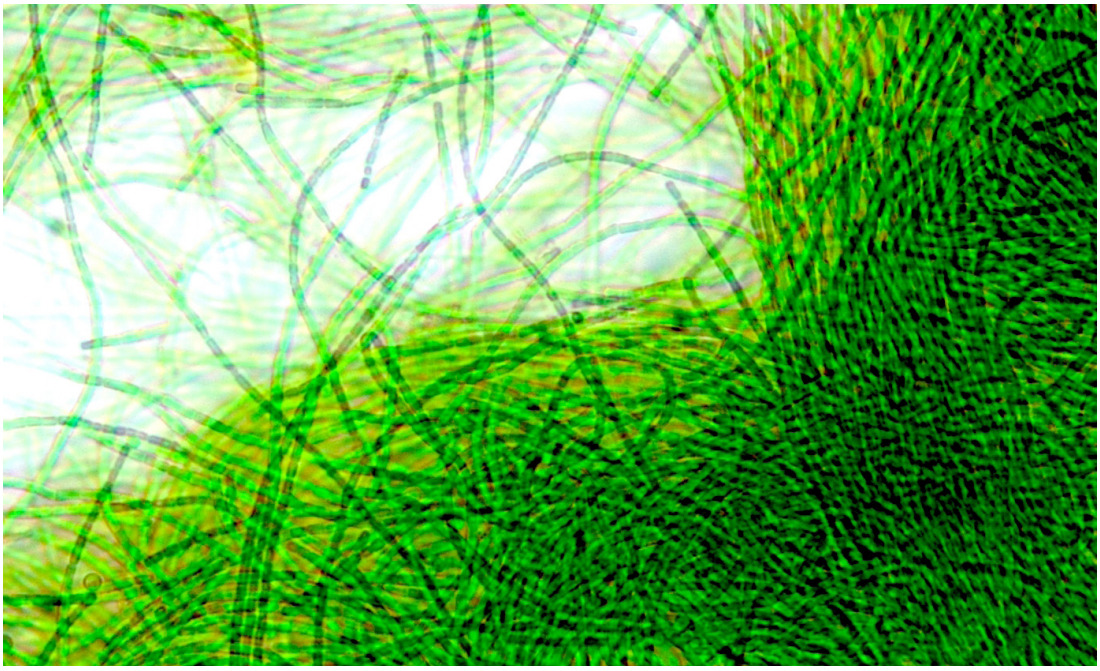
يعد عزل السلالات الجديدة وتحديد أنواعها أمرًا ضروريًا للتوجه إلى عملية تطوير قيمة مضافة من خلال الطحالب. ستتميز السلالة الأفضل لعملية الإنتاج، بناءً على مواقع الاستزراع وكذلك على المنتجات النهائية المستهدفة. في الفصل الثاني، تم فحص سلالات الطحالب الدقيقة والبكتيريا الزرقاء الجديدة المعزولة من الخليج العربي وتم دراسة قدرتها على احتجاز الكربون ومن ثم إمكانية الحصول على منتج ذو قيمة إقتصادية في ظل الظروف الصناعية ذات الصلة بدرجات حرارة مرتفعة (تصل إلى 40 درجة مئوية) وتراكيز عالية من غاز ثاني أكسيد الكربون (تصل إلى 30٪). تم تحديد سلالتين هما، *Leptolyngbya* sp. و*Picochlorum* sp.، والتي أظهرتا نمواً جيدًا في درجات حرارة تصل إلى 40 درجة مئوية ، وأظهرتا أيضًا تحملاً لتراكيزعالية من غاز ثاني أكسيد الكربون. تمكنت كل من الطحالب الدقيقة المعزولة، *T. subcordiformis* و*Picochlorum* sp.، من تكوين كميات كبيرة من الدهون، بما في ذلك أحماض أوميغا 3 الدهنية عالية القيمة EPA وDHA من ناحية آخرى فإن كلا من البكتيريا الزرقاء، *Leptolyngbya* sp. و*Chroococcidiopsis* sp.، كونت مستويات من البروتينات النباتية ، وأظهرت السلالة الأخيرة أيضًا مؤشرات على خصائص محتملة لتثبيت النيتروجين. أظهرت جميع السلالات المتنوعة تباين كبير في الاستجابة والمنتجات المحتملة، كمؤشر لخصائص واعدة، مما يجعلها سلالات قيّمة لمزيد من التحقيق في التطبيقات التجارية وتقليل انبعاثات غاز ثاني أكسيد الكربون.

## الفصل الخامس: الجدوى التقنية والاقتصادية لإنتاج الطحالب في شبه الجزيرة العربية

باستخدام بيانات الإنتاج في الزراعة الخارجية من الفصل الرابع، وبالإضافة إلى النتائج التي تم الحصول عليها سابقا لسلالات أخرى، تمت دراسة الجدوى التقنية والاقتصادية المحتملة لإنتاج الطحالب في شبه الجزيرة العربية (الفصل 5). كما جرى تقييم مختلف نظم الزراعة ومواقع الإنتاج وحجم الإنتاج من حيث تأثيرها على تكاليف إنتاج الكتلة الحيوية. وأظهرت أنظمة زراعة الألواح المسطحة والأحواض المائية المفتوحة أقل تكاليف لإنتاج الكتلة الحيوية المتوقعة، عند 3.0 و2.9 يورو/ كجم على التوالي، على مقياس 100 هكتار. بالإضافة الى ذلك، كانت تكاليف إنتاج الكتلة الحيوية في الأنظمة الأنبوبية، الأفقية والرأسية، أكثر تكلفة بمقدار 1.5 مرة. بينما كان تأثير اختلاف الموقع في جميع أنحاء المنطقة على تكاليف الإنتاج ضئيلة جدا. بين السعة الإنتاجية من 1 هكتار إلى 100 هكتار، ظهرت أكبر تخفيضات في تكاليف الإنتاج على مستوى 10 هكتار بنسبة (67٪)، ومع زيادة أخرى في سعة الإنتاج أدى إلى تخفيضات إضافية في التكاليف بنسبة 13٪. فضلاً عن ذلك، كان من المتوقع أن يؤدي تحسين عوامل عمليات الإنتاج، مثل مضاعفة كفاءة التمثيل الضوئي، وزيادة التحمل لدرجة الحرارة العالية لتقليل احتياجات الطاقة في عمليات التبريد ، وأساليب الحصاد البديلة، وإعادة تدوير غاز ثاني أكسيد الكربون ، إلى خفض تكاليف الإنتاج، مع انخفاض تكلفة إنتاج الكتلة الحيوية المتوقعة في أنظمة الاستزراع للأحواض المائية المفتوحة والألواح المسطحة عند 0.82 و0.89 يورو/ كجم على التوالي. وكان لزيادة كفاءة التمثيل الضوئي ودرجة الحرارة المثلى أكبر تأثير على التكاليف المتوقعة، وهذا هو السبب في أن الجهود المبذولة لإنتاج سلالات متحملة للحرارة والضوء، مثل *Leptolyngbya* sp.، يمكن أن تكون البداية لتحقيق الجدوى التجارية للطحالب في المنطقة.

## مناقشة عامة: الطحالب كمواد أولية مستدامة لشبه الجزيرة العربية

في الفصل السادس، تم استخدام نتائج الفصل السابق كأساس لمناقشة المنظور العلمي والاجتماعي والاقتصادي لإمكانات الطحالب كمادة أولية مستدامة لدول شبه الجزيرة العربية، بما في ذلك قطر. وجرى تقييم الجوانب الإقليمية، مثل المناخ المناسب والتنوع البيولوجي المحلي الكبير، باعتبارهما من المساهمين الواعدين في تحقيق عملية مجدية تقنيا واقتصاديا في إنتاج الطحالب. وقد ساهم في ذلك أيضا توافر الأراضي غير الصالحة للزراعة، ومياه البحر، ومصادر صناعية عديدة لغاز ثاني أكسيد الكربون. علاوة على ذلك، فإن المتطلبات الإقليمية لمصادر الأعلاف والأغذية المستدامة المنتجة محليا، والطموح إلى التنويع الاقتصادي الذي يتجه بعيدا عن الصناعات القائمة على الوقود الأحفوري، يمكن أن تكون المحرك الذي تحتاجه صناعة الطحالب للنمو لتصبح سلسلة قيمة ناجحة تجاريا داخل المنطقة. ويمكن تجنب المخاطر، مثل عدم اليقين في عمليتي توسيع نطاق الإنتاج والتكامل، وتوافر الموارد البشرية المحدود على المستوى الإقليمي، من خلال الإدارة الريادية المدروسة، وهناك سوف تفتح الأبواب نحو قطر "زرقاء-خضراء".



# Nomenclature

# NOMENCLATURE

| | |
|---|---|
| $a_X$ | Wavelength Dependent Dry Weight Specific Absorption Coefficient ($m^2 \cdot kg^{-1}$) |
| $C_{PC}$ | Phycocyanin Concentration ($mg_{PC} \cdot L^{-1}$) |
| $C_N$ | Nitrogen Concentration ($g_N \cdot L^{-1}$) |
| $C_X$ | Biomass Concentration ($g_X \cdot L^{-1}$) |
| $C_{Chl}$ | Chlorophyll Concentration ($mg_{Chl} \cdot L^{-1}$) |
| $d$ | Depth (cm or m) |
| $E_{PAR}$ | Energetic content of the PAR fraction of sunlight ($mol \cdot J^{-1}$) |
| $EP$ | Extract Purity (-) |
| $\varepsilon$ | Molar extinction Coefficient ($L \cdot mol^{-1} \cdot cm^{-1}$) |
| $F_H$ | Harvest Volume ($L \cdot d^{-1}$) |
| $\Delta H_C^0$ | Enthalpy of biomass combustions ($KJ \cdot g^{-1}$) |
| $I$ | Light intensity ($\mu mol\ photons \cdot m^{-2} \cdot s^{-1}$) |
| $I_{day}$ | Average areal daily photon flux density ($mol\ photons \cdot m^{-2} \cdot d^{-1}$) |
| $M_C$ | Molecular weight of Carbon ($g \cdot mol^{-1}$) |
| $M_{CO_2}$ | Molecular weight of $CO_2$ ($g \cdot mol^{-1}$) |
| $M_{PC}$ | Molecular weight of Phycocyanin ($g \cdot mol^{-1}$) |
| $P_{PC}$ | Phycocyanin Productivity ($mg_{PC} \cdot L^{-1} \cdot d^{-1}$) |
| $P_X$ | Biomass Productivity ($g_X \cdot L^{-1} \cdot d^{-1}$) |
| $P_{X,areal}$ | Areal Biomass Productivity ($g_X \cdot m^{-2} \cdot d^{-1}$) |
| $PE$ | Photosynthetic Efficiency (%) |
| $R_{CO_2}$ | Carbon Capture Rate ($mg\ CO_2 \cdot L^{-1} \cdot d^{-1}$) |
| $T$ | Temperature (˚C) |
| $t$ | time (d) |
| $\mu$ | Specific growth rate ($d^{-1}$) |
| $V_R$ | Reactor Volume (L) |
| $X_C$ | Carbon Content ($g_C \cdot g_X^{-1}$) |
| $X_{Chl}$ | Chlorophyll content ($mg_{Chl} \cdot g_X^{-1}$) |
| $X_N$ | Nitrogen Content ($g_N \cdot g_X^{-1}$) |
| $X_{PC}$ | Phycocyanin content ($mg_{PC} \cdot g_X^{-1}$) |

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# About the author

# ABOUT THE AUTHOR

Kira Schipper was born on September 14th 1987 in San Diego, California (USA). After moving to the Netherlands in her early childhood, she completed her secondary school at the Wolfert van Borselen in Rotterdam in 2006. In that same year she started with a Bachelor in Life Science and Technology at Delft Technical University and Leiden University (The Netherlands), followed by a Master degree in Biochemical Engineering from Delft Technical University in 2009.

During her Master, she worked on isolating novel extremophilic microorganism from ultra-basic serpentinizing springs at the J. Craig Venter Institute (San Diego, USA), as well as competed in MIT's iGEM competition, where her team made it to the finals. Furthermore, she also completed an internship at TNO in Delft, looking into methods the use of membrane pertraction for the separation and recovery of high-value fine chemicals from algae. After completing her Master in 2011, she moved to Qatar, where she worked as a Bioprocess Engineer at TNO's Middle East Branch office, and later as the office's Operations and Marketing Manager.

In 2014 she started working at Qatar University's Algal Technologies Program (ATP) as a Research Associate, where she is responsible for managing a number of the program's industry funded research projects, as well as involved in business development and project acquisition. In 2016 she started her PhD as a collaboration between Wageningen University & Research (department of Bioprocess Engineering) and Qatar University (ATP), the results of which are described in this thesis.

She is currently still active at Qatar University, in business development, project management, as well as exploring options for scale-up and commercialization of algae-based technologies.

## LIST OF PUBLICATIONS

**K. Schipper**, H.M.S.J. Al Jabri, R.H. Wijffels, M.J. Barbosa, Techno-Economics of Algae Production in the Arabian Peninsula. *Bioresource Technology* **331** 125043 (2021)

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# OVERVIEW OF COMPLETED TRAINING ACTIVITIES

## *DISCIPLINE SPECIFIC ACTIVITIES*

| | | |
|---|---|---|
| Algae Europe 2015 (Lisbon, Portugal) | EABA | 2015 |
| European Algae Biomass 2015 (Amsterdam, The Netherlands) | ACI | 2015 |
| Annual Research Conference (ARC'16) (Doha, Qatar) | QF | 2016 |
| Microalgae Process Design: from cells to photobioreactors (4th edition) (Wageningen, The Netherlands) | VLAG | 2016 |
| ABO Algae Biomass Summit (Phoenix, AZ, USA)[1] | ABO | 2016 |
| International Conference on Sustainable Development "Achieving Food Security in Arid Lands" (Doha, Qatar) | QU | 2016 |
| QU Life Science Symposium 2016 (Doha, Qatar)[1] | QU | 2016 |
| Qatar Foundation Annual Research Conference '18 (Doha, Qatar)[2] | QF | 2018 |
| Qatar University Annual Research Forum 2018 (Doha, Qatar)[1] | QU | 2018 |
| AlgaEurope (Amsterdam, The Netherlands)[1] | EABA | 2018 |
| QU Life Science Symposium 2018 (Doha, Qatar)[1] | QU | 2018 |

## *GENERAL COURSES*

| | | |
|---|---|---|
| Arabic for Non-Native Speakers Level 1-3 (Doha, Qatar) | QU | 2015 |
| ERADA Training: Entrepreneurship Building Blocks (Doha, Qatar) | QU | 2016 |
| Statistics for Graduate Students (Doha, Qatar) | QU | 2018 |
| Qatar University Author Workshop: How to Write a Great Research Paper and Get it accepted by a Reputable Journal (Doha, Qatar) | Elsevier | 2018 |
| Persoonlijke Effectiviteits Cursus (Utrecht, The Netherlands) | TNO | 2012 |

## *OPTIONALS*

| | | |
|---|---|---|
| Preparation of research proposal | BPE | 2016 |
| Scientific Visit to Algae Facilities in USA | QU | 2016 |
| Scientific Visit to Algae Facilities in Europe | QU | 2015 |

[1]Poster [2]Oral

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