

***Operating Strategy to Reduce the Energy Consumption of  
Flat-Panel Airlift Photobioreactors with Respect to  
Mixing of Thermosynechococcus elongatus Suspension Cultures  
- Light-specific Adaptation of the Superficial Gas Velocity -***

**Dissertation for Obtaining the Doctoral Degree  
of Natural Sciences (Dr. rer. nat.)**

**Faculty of Natural Sciences  
University of Hohenheim**

Institute of Food Science and Biotechnology

submitted by  
*Peter Bergmann*

from *Rostock, Germany*  
*2018*

Dean : Prof. Dr. rer. nat. Uwe Beifuß  
1<sup>st</sup> reviewer : Prof. Dr. rer. nat. Walter Trösch  
2<sup>nd</sup> reviewer : Prof. Dr. rer. nat. Lutz Fischer  
Date of submission : January 25<sup>th</sup> 2018  
Date of oral examination : October 26<sup>th</sup> 2018

This work was accepted by the Faculty of Natural Sciences at the University of Hohenheim on August 14<sup>th</sup> 2018 as “Dissertation for Obtaining the Doctoral Degree of Natural Sciences”.

*"Light thinks it travels faster than anything but it is wrong. No matter how fast light travels, it finds the darkness has always got there first, and is waiting for it."*

- Terry Pratchett in *Reaper Man*, 1991 -



---

# Contents

---

<b>Acknowledgements</b>	<b>III</b>
<b>List of Abbreviations</b>	<b>V</b>
<b>1 Summary</b>	<b>1</b>
<b>2 Zusammenfassung</b>	<b>3</b>
<b>3 Introduction</b>	<b>5</b>
3.1 Microalgae in General . . . . .	5
3.1.1 Cyanobacteria in General . . . . .	6
3.1.2 <i>Thermosynechococcus elongatus</i> BP-1 . . . . .	7
3.2 Cyanobacteria in Biotechnology . . . . .	9
3.3 Photosynthetic Production of Bulk - Economics . . . . .	10
3.3.1 Low Productivity and Cell Density as Major Cost Driver . . . . .	11
3.3.2 Mixing Energy as Major Cost Driver . . . . .	12
3.3.3 Cooling Necessity as Major Cost Driver . . . . .	13
3.4 Mass Transfer in Microalgal Cultures . . . . .	14
3.5 Research Objective . . . . .	16
<b>4 Publications</b>	<b>17</b>
4.1 Suspension Culture of Phototrophic Algae and Cyanobacteria . . . . .	17
4.2 Repeated Fed-batch Cultivation of <i>Thermosynechococcus elongatus</i> . . . . .	44
4.3 Operating Strategy to Reduce the Energy Consumption of a FPA-PBR . . . . .	53
<b>5 Patent Application</b>	<b>61</b>

5.1 Bioreactor . . . . . 61

**6 Final Discussion and Remarks 63**

**7 Bibliography 67**

---

## Acknowledgements

---

An experimental scientific work will never be the labor of a single person. It is for this reason that time has come to express my gratitude to people who have supported me during the last years. First of all, I would like to express my explicit gratitude and deepest appreciation to Prof. Dr. Walter Trösch and Dr. Peter Ripplinger for their patient guidance and mentorship throughout the years. Your trust enabled me to further develop my scientific skills and social competence by working with people all over the world, having numerous professional, cultural and religious backgrounds. It has not only been a great pleasure traveling and meeting aforementioned people but also encouraged me to take every effort necessary to make this world a better place. I would also like to express my gratitude to Prof. Dr. Lutz Fischer from the University of Hohenheim, Germany, who agreed to act as co-supervisor and Prof. Dr. Gerd Klöck from the University of Applied Sciences Bremen, Germany, without whom I'd probably never have come in contact with microalgae biotechnology at all. Another word of thanks which I must express goes to Valentina Ruppel and Carolin Koc who, despite my lack of time for management operations, always succeeded in realizing studies as desired and actually did a lot of the laboratory hands-on work. I'd be remiss if I didn't also acknowledge the rest of my colleagues who always supported me with hands-on work, thought-provoking discussions and general collegiality that each of them offered over the years.

My heartfelt thanks go out my family, especially my beloved parents and brother, for supporting me during all these years of studying. Special thanks go to my girlfriend Stefanie for being that patient with and not distracted by me spending quite some time abroad or in the laboratory. Last but not least I want to thank my toddler Lias whose involuntary sleepless nights finally made me get through the paperwork. All of you will always have a very special place in my heart - I love you.



---

## List of Abbreviations

---

<b>CAGR</b>	Compound Annual Growth Rate
<b>CAPEX</b>	capital expenditures
<b>CCM</b>	carbon concentrating mechanism
<b>CO<sub>2</sub></b>	carbon dioxide
<b>DIC</b>	dissolved inorganic carbon
<b>DW</b>	dry weight
<b>FPA-PBR</b>	flat-panel airlift loop photobioreactor
<b>GWP®</b>	Green Wall Panel®
<b>HPS</b>	high-pressure sodium
<b>LED</b>	light-emitting diode
<b>MFC</b>	mass flow controller
<b>OPEX</b>	operational expenditures
<b>PBR</b>	photobioreactor
<b>PFD</b>	photon-flux density
<b>PHB</b>	polyhydroxybutyrate
<b>PI</b>	photosynthesis-irradiance
<b>PS</b>	photosystem
<b>TRL</b>	Technology Readiness Level



# CHAPTER 1

---

## Summary

---

Photoautotrophic microalgae mass production is limited by light availability due to effects of absorption and reflection, especially throughout outdoor cultivation prohibiting the adjustment of photon-flux density (PFD). Generating turbulence within the cultures in order to minimize photolimiting and photoinhibitive effects is the method of choice to overcome that obstacle. Then again, energy required for its generation represents one of the major drivers contributing to overall production costs of microalgae biotechnology.

The present work describes the development of an advanced operating strategy for the mixing of flat-panel airlift loop photobioreactors (FPA-PBRs) that through its application decreases the specific energy consumption, thus the energy requirement per unit of biomass produced, when cultivating phototrophic microorganisms. Experiments were carried out with the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 utilizing distinct FPA-PBRs equipped with culture-flow directing installations and illuminated by high-pressure sodium (HPS) lamps.

In the first paper, the impact of utilizing respective FPA-PBRs is investigated. Preliminary experiments were performed in order to eliminate any limitations beyond the sphere of influence of photobioreactor (PBR) design. Apart from the  $\text{NO}_3^-$  concentration which had to be retained at  $2000 \text{ mg L}^{-1}$  to sustain non-limited growth, special attention was paid to the administration of dissolved inorganic carbon (DIC), inter alia in the form of hydrogen carbonate as  $\text{CO}_2$  gas solubility was limited by the applied cultivation temperature of  $55^\circ\text{C}$ . It is for this reason, in conjunction with a short residence time of the  $\text{CO}_2$ -enriched air bubbles that an increase in  $\text{CO}_2$  concentration showed only minor effects compared to increasing carbonate

concentration that directly correlated to maximum productivity attaining  $2.9 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ d}^{-1}$ , the highest to be reported for *T. elongatus* BP-1, using  $0.04 \text{ g L}^{-1} \text{ Na}_2\text{CO}_3$ . When comparing PBRs with and without culture-flow directing installations, e.g. static mixers, it was found that the former outperformed the latter as an increase in maximum volumetric productivity and final biomass concentration by a factor of 3.4 and 2.0 was recorded, respectively, whilst the energy input in the form of superficial gas velocity remained unchanged. The enhanced growth performance was attributed to improved specific light availability due to the formation of eddies within cultures induced by static mixers. Thereby, light-dependent downregulation of quantum-yield and respiratory losses were reduced, ultimately allowing for a more efficient photon-utilization towards assimilatory photochemistry when compared to randomly mixed cultures.

In the second study, the joined impact of PFD, biomass concentration and superficial gas velocity is investigated and an operating strategy for FPA-PBRs deduced. Preliminary experiments were performed in order to establish a modified photosynthesis-irradiance (PI) curve at default mixing settings which defined the light compensation point and the irradiance of saturation with  $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$  and  $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , respectively. Cultivations were then performed at sub-, quasi-, and supra-saturating PFDs ( $180 \dots 780 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) utilizing multiple gas flow velocities ( $0.11 \dots 0.83 \text{ vvm}$ ). It was found that at a given velocity, productivity and final biomass concentration increased with increasing PFD. Moreover, it was found that in comparison with default mixing settings, the superficial gas velocity during sub-saturating PFD and/or biomass concentrations  $< 3 \text{ g}_{\text{DW}} \text{ L}^{-1}$  can be reduced to cut operational expenditures (OPEX) on mixing, whilst an increase during supra-saturating PFD and/or higher biomass concentrations enhances productivity and final biomass yield. An operating strategy based on the PFD-triggered adjustment of the superficial gas velocity is proposed and results were mathematically translated to exemplary outdoor diurnal cycles of PFD. By applying the strategy on sunny days, productivity is increased by 24%, while reducing not only energy input but also  $\text{CO}_2$ -demand by 11%. On cloudy days, productivity is only slightly increased but energy input and  $\text{CO}_2$ -demand reduced by 37%. Consequently, the specific energy requirement of FPA-PBRs when cultivating phototrophic microorganisms is reduced significantly, especially at locations with only stochastic light supply, e.g. in temperate latitudes.

# CHAPTER 2

---

## Zusammenfassung

---

Die industrielle, photoautotrophe Produktion von Mikroalgen ist auf Grund von Absorption und Reflexion durch eine eminente Limitation der Lichtverfügbarkeit gekennzeichnet. Diese manifestiert sich primär während Freilandkultivierungen, da hier eine Anpassung der Photonenflussdichte (PFD) unmöglich ist. Um dieses Hemmnis zu überwinden ist die Erzeugung von gerichteter Turbulenz zur Minimierung photolimitierender und photoinhibierender Effekte das Mittel der Wahl. Andererseits stellt die Prozessenergie, welche hierfür aufgewendet werden muss, einen der Hauptkostenfaktoren innerhalb der Mikroalgenbiotechnologie dar.

Die vorliegende Arbeit beschreibt die Entwicklung einer modernen Betriebsstrategie zur Durchmischung von Flat-Panel Airlift Photobioreaktoren (FPA-PBRs), welche während der Kultivierung den spezifischen Energieeintrag lichtabhängig reduziert. Experimente wurden mit dem thermophilen Cyanobakterium *Thermosynechococcus elongatus* BP1 unter Verwendung spezieller FPA-PBRs durchgeführt, welche strömungsführende Einrichtungen enthielten, und mit Natriumdampf-Hochdrucklampen betrieben wurden.

In der ersten Veröffentlichung wird der Effekt der Verwendung besagter FPA-PBRs untersucht. Einleitende Versuche dienten zum Ausschluss potentieller Limitationen jenseits des Einflusses der Reaktorgeometrie. Neben der Konzentration von  $\text{NO}_3^-$ , welche bei  $2000 \text{ mg L}^{-1}$  gehalten werden musste um unlimitiertes Wachstum aufrechtzuerhalten, wurde besonderes Augenmerk auf die Bereitstellung von anorganischem Kohlenstoff, unter anderem in Form von Hydrogencarbonat, gelegt. Auf Grund der hohen Kultivierungstemperatur von  $55^\circ\text{C}$ , im Zusammenspiel mit einer kurzen Verweilzeit der mit  $\text{CO}_2$  angereicherten Luftblasen, zeigte die Veränderung der  $\text{CO}_2$ -Konzentration kaum einen Einfluss, während die Carbonatkonzen-

tration direkt mit der maximalen Produktivität korrelierte. Unter Verwendung von  $0,04 \text{ g L}^{-1}$   $\text{Na}_2\text{CO}_3$  erreichte diese  $2,9 \text{ g}_{\text{TS}} \text{ L}^{-1} \text{ d}^{-1}$ , die höchste welche jemals für *T. elongatus* BP1 beschrieben wurde. Der Vergleich der PBRs mit und ohne strömungsführenden Einrichtungen zeigte, dass bei identischem Energieeintrag (gleiche Gasleerrohrgeschwindigkeit) ersterer eine um den Faktor 3,4 gesteigerte maximale Produktivität aufwies und die Biomasseausbeute verdoppelte. Dies wurde der verbesserten Lichtverfügbarkeit zugesprochen, welche aus der Bildung von Wirbeln, induziert durch statische Mischer, resultierte. Limitierende und inhibierende Effekte wurden minimiert und Photonen effizienter der assimilatorischen Photochemie zur Verfügung gestellt.

In der zweiten Veröffentlichung wird der kombinierte Einfluss von PFD, Biomassekonzentration und appliziertem Gasvolumenstrom untersucht und eine Betriebsstrategie für FPA-PBRs abgeleitet. Einleitende Untersuchungen dienten der Erstellung einer modifizierten Photosynthese-Lichtintensitäts-Kurve unter Standardbedingungen, welche in einer PFD von  $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$  für den Lichtkompensationspunkt und  $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$  für den Lichtsättigungspunkt resultierte. Weitere Untersuchungen wurden bei sub-, quasi- und suprasättigender PFD ( $180 \dots 780 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) unter Applikation verschiedener Gasvolumenströme ( $0,11 \dots 0,83 \text{ vvm}$ ) durchgeführt. Hierbei erhöhten sich sowohl maximale Produktivität als auch Biomasseausbeute bei gegebenem Gasvolumenstrom mit steigender PFD. Im Vergleich zur konventionellen Strategie kann die Gasleerrohrgeschwindigkeit bei subsättigender PFD und/oder Biomassekonzentrationen von  $< 3 \text{ g}_{\text{TS}} \text{ L}^{-1}$  reduziert werden, um Betriebskosten zu minimieren. Während suprasättigender PFD und/oder höheren Konzentrationen steigert ein erhöhter Gasvolumenstrom sowohl die maximale Produktivität als auch die Ausbeute. Eine auf der PFD-gesteuerten Anpassung der Gasleerrohrgeschwindigkeit basierende Betriebsstrategie wird vorgeschlagen und Ergebnisse mathematisch auf beispielhafte Tagesgänge der PFD übertragen. Es zeigte sich, dass so die Produktivität an sonnigen Tagen um 24% gesteigert wird, während sowohl der Energieeintrag als auch der  $\text{CO}_2$ -Bedarf um 11% minimiert werden. An bewölkten Tagen steigt die Produktivität nur marginal, jedoch werden sowohl Energieeintrag als auch  $\text{CO}_2$ -Bedarf um 37% minimiert. Folgerichtig wird der spezifische Energieeintrag von FPA-PBRs während der phototrophen Kultivierung von Mikroorganismen signifikant reduziert, im Speziellen in Regionen mit lediglich stochastischem Lichtangebot, wie z.B. gemäßigten Klimazonen.

# CHAPTER 3

---

## Introduction

---

The following sections aim at describing the theoretical principles necessary for a better understanding of this work. The cultivated organism, namely *Thermosynechococcus elongatus* BP-1, will be presented in particular and relevant process-design aspects towards the industrial value creation from photosynthetic microorganisms elaborated. Here, mass productions performed outdoors, thus cultivations subjected to ever changing environmental factors, in particular temperature and light intensity, are in the focus of consideration.

### 3.1 Microalgae in General

Microalgae are a group of organisms that comprises prokaryotic and eukaryotic microorganisms capable of performing photosynthesis. Thus, microalgae can, alike higher plants, build up organic compounds and reproduce on the basis of utilizing (sun)light as source of energy and carbon dioxide (CO<sub>2</sub>) as C-source (Lee, 2008).

Down to the present day, microalgae taxonomy, historically based on limited morphological characteristics, has undergone constant changes which is due to the increasing availability of sophisticated molecular methods giving insights into the species' phylogenetic relationships leading to continuous reclassification of strains on species and even genus level (Borowitzka, 2016). According to Graham *et al.*, 2000, there are nine major algal phyla based on the cellular structure and biochemistry of respective algal cells that may be differentiated e.g. by their photosynthetic pigments, storage products and cell surface structures. Amongst them the prokaryotic cyanobacteria, colloquially called blue-green algae, can be found to which

the organism examined in the present work, *Thermosynechococcus elongatus*, is assigned.

### 3.1.1 Cyanobacteria in General

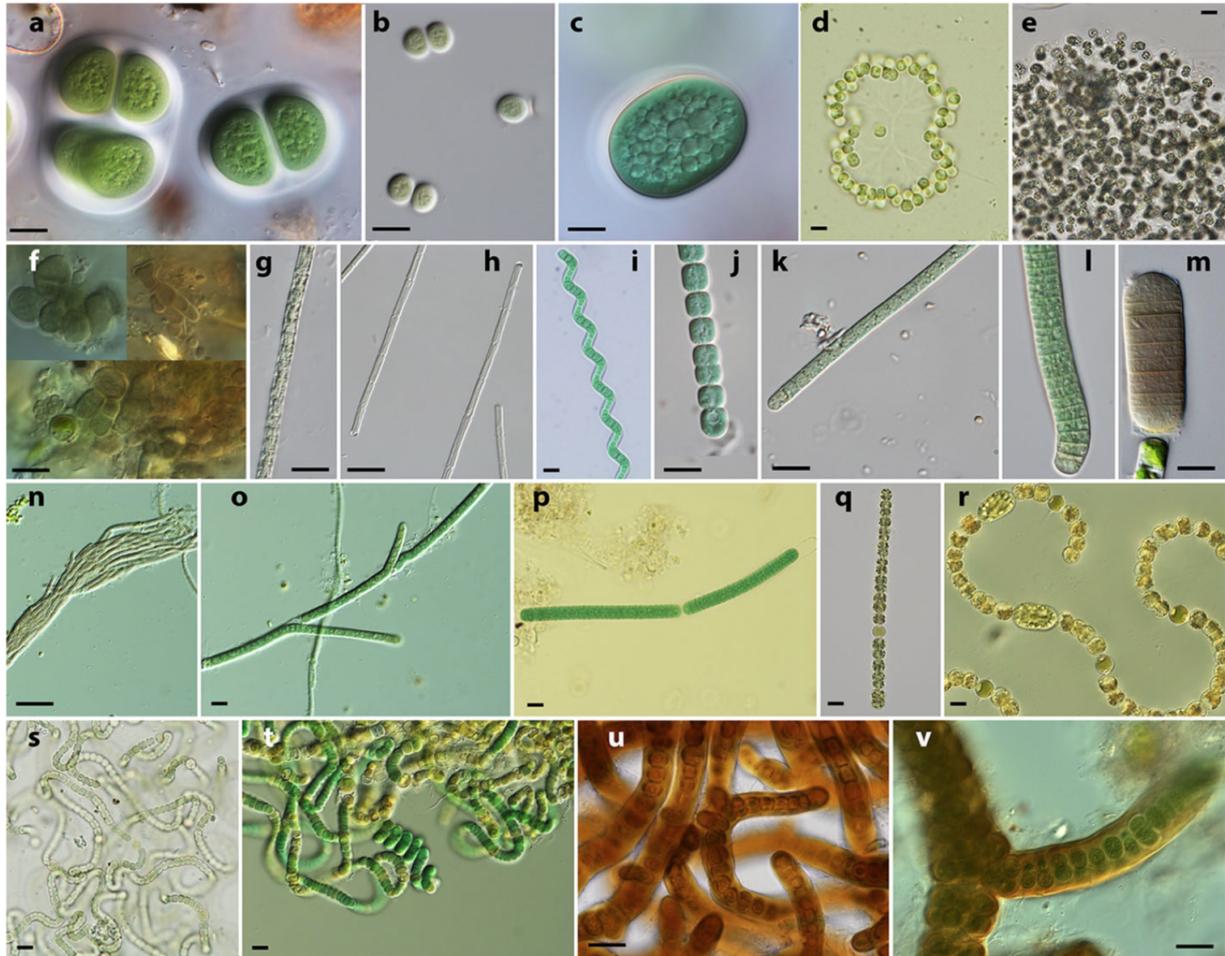
Cyanobacteria are prokaryotic microorganisms capable of performing oxygenic photosynthesis. They are believed to be one of the phylogenetically oldest organisms on earth and can be traced back about 3.5 to 2.7 billion years (Lee, 2008; Pulz *et al.*, 2004) to a time, when no traces of oxygen were within the atmosphere. Thus, they are of eminent ecological significance as they were responsible of shaping the planet's atmosphere (Hamilton *et al.*, 2016). Ancestors of today's cyanobacteria are believed to be omnipresent in the form of plant chloroplasts incorporated by a single endosymbiotic event (Oren, 2014).

Over the millennia, due to evolutionary pressure, today's cyanobacteria, comprising around 8000 species (Guiry, 2012), populate every imaginable biotope on earth including Antarctic regions, deserts and salt lakes (Whitton *et al.*, 2012). They are characterized by a morphological divergence from unicellular to filamentous and mat-forming appearance (see Fig. 3.1) with certain genera showing some degree of cell specialization. *Anabaena* sp. for example is capable of biological fixation of atmospheric nitrogen due to the presence of heterocysts (Kaneko *et al.*, 2001). The same species are also capable of forming akinetes being resting stage cells (Hori *et al.*, 2003).

Cyanobacteria have Gram-negative like structured didermic cell envelopes (Flores *et al.*, 2014) with photosynthesis taking place in internal thylakoid membranes that host both, photosystem (PS) I and PS II. Photosynthesis is assisted by the presence of phycobilisomes, large protein antenna complexes containing phycobiliproteins accommodating accessory chromophores (Grossman *et al.*, 1993). Phycobiliproteins are light absorbing complexes such as phycoerythrin, phycocyanin and allophycocyanin, enabling cells to efficiently absorb and utilize light in the so called "green-gap" of photosynthesis, being wavelengths in the range of 490 to 620 nm not efficiently absorbed by neither chlorophyll. Many cyanobacteria are capable of locomotion by gliding (Hoiczyk, 2000), even directed movement as a function of light availability called phototaxis (Kim, 2017).

Not only their natural diversity makes cyanobacteria a promising source for the biotechnological conversion of CO<sub>2</sub> in added-value products, base chemicals and even fuels. An ever increasing number of complete genomes (Fujisawa *et al.*, 2016) and molecular tools

available (Hagemann *et al.*, 2018) further promotes their potential for industrial utilization as green cell factories.



**Fig. 3.1:** Illustration of morphological diversity in cyanobacteria. Groups (orders) follow Rippka *et al.*, 1979. I. **Chroococcales:** **a** *Chroococcus subnudus*, **b** *Chroococcus limneticus*, **c** *Cyanotheca aeruginosa*, **d** *Snowella litoralis*, **e** *Microcystis aeruginosa*. II. **Pleurocapsales:** **f** *Pleurocapsa minor*. III. **Oscillatoriales:** **g** *Planktothrix agardhii*, **h** *Limnothrix redekei*, **i** *Arthrospira jenniferi*, **j** *Johanseninema constrictum*, **k** *Phormidium* sp., **l**, **m** *Oscillatoria* sp., **n** *Schizothrix* sp., **o** *Tolypothrix* sp., **p** *Katagnymene accurata*. IV. **Nostocales:** **q** *Dolichospermum planctonicum*, **r** *Dolichospermum* sp., **s** *Nostoc* sp., **t** *Nodularia moravica*. V. **Stigonematales:** **u**, **v** *Stigonema* sp. Scale bar a–u = 10  $\mu\text{m}$ , v = 20  $\mu\text{m}$ . Reproduced from Dvořák *et al.*, 2015.

### 3.1.2 *Thermosynechococcus elongatus* BP-1

*Thermosynechococcus elongatus* is the type species of the genus *Thermosynechococcus* belonging to the phylum Cyanobacteria (Guiry *et al.*, 2018). Its full phylogentic classification is listed in Tab. 3.1.

The strain *T. elongatus* BP-1 was isolated from a hot spring in Beppu, Kyushu, Japan and has an optimal growth temperature of 55°C (Yamaoka *et al.*, 1978). *T. elongatus* cells are unicellular, obligately autotrophic and rod-shaped with about 5 - 10 µm in length and 1.5 µm in width (Nakamura *et al.*, 2002; see Fig. 3.2). They divide by binary fission perpendicular to the longitudinal axis.

**Tab. 3.1:** Phylogenetic classification of *Thermosynechococcus elongatus*.  
Adapted from Guiry *et al.*, 2018.

<b>Taxon</b>	<b>Name</b>
Empire	Prokaryota
Kingdom	Eubacteria
Phylum	Cyanobacteria
Class	Cyanophyceae
Order	Synechococcales
Family	Synechococcaceae
Genus	<i>Thermosynechococcus</i>
Species	<i>Thermosynechococcus elongatus</i>

Cells contain chlorophyll *a*, carotenoids and phycocyanobilin and, due to their thermophilic character, have widely been used as model for studies on the photosynthetic apparatus (Kölsch *et al.*, 2018; Kern *et al.*, 2005), also as its genome structure is readily available (Nakamura *et al.*, 2002). Besides structural studies on its photosystems, only limited work has been performed on its metabolic pathways. Eberly *et al.*, 2012 found that *T. elongatus* BP-1 accumulates up to 14.5% (w/w) polyhydroxybutyrate (PHB) when grown at limited CO<sub>2</sub> availability, thus making it a potential candidate for the photosynthetic production of bioplastic precursors.



**Fig. 3.2:** Light microscopic picture of *T. elongatus* BP-1, kindly supplied by Dr. Tatyana Darienko, Culture Collection of Algae at Göttingen University, Göttingen, Germany.

## 3.2 Cyanobacteria in Biotechnology

Cyanobacteria undoubtedly bare an enormous biotechnological potential, which is yet to be exploited. The traditional use of the edible cyanobacterium *Arthrospira* sp., formerly classified as *Spirulina*, dates back to the 16<sup>th</sup> century when its biomass has already been used as food by the Aztecs in Mexico (Habib *et al.*, 2008). Nowadays, biomass of *Arthrospira* sp. is commercialized worldwide. According to García *et al.*, 2017 and based on a market report published by Credence Research (Credence Research, 2017), the global *Arthrospira* market is expected to grow at 10% Compound Annual Growth Rate (CAGR) from 2016 to 2026 reaching a global value of US\$ 2,000 million which is attributed to an increased utilization of *Arthrospira* as superfood (Seyidoglu *et al.*, 2017) and in cosmetics (Ovando *et al.*, 2018), due to its high nutritional value (Clement *et al.*, 1967) and functional properties (Ovando *et al.*, 2018). For the same reasons, *Arthrospira* may also be applied as supplemental feed for aquaculture (Plaza *et al.*, 2018) and livestock (Selim *et al.*, 2018), increasing survival rates and food quality along the food chain, respectively. Even its utilization within life support systems in space is in the focus of present research activities (Mao *et al.*, 2018). An additional

industrial utilization of cyanobacteria is as biofertilizer that, through nitrogen-fixing species (e.g. *Nostoc* sp.), contributes to soil fertility (Lem *et al.*, 1985).

Applied research for the future commercialization of further products from cyanobacteria is on its way. These include, amongst many more, nutraceuticals with antimicrobial activity for human and animal consumption (Panjar *et al.*, 2017), further natural pigments as antioxidants and colorants (Gris *et al.*, 2017), pharmaceuticals including anti-cancer drugs (Nowruzzi *et al.*, 2018) and diverse biofuels, e.g. ethanol (Liang *et al.*, 2018).

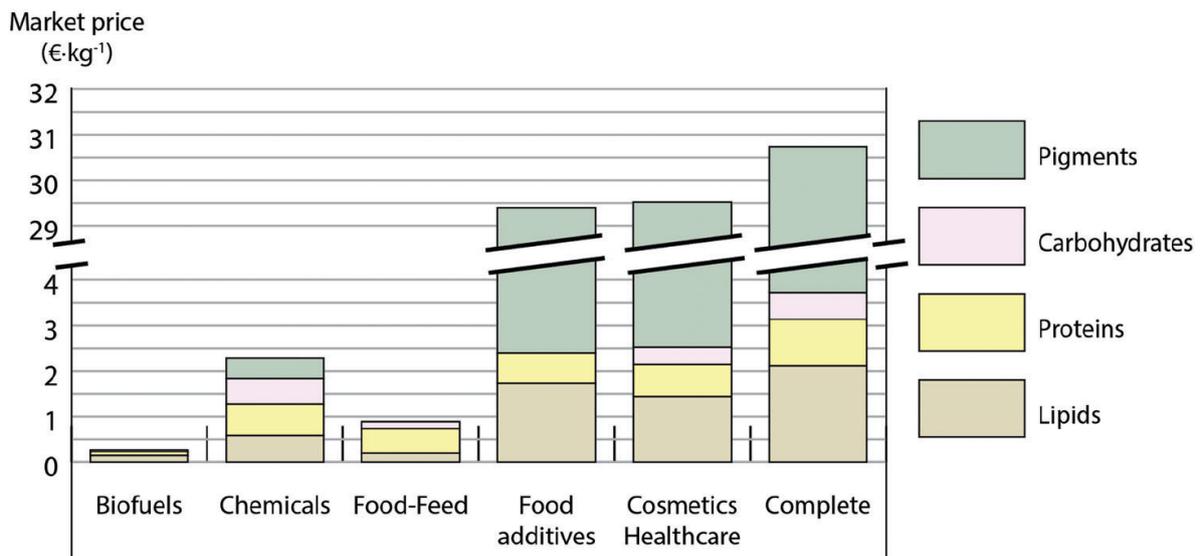
Despite the biotechnological potential of cyanobacteria, large-scale exploitation is restricted to a confined number of applications with only limited quality requirements. Besides political hurdles (Trentacoste *et al.*, 2015) as a result of acting within a young but ever growing sector of biotechnology, the comparably high production costs are yet limiting the penetration of bulk markets (see Section 3.3). This is especially true for mesophilic strains that may not be mass produced in open systems that are relatively inexpensive to deploy and operate. Here, the lack of an applicable selective pressure, such as high salinity or pH, does significantly increase the risk of contamination when compared to extremophilic species, thus necessitating the use of PBR technology.

### 3.3 Photosynthetic Production of Bulk - Economics

Whilst the production of high-value products (e.g. pigments and food supplements) from photosynthetic microorganisms, predominantly eukaryotic microalgae, is already economically feasible and state-of-the-art, the production of commodities such as food, feed and fuel requires a significant reduction in production costs (Ruiz *et al.*, 2016). The same authors state a present production cost of 28.4 € kg<sup>-1</sup> algal biomass for cultivation and harvest excluding further downstream processing. For their calculation, they considered the production in PBRs on a scale of 1 ha which appears to be a conservative but reasonable basis considering the Technology Readiness Level (TRL) of microalgal biotechnology, at least when it comes to large scale applications towards the production of commodities. Here, the current TRL does not exceed prototype deployment (Freeman *et al.*, 2011). Other studies estimate production costs to be in the range of 4.15 € kg<sup>-1</sup> (Norsker *et al.*, 2011) and 9 € kg<sup>-1</sup> algal biomass (Wijffels *et al.*, 2010b). Elicitations of production costs during actual plant opera-

tion result in 69 € kg<sup>-1</sup> (Acién *et al.*, 2012) or even 600 US\$ kg<sup>-1</sup> algal biomass (Borowitzka, 1997).

Therefore, effective present production costs are at least one order of magnitude higher than the market revenue of microalgae for food/feed ingredients and bulk chemicals as production costs of well below 10 € kg<sup>-1</sup> algal biomass are required (Ruiz *et al.*, 2016). Microalgae based biofuels are another order of magnitude away as here, production costs of well below 1 € kg<sup>-1</sup> algal biomass are required (Wijffels *et al.*, 2010a) and therefore, for the time being, only conceivable in a biorefinery concept, thus assuring profitability by commercial exploitation of valuable substances with downstream energy-related utilization of residual biomass or fractions thereof. Figure 3.3 compares the market value of microalgae and their ingredients comparing them to attainable sales revenues in different industrial sectors. It again becomes clear that the penetration of bulk markets necessitates a significant decrease in production costs.



**Fig. 3.3:** Market value of microalgae and their ingredients in comparison to attainable sales revenues in different industrial sectors according to Ruiz *et al.*, 2016. The complete scenario aims at allocating ingredients to respective markets with the highest achievable revenue per unit of biomass.

### 3.3.1 Low Productivity and Cell Density as Major Cost Driver

Although the utilization of phototrophically grown microalgae as potential renewable feedstock for a multitude of applications attracts increasing attention, cultivation efficiency, thus

productivity (biomass production per unit of time and volume) and yield (final biomass concentration to be harvested) remains one of the main bottle-necks for large-scale industrial deployment. This is because of the fact that light availability becomes limiting in dense cultures, thus being the primary factor influencing the process' efficiency (Richmond, 2004). This is especially true for outdoor cultivations where fluctuating conditions dominate tight process control.

Compared to conventional biotechnology (e.g. *S. cerevisiae*) for which high-productivity fermentations reaching densities of more than 120 g L<sup>-1</sup> at greater 25 g L<sup>-1</sup> h<sup>-1</sup> were reported decades ago (Shay *et al.*, 1987), production of phototrophic microalgae is indeed slow, even when taking into consideration outstanding maximum performances. The highest reported productivity of a microalga cultivated in a PBR is 0.5 g L<sup>-1</sup> h<sup>-1</sup> achieved with *Chlorella sorokiniana* CCAP 211/8k cultivated in continuous mode in a laboratory short light-path (14 mm) PBR under high irradiance (2100 μmol m<sup>-2</sup> s<sup>-1</sup>) at a fairly low biomass concentration of 2.1 g L<sup>-1</sup> (Cuaresma *et al.*, 2009). The highest reported biomass concentration reached is 40 g L<sup>-1</sup> achieved with *Chlorella* sp. cultivated in fed-batch mode in a thin-layer (6 mm) outdoor open PBR operated in Třeboň, Czech Republic (Doucha *et al.*, 2006).

In order to reduce limiting and inhibitive effects of too little or excess light, different methods have been put into practice. These include e.g. the exploitation of short light paths as outlined above, or the incorporation of culture-flow directing installation resulting in a movement of algal cells within the different photic zones of the culture (Huang *et al.*, 2017).

In any case, maximizing productivity (at a given amount of process energy spent) will generally increase process economics by reducing process duration. Maximizing biomass yield will significantly reduce biomass specific harvesting costs, which were reported to contribute 20 - 30% to the total biomass productions costs (Gudin *et al.*, 1986). A recent study conducted by Fasaei *et al.*, 2018 showed, that by increasing the biomass concentration from 0.5 g L<sup>-1</sup> (open pond) to 2.5 g L<sup>-1</sup> (PBR), biomass specific harvesting and de-watering costs were reduced from 15 to 3%.

### 3.3.2 Mixing Energy as Major Cost Driver

Whilst microalgae valorization in high-value markets allows for the utilization of artificial light sources such as light-emitting diodes (LEDs) in order to compensate for fluctuating

light conditions assuring continuous product output at constant quality (Blanken *et al.*, 2013; Schulze *et al.*, 2014), this approach is financially prohibitive when addressing bulk markets. Here, light energy must be provided by means of sunlight which is free and abundant. Anyhow, besides raw materials for e.g. cleaning and disinfection, process-energy for culture mixing, thus light supply, remains one of the main cost drivers contributing to 14-17% of the overall production costs, even when capital expenditures (CAPEX) are included in the calculations (Ruiz *et al.*, 2016). This awards process-energy an even greater impact when only considering OPEX. Here, in turn, 80% of the total energy consumption is required for adequate culture mixing (Ruiz *et al.*, 2016) in order to reduce growth-limiting and growth-inhibitive effects (photolimitation and photoinhibition) as a result of sparse or excess light availability. Another study conducted by Norsker *et al.*, 2011 resulted in a proportion of the process-energy on total biomass production costs of up to 23% when only considering the energy required for mixing. Therefore, reducing specific energy consumption, thus the energy requirement per unit of biomass produced, represents a significant lever to cut OPEX and thus, total production costs. Hereby, mixing is achieved by different PBR propulsions whereby mainly pump- and airlift-driven systems are distinguished.

#### 3.3.3 Cooling Necessity as Major Cost Driver

Although sunlight is free and abundant, it does come at costs as PBRs act as thermal heat collectors focusing additional heat on them, especially in dense (dark) cultures with high specific heat capacity. These circumstances require energy and/or water consuming cooling measures as temperatures of only 2-4°C above optimum may be detrimental, if not even lethal, for many species (Mata *et al.*, 2010). Considering a production plant of 1 ha utilizing Green Wall Panel® (GWP®) PBRs (315 m<sup>3</sup>) with culture cooling performed in external heat exchangers, Tredici *et al.*, 2016 calculated that around 14.5% of the capital costs per year are represented by the cooling infrastructure. Here, only the piping, fittings and valves were considered neglecting the centrifugal pumps, that, in turn, are responsible for 16.3% of the annual energy consumption. In order to decrease cooling expenses during outdoor cultivations, thermotolerant or thermophilic strains, as the organism examined in the present work, *Thermosynechococcus elongatus*, may be applied (Hanagata *et al.*, 1992; Béchet *et al.*, 2013). Then again, availability of DIC may become a limiting factor due to the reduced

solubility of CO<sub>2</sub> at elevated temperatures.

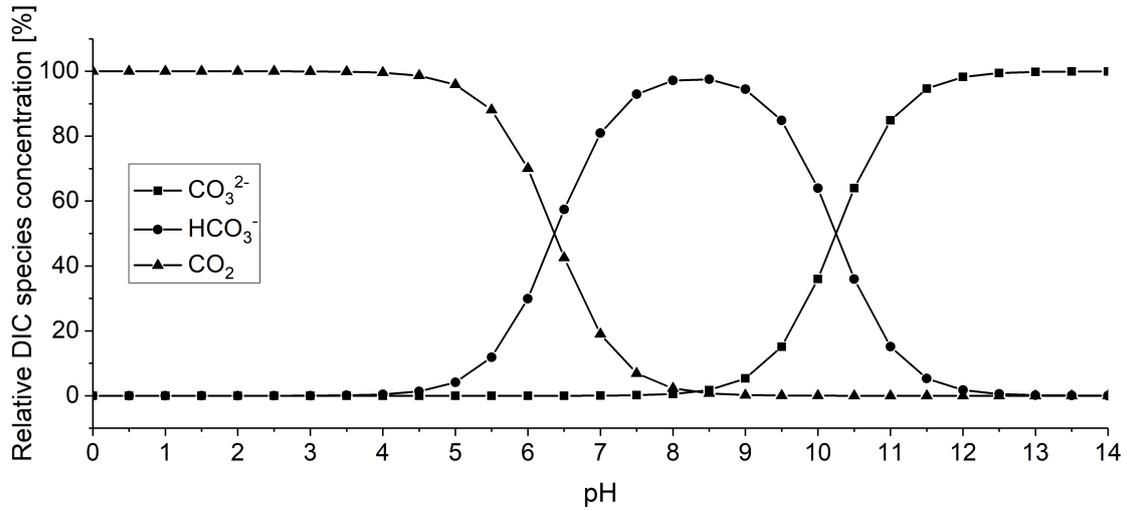
### 3.4 Mass Transfer in Microalgal Cultures

Aside from its influence on cooling efforts and biological performance (e.g. enzyme activity), temperature also has a major impact on the availability of DIC which is essential in microalgae mass cultivation. As DIC serves as sole carbon source during phototrophic cultivation, it may directly be limiting the overall process (Varshney *et al.*, 2015).

At a given medium composition and partial pressure of CO<sub>2</sub>, solubility is decreased with increasing temperature. Carbon dioxide solubility is furthermore influenced by the media's composition, mainly its salinity, with the solubility decreasing with increasing salinity (Knoche, 1980). Evolving from atmospheric CO<sub>2</sub>, carbon in aqueous solutions exists in three DIC species being CO<sub>2</sub> (H<sub>2</sub>CO<sub>3</sub>), HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> (see Equations 3.1 - 3.4).

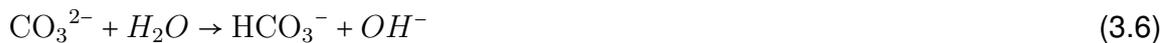


The proportion at which these species are present severely depends upon the culture's pH value following the carbonic acid equilibrium (see Fig. 3.4).



**Fig. 3.4:** Representation of the DIC species vs. pH response. The proportion of different DIC species changes as a function of the media's pH value.

Depending on the cultivated microalgae species as well as the presence and activity of carbon concentrating mechanisms (CCMs), microalgae will use either use atmospheric CO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup> or both (Giordano *et al.*, 2005). The uptake of either species results in an increase in pH, thus, if not counteracted, in decreased absolute DIC concentration readily available for cells. This is due to the fact that the equilibrium is re-adjusted following the uptake of CO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup>, both resulting in the formation of hydroxide ions (see Equation 3.5 and 3.6).



In dense microalgal cultures exhibiting intense photosynthesis, either buffering systems or continuous CO<sub>2</sub> supply are necessary. The latter can be achieved by utilizing CO<sub>2</sub>-enriched (industrial waste) gases or by the utilization of media enriched with carbonates (e.g. CaCO<sub>3</sub>, MgCO<sub>3</sub>). Hereby, the carbonate and bicarbonate are released into the medium and become part of the above mentioned equilibrium increasing the pH by the introduction of hydroxide ions (see Equation 3.7 and 3.8). At the same time, the overall buffering capacity of the medium is raised.



The method of choice to increase the DIC concentration is dependent on the alga under examination (e.g. mesophile vs. thermophile), the co-location of the production facility (e.g. the presence of CO<sub>2</sub> generating industry) and the cultivation technology used (e.g. residence time of gaseous CO<sub>2</sub>). Naturally, either strategy has its limitations. Whilst the injections of gaseous CO<sub>2</sub> is limited by its solubility as a functions of temperature and residence time, the provision of DIC via carbonates increases the pH value ultimately exceeding 10.5 reducing the DIC concentration readily available for microalgal proliferation.

### 3.5 Research Objective

The overarching objective of the work performed was to decrease the OPEX, particularly the specific energy consumption, thus the energy requirement per unit of biomass produced, when cultivating a thermophilic cyanobacterium in distinct FPA-PBRs (see Section 4.2). In addition, it was to be evaluated whether or not the specific energy consumption may be minimized by reconsidering the mixing strategy of respective FPA-PBRs. For this purpose, the present study aimed towards generating a deep insight and understanding of the complex interplay between hydrodynamics and specific light availability, thus the joined influence of PFD, biomass concentration and superficial gas velocity, on the growth kinetics of phototrophic microorganisms. Ultimately, the study aimed towards the development of an advanced control-command strategy enabling an automated adjustment of the volumetric gas flow velocity to actual prevailing specific PFD (see Section 4.3).

# CHAPTER 4

---

## Publications

---

### 4.1 Suspension Culture of Phototrophic Algae and Cyanobacteria

Published as Book Contribution:

Bergmann, Peter, Nissen, Astrid, Beyer, Lars, Ripplinger, Peter and Trösch, Walter (2014). "Suspension Culture of Microorganisms (Algae and Cyanobacteria) Under Phototrophic Conditions". In: *Industrial Scale Suspension Culture of Living Cells*. Ed. by H.-P. Meyer and D. R. Schmidhalter. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA, Chap. 6, pp. 198-223.

Invited book contribution reproduced with kind permission of John Wiley and Sons under license number 4234730835428. ©2014 Wiley-VCH Verlag GmbH & Co. KGaA, Boschstr. 12, 69469 Weinheim, Germany.

The publication describes the basics of microalgal biology, the history and fundamentals of microalgal biotechnology as well as basic and advanced process-design aspects of the latter. Industrially relevant open and closed cultivation systems, namely open ponds and photobioreactors, are elucidated and compared in terms of design, costs and process stability as well as performance, energy consumption and potential products. The conclusion gives an overview of prospects and future challenges.

# Industrial Scale Suspension Culture of Living Cells

Edited by  
Hans-Peter Meyer and  
Diego R. Schmidhalter

WILEY Blackwell

## Summary Box: Characteristics of Production System

Production systems, cells and products	Expression systems Type of cells	Open towards the environment (ponds) and closed (photobioreactors) systems in multiple designs; cultivation of prokaryotic and eukaryotic photosynthetic microorganisms
	Typical products Preferred for the production of	Biomass (food, food supplement, health supplement, feed), lipids (biofuel), fatty acids (omega-3 and omega-6), pigments (astaxanthin, beta-carotene)
	Special products	Pharmaceuticals, cosmeceuticals
	System maturity Products on the market	Multiple open systems on the market (biomass, astaxanthin, beta-carotene), three tubular systems on the market (food and health supplements), flat-plate systems in multiple pilot plants
Characteristics affecting product quality	Secretion, product location, proteolysis	Hardly any secretion, mainly intracellular product formation
	Post-translational modifications (PTMs) as disulfide bonds, glycosylation, protein multimers, product size	PTM not of importance for current products, nevertheless possible
	System robustness, solubility issues, endotoxins	System can generally be regarded as robust towards biotic and abiotic factors; no solubility issues; hardly any endotoxins in currently used strains detectable (food-grade products)
Systems characteristics concerning growth and productivity	Maximum specific growth rate, $\mu_{\max}$ ( $\text{h}^{-1}$ )	0.2
	Maximum dry biomass concentration, $x_{\max}$ ( $\text{g l}^{-1}$ )	0.5 (open systems), 20 (closed systems)
	Maximum specific product production rate, $q_p$ ( $\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ )	Not relevant, biomass is the product
	Maximum volumetric product production rate, $r_p$ ( $\text{g} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ )	0.2
	Maximum product titres, $c_{p,\max}$ ( $\text{g l}^{-1}$ )	0.2 (open ponds), 20 (closed systems)
	Specific maintenance, $m_s$ ( $\text{g g}^{-1} \text{h}^{-1}$ )	Not relevant for photoautotrophic systems
	Yield coefficients $Y_{x/s}$ and $Y_{p/s}$ ( $\text{g}^{-1} \text{g}^{-1}$ )	Not relevant for photoautotrophic systems
	Energy and carbon source Special nutritional requirements	Energy: light (natural and/or artificial), carbon: dissolved inorganic carbon ( $\text{CO}_2$ , $\text{HCO}_3^-$ )
	Maximum oxygen uptake rate, $\text{OUR}_{\max}$ ( $\text{mmol l}^{-1} \text{h}^{-1}$ )	Not relevant for photoautotrophic systems
	Heat production rate ( $\text{W m}^{-3}$ )	Not relevant for photoautotrophic systems
	Typical duration from inoculation of a production culture to harvest (days)	56 (open systems), 21 (closed systems)
Systems shear sensitivity	Utilization of centrifugal pumps might result in shear stress, hardly any shear stress using airlift systems	
Cost and performance aspects	Preferred bioreactor design Equipment standard and typical production scale	Open ponds of several hectares for extremotolerant species, PBRs (photobioreactors) for moderate species at several $\text{m}^3$
	Most important production cost drivers of production culture	Energy for culture mixing (convective mass transfer)
	Process development cost aspects Duration of process development	Not predictable; dependent on desired product and its quality, strain used, locality of production and degree of process integration
	Key strengths of system	Production of organic compounds by utilization of inorganic carbon sources and energy derived from sunlight
	Key weaknesses of system	Little productivity (emerging technology) at high energy consumption
	Issues to be addressed in the future	Increase in productivity and decrease in energy consumption by means of biological and engineering aspects: isolation of new strains, genetic engineering of strains, PBR design, development of innovative mixing strategies, integration of local resources (water, flue-gases), integration of photovoltaic energy recovery for mixing energy demand

## 6 Suspension Culture of Microorganisms (Algae and Cyanobacteria) Under Phototrophic Conditions

*Peter Bergmann, Astrid Nissen, Lars Beyer, Peter Ripplinger, and Walter Trösch*

### Abstract

Global net primary production of biomass has faded dramatically over the past few decades as a consequence of the increasing population and its infrastructure demands. Photosynthetic microorganisms constitute the substructure of the water based food chain and are characterized by a five- to tenfold higher surface area productivity in comparison to traditional terrestrial crops. Based on their larger areal productivity they are an expandable and so far sparsely utilized resource in terms of biotechnology. In addition, extended utilization in a sustainable form decreases anthropogenic carbon dioxide emission. Furthermore, the production of valuable compounds, for example, for pharmaceutical and cosmetically applications without genetic modification, is broadly possible by simple modification of cultivation parameters. For application of these benefits to the preservation of the livelihood of mankind, sustainable (phototrophic) production and utilization paths need to be designed beyond arable land use for food production. The present chapter describes the basics of microalgal biology as well as basic and advanced process design aspects. Industrially relevant open and closed cultivation systems, namely, open ponds and photobioreactors, are elucidated and compared in terms of design, costs, and process stability as well as performance, energy consumption, and potential products. The conclusion gives an overview of prospects and future challenges.

### 6.1 Introduction

Photosynthetic microorganisms constitute the substructure of the water-based food chain and are characterized by a five- to tenfold higher surface area productivity in comparison to traditional terrestrial crops [1]. Owing to an increasing global population and the continuously rising demand for nourishment rich in animal protein, the demand for biomass as nutrition for man and livestock has

*Industrial Scale Suspension Culture of Living Cells*, First Edition.  
Edited by Hans-Peter Meyer and Diego R. Schmidhalter.  
© 2014 Wiley-VCH Verlag GmbH & Co. KGaA. Published 2014 by Wiley-VCH Verlag GmbH & Co. KGaA.

increased severely in recent years. Further increasing demand for biomass arises from the continuously growing need for chemical precursors (e.g., for commodities and biofuels) due to shortage of fossil resources, political discussions on “food versus fuel” (first-generation biofuels), the enacted “energy turnaround” following nuclear catastrophes, and aspired carbon footprint reduction in all social sectors. Coincidentally, the global net primary production of biomass faded dramatically (10%) [2]. This is in consequence of increasing areal requirements for infrastructure of man and livestock (e.g., forest clearings), soil erosion of extensively used agricultural lands, and advancing climate change (+2–4 °C) leading to enhanced water stress in agronomical production with an upward tendency. Owing to their greater areal productivity photosynthetic microorganisms can help to reduce losses in net primary production and therewith decrease anthropogenic carbon dioxide emission. To put this benefit to use, sustainable (phototrophic) production and utilization paths need to be designed.

### 6.1.1

#### Photosynthetic Microorganisms (Algae and Cyanobacteria) in General

The scientific study of photosynthetic microorganisms is called phycology, a term derived from the Greek word *phykos* meaning seaweed [3]. Hereby, the study equally includes eukaryotic and prokaryotic photosynthetic microorganisms, often referred to as microalgae and cyanobacteria, respectively. Nevertheless, the term microalgae itself does not describe a distinct taxon in biological systematics but derives from common language and relates to groups of organisms that share some characteristics but are very distinct from each other. It is, therefore, not incorrect to use the term microalgae when talking about both eukaryotic and prokaryotic photosynthetic microorganisms and this nomenclature will be used throughout the present chapter, especially since process design aspects are the same for both.

One common feature of microalgae is their microscopic appearance, which disallows imaging a single cell with the naked eye. Microalgae are thallophytes, meaning they are plant-like organisms possessing similar nutritional requirements but lacking differentiated tissues. All microalgae possess chlorophyll *a* (chlorophyll *b*, *c*, *d* and other photosynthetic active pigments might be present in various algal groups in various ratios), which makes them capable of exhibiting photosynthesis [3]. Microalgae are therefore able to convert solar energy into chemical energy by assimilating (in)organic carbon from the environment. Since these few universal characteristics do not closely define microalgae it is not surprising that they encompass groups of organisms that are not necessarily closely related. This is especially true for above-mentioned cyanobacteria (often referred to as blue-green algae), which are prokaryotic and therefore more closely related to bacteria than to other algae. Microalgae belong to the phylogenetically oldest organisms on earth. Cyanobacteria can be traced back about 3.5 to 2.7 billion years [3,4] to a time when no traces of oxygen were within the atmosphere. Not until these ancient cyanobacteria produced oxygen was heterotrophic life, as we know it today, possible. Besides the few general

characteristics, microalgae are an extremely heterogeneous group of organisms and it is estimated that between 40 000 and several million [5–7] species exist. Their abundance is of environmental importance, especially in the oceans, which cover 71% of the Earth's surface, where they constitute the only primary producer of biomass and natural gas. Furthermore, it is estimated that microalgae contribute to approximately 40–50% of the oxygen in the atmosphere [7]. Over the millennia, due to evolutionary pressure, today's microalgae populate nearly every imaginable biotope on earth. Microalgae can virtually be found in any terrestrial and oceanic habitats, from hot, dry deserts to the ice cold Antarctic Ocean, from the illuminated surface to the soil under aphotic (non-illuminated) conditions, from freshwater to hypersaline salt-lakes (salinity of up to 35%), from highly acidic to highly alkaline habitats, [8].

### 6.1.2

#### Microalgal Evolution and Taxonomy

As stated above, microalgae have a very long history of evolution. During the past few billions of years two major and one “minor” events of endosymbiosis led to today's microalgal biodiversity. Endosymbiosis is called the event of incorporation of an organism by another through phagocytosis (without digestion).

During primary endosymbiosis an ancient cyanobacterium was taken up by a eukaryotic, heterotrophic (organic compounds as carbon and energy source) cell resulting in the first eukaryotic microalga containing a plastid named chloroplast, the site of photosynthesis in eukaryotic algae. This inclusion was followed by a successive gene transfer from the plastid to the nucleus of its host. Ultimately, only a few genes remained in the plastid. Primary endosymbiosis resulted in the so-called Archaeplastida consisting of three lineages, namely, green algae (chlorophytes, evolutionary ancestor of land plants), red algae (rhodophytes), and glaucophytes. Secondary endosymbiosis resulted in a further boost in algal diversity. Hereby, members of the Archaeplastida (mainly red algae) were taken up by a eukaryotic, heterotrophic cell resulting in multiple microalgal lineages. Some of these abandoned their previously acquired plastids and acquired new ones (tertiary endosymbiosis). These three events of endosymbiosis may be the reason for the evolutionary success of microalgae and their almost inexhaustible assortment of potentially exploitable products [9].

### 6.1.3

#### Microalgae in Biotechnology

The enormous diversity of microalgae promises to provide an opulent source for a multitude of products for various applications. Depending on the examined species microalgae of prokaryotic as well as eukaryotic nature possess a surface to volume ratio of about 400 : 1, which is an order of magnitude higher than that of leaves of terrestrial crops. This not only allows a much more efficient transport between biocatalyst (microalgae cells) and dissolved substrates but also a much

more efficient absorption of the “substrate” light which is, due to self-shading, growth limiting in dense cultures. Microalgae can be cultivated on non-arable land (water-based) and their production therefore does not compete with the production of food crops. Alterations in process conditions (e.g., light intensity, nutrient supply, salinity, and/or pH) often allowing to “design” the final biomass composition of many algal species to a certain extent depending on the desired exploitation. Thus, for example, can the protein rich green alga *Chlorella vulgaris* be triggered to accumulate high amounts of lipids as biofuel precursors when exposed to nutrient and light stress [10]. A further characteristic of industrial microalgae cultivation is the ease of processing the resulting biomass due to the lack of lignocellulose. Downstream processes are therefore much more straightforward than those of terrestrial crops. Despite all these facts microalgae are on a biotechnological view still not a well-studied group [11]. This is because only a few thousand microalgae species have been investigated concerning their production of valuable chemical compounds and up to now not more than 15 species have been cultivated on a large scale for industrial purposes – mainly in Asian countries such as China, Japan, and Taiwan where *Chlorella* and *Spirulina* species are mainly used as “health food” and cosmetics. In Australia, India, and the USA *Dunaliella* is cultivated for the production of beta-carotene [11,12]. Some additional utilization of microalgal biomass is in animal feed, biofertilizer, feed for aquacultures, their role in wastewater treatment, the production of valuable compounds (polyunsaturated fatty acids, antioxidants, vitamins, pharmaceuticals), and their precursors as well as biofuel production. The turnover of algae biomass was about 5000 t in 2005 with an estimated sales volume of US\$1.25 billion [12]. Today’s turnover is estimated to be above 10 000 t [13]. These numbers and facts illustrate the high potential of microalgae biotechnology and therewith potential that arises from these barely known organisms concerning new product discoveries.

### 6.1.4

#### Industrial Microalgae Biotechnology – A Brief History

Despite the long history of cultivation of microalgae, industrially relevant cultivation, hence microalgae mass cultures, is a relatively young field of microbiology. Most methods used for microalgae isolation, separation, purification, and cultivation as well as development of culture media were already established in the late 1800s and early 1900s. The first report on microalgae cultivation was published in 1850 by the German botanist and microbiologist Ferdinand Julius Cohn (Cohn and Heinrich Herman Robert Koch are generally regarded as the founders of modern bacteriology), who succeeded in cultivating the green alga *Haematococcus* for a period of time. As with terrestrial plants, microalgae need among others nitrogen, phosphor, calcium, potassium, and several trace elements. The first efforts to cultivate microalgae using artificial media composed of several inorganic salts were conducted by the Russian plant physiologist Andrei Sergeevich Famintzin in 1871. Axenic cultures of microalgae were reported as early as 1890 when

the Dutch microbiologist Beijerinck managed to isolate, separate, and purify the green algae *Chlorella* and *Scenedesmus* from environmental samples using gelatin solidified sampled water [14]. This pioneering work was followed by knowledge acquisition by a multitude of scientists. New isolation techniques and media recipes were developed, fresh and sea water species cultivated and described, and toxic substances and nutrient concentrations identified, ultimately resulting in the foundation of numerous cultures collections, with the largest ones being the Culture Collection of Algae and Protozoa (CCAP) in Argyll, Scotland, the Culture Collection of Algae at Göttingen University (SAG) in Germany, the National Center for Marine Algae and Microbiota (NCMA) in East Boothbay, USA, and the Culture Collection of the University of Austin (UTEX) in the USA temporarily depositing 2000, 2400, 2700, and 3000 purchasable strains, respectively. Although dense and large quantities of laboratory cultures of algae were already reported in 1919 and 1938 [14] scientific focus was laid on mass cultivation of microalgae only since the late 1940s at Stanford (USA) [15], Essen (Germany) [16], and Tokyo (Japan) [17]. Henceforth laboratory methods were translated into engineering attempts for large scale outdoor microalgae cultivation. The results were summarized in a classic report by Burlew (USA) [18].

## 6.2 Basic Process Design Aspects

During the short history of microalgae mass cultivation a number of production systems and techniques were developed. Several considerations have to be taken into account and the cultivation system and technique used highly depend on the cultivated alga and the end product desired.

Generally, algae can be grown in open (towards the environment) systems or in enclosed photobioreactors. Open systems can be subject to contamination with other algae, grazers, fungi, bacteria, and viruses that might outcompete/feed on/kill the intended alga species, hence leading to culture loss possibly within a few days, complicating product purification or making it futile, for example, for pharmaceutical purposes [1,3,4]. Furthermore, reproducible results are rarely achievable as the control of parameters such as temperature, pH, and nutritional composition is difficult. Nevertheless, open systems are the cheapest way for algae cultivation. Enclosed bioreactors on the other hand give the opportunity to control the process parameters favored. Biomass production per volume is higher compared to open systems and the total area needed is smaller [2]. In addition, photobioreactors can, depending on their layout, be sanitized by means of chemicals (e.g., hydrogen peroxide), ozone, gamma radiation, or steam, minimizing the risk of contamination.

Cultivation can be performed using different “trophy” modes. The two main modes are photolithoautotrophy (feeding by inorganic compounds and energy acquisition by light) and heterotrophy (feeding and energy acquisition by organic compounds). Few microalgae are obligate heterotrophs. A common feature of

microalgae is their capability to use both feeding methods, for example, using photolithoautotrophy as primary trophic and heterotrophy during phases of insufficient light intensity. Any constellation of these possibilities has its positive and negative aspects. The choice of algae and/or its carbon source depends on the desired product. Sole photoautotrophic growth leads to a decrease in maximum cell concentration since the availability of (sun)light, the only energy source, becomes the limiting factor at one point due to self-shading within cultures becoming denser. Furthermore, more space is needed to produce an equal amount of product compared to a mixo- or heterotrophic growth. Mixotrophic growth was shown, for example, for *Chlorella vulgaris* to yield in a maximum specific growth rate that equals the sum of photoautotrophic and heterotrophic growth rates of other microalgae [3]. In addition, mixotrophically cultivated algae can overcome stress situations in dim lighting times by feeding from organic carbon sources. Nevertheless, feeding of organic nutrients on an industrial scale is costly and hence one of the disadvantages of heterotrophic growth. In addition, substrate concentrations must be kept at a low level since high concentrations can have inhibitory effects on cell growth [19]. Furthermore, heterotrophic cultivation lacks the opportunity to produce photo-induced substances such as pigments.

Besides the supply of adequate media (macro- and micro-nutrients) and the adjustment and retention of proper temperature (enzyme activity) and pH (enzyme activity, cell permeability, and availability of dissolved inorganic carbon (DIC) species) regimes light is the most crucial factor in microalgae mass cultivation. For cultivations taking place solely photoautotrophically producers have to take care that light is the only “substrate” limiting the growth and therefore determines the cultivation’s performance. Prerequisite for maximum areal productivity and therewith economic as well as ecologic efficiency is the possibility for microalgae to absorb locally available light with the highest conceivable yields in order to convert solar energy (light) into biochemical energy, that is, microalgal biomass, with the utmost efficiency. As solar intensity undergoes seasonal and diurnal changes between 0 and  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (photosynthetically active radiation, 400–700 nm wavelength) photons both phenomena, photolimitation (light intensity below compensation point) and photoinhibition (light intensity above saturation point), can minimize the productivity and even irreversibly damage microalgae cultures. These inhibiting effects occur especially in outdoor cultivations, during which cultures often face sub- and supra-optimal levels of light intensity resulting from circadian and seasonal changes in available light depending on the latitude of the production site. Even during supra-optimal levels the culture suspension is efficiently supplied with light to no more than 1 cm in depth and, thus, may suffer from severe photoinhibition and is therefore unable to convert incoming photons efficiently. Other areas of the culture may use incoming photons efficiently but may suffer from photolimitation since cells face zones of darkness, depending on the cell density, the cultivation system, and its light path. The primary goal is to eliminate these inhibiting phenomena, avoiding limiting and inhibiting light conditions, and gain successive increases in productivity, which can be reached through several basic approaches.

Biologically, the propagation or genetic modification of high light adapted cells will lead to minimized light harvesting complexes within their photosynthetic apparatuses. Light can yet be used efficiently, photoinhibition is minimized, and due to reduced absorption light can penetrate the culture more deeply.

From the biotechnology-process engineering point of view the depth (ponds) or layer thickness (closed systems) can be minimized. This will most probably lead to reduced outputs of biomass and/or desired product per unit area. Light can be directed to the inner zones of the pond or cultivation vessel using optical light guides, which, however, increase engineering expenditure, increase capital expenditure, and may disturb fluid dynamics. In addition, both attempts do not address the problem of photoinhibition at all.

Convective mass transfer (ideal mixing, “light dilution”) can be used to offset light inhibition by directed allocation of biocatalysts within the culture volume and furthermore to enhance mass transfer. Photosynthetic photon capture occurs much faster than actual carbon fixation, during which electron transport might be saturated and further impinging photons are therefore wasted. By applying a directed flow of culture suspension with a short residence time in photic zones and prolonged residence in aphotic zones, carbon capture can catch up, leading to more efficient photon utilization by this so-called flashing-light effect.

Further “unit operations” of process engineering leading to optimal biomass productivity can be extracted from the era of “single cell protein” (SCP) production. During the 1970s SCP was the vision behind the solution portfolio of science and industry to cope with the increasing protein demand of mankind. The idea was abandoned because the resulting biomass contained far too high concentrations of nucleic acids. However, the keynote, the basic visionary thought behind the idea, is still valid. The bioreactors developed for SCP production were optimized to allow high growth rates for aerobic bacteria in a three-phase system (gas, liquids, solids (biocatalyst)). Convective mass transfer is the method of choice to increase oxygen transfer and supply to aerobic bacteria during production processes in which oxygen is growth limiting, due to its poor solubility. This can analogously be applied for photolithoautotrophic cultivation of microalgae during which, along with light, dissolved inorganic carbon in the form of  $\text{CO}_2$  or  $\text{HCO}_3^-$  is a substrate and of utmost importance. Therefore, in general the performance of microalgae cultivation depends on four phases, namely, gas ( $\text{CO}_2$  in;  $\text{O}_2$  out), liquid (medium), and solid (biocatalyst) coupled with the light as the fourth phase (i.e., a g,l,s-system). Thereby, the efficiency is defined by the mixing time/energy input required per volume for a growth limiting substrate and the resulting volumetric productivity in  $\text{g}_{\text{dry weight}} \text{l}_{\text{reactor volume}}^{-1} \text{t}_{\text{ime}}^{-1} (P_{\text{vol}})$ .

The quantitatively highest energy effort during photoautotrophic microalgae cultivations in a four-phase system is, for reasons mentioned above, spent on the generation of turbulence (convective mass transfer). To classify the regeneratively produced biomass as carbon neutral the energy needed to create one quantum of biomass must be less than the energy biochemically stored in the same quantum of microalgae. If less energy is needed and under the precondition that waste carbon dioxide and technical (recycling) media with sufficient

nitrogen and phosphorous are used biomass production using microalgae can even decrease the carbon dioxide concentration resulting from civilization's progression based on fossil resources.

Biomass production in general can take place using different cultivation strategies that are analogous to conventional fermentation modes in biotechnology. Thereby, batch (no addition to or removal from the suspension after inoculation), semi-continuous (partial harvest, e.g., 30% (v/v) of culture and replenishment with fresh medium), and continuous (supply of fresh medium and withdrawal of culture at the same rate) modes are discerned. The continuous mode is either run as chemostat (monitoring of nutrient concentration) or turbidostat (monitoring of biomass). The latter can, for example, be done by multi-angle stray-light measurements for which, due to the disturbance of changing light intensities especially during outdoor cultivations, partial darkening of the reactor needs to be considered (e.g., by constructing a non-transparent bypass).

Sterility is a crucial factor in industrial (large-scale) cultivations of phototrophic microorganisms. Although sterilization or hygienization may be applied for photobioreactors, depending on their geometry and/or construction material, long-term asepsis is hardly achievable. As is true for other biotechnological cultivations, asepsis can only be achieved during batch cultivations and not during continuous operation. Most cultivations describe a consortium of desired microorganisms and (other) bacteria. Hereby, no hazard is generated for the phototrophic organisms because of the much lower number of contaminating bacteria. The non-photosynthetic bacteria cannot outcompete the microalgae since growth by heterotrophy requires an organic carbon source that is not present in media used for phototrophic microalgae proliferation (only resulting from cell lysis of microalgae). Heterotrophic metabolism decreases oxygen levels while increasing carbon dioxide levels, resulting in a growth advantage for the microalgae. Nevertheless, sterilization or hygienization is important. For most applications it is of utmost importance to retain mono-algal cultures (cultures with only one phototrophic microorganism present). Potential algal contaminants are efficiently depleted by hygienization procedures. These procedures also address the possible presence of aquatic fungi and viruses.

### 6.3 Large-Scale Cultivation Systems

During the past 50 years different systems for the mass cultivation of microalgae evolved from applied algological research resulting in (pilot) plants capable of producing quantities from kilograms to tons per month. In this sense, the engineering layout is dictated by the desired product. Plants for the production of food and food additives must be cheap and easy to maintain whereas plants aiming for high value products allow more sophisticated equipment and therewith associated higher capital and operational expenditures [20]. Requirements for all cultivation system are alike. These are maximization of surface area to volume ratio

(photosynthetically active area) using transparent and durable (especially UV-resistant) construction materials, agitation/mass transfer, sufficient carbon dioxide supply (carbon source) and excess oxygen discharge (photorespiration), minimization of water losses (evaporation), process control (pH and temperature) [21–23], and their costs/scalability for industrial application. Subsequent sections will describe, compare, and discuss representatively available cultivation systems. Open systems for microalgae proliferation are included briefly since their industrial impact still is a benchmark for other cultivation systems.

### 6.3.1

#### Open Ponds – Technology Overview

Introduced in the 1960s [21], open ponds are the systems used longest for microalgae large-scale cultures for feed and food production [24]. Besides natural, unmixed ponds this type of cultivation system can be differentiated into three major concepts: (i) circular ponds with agitation provided by a mechanically driven, rotating arm; (ii) stuck oval ponds (raceway ponds) that can be constructed either individually or modularly (meander-formed) by joining several units with agitation provided by a mechanically driven paddle wheel; and (iii) sloping ponds (often meander formed) with agitation supplied by pumping and gravity flow [20,25,26]. The power required for mixing of the culture increases as the cube of velocity applied [27] and therefore the lowest velocity providing sufficient mixing to keep the algae in suspension is selected and flow velocities as low as  $1\text{--}5\text{ cm s}^{-1}$  were reported [28]. Baffles in the channel guide the flow around the bands to minimize space. Newer pond constructions are endowed with more sophisticated equipment such as sparging (areal or punctual as risers) of carbon dioxide, carbon dioxide enriched air, or flue gas rich in carbon dioxide in order to increase volumetric productivity [29]. Other ponds are covered with Plexiglass or foil to prolong the growing season, which is dependent on the site of production, and to increase carbon dioxide transfer rates [25]. Attempts have been undertaken to improve light utilization at greater depth by incorporation of transparent rectangular chambers [21]. Although introduced in pioneering times and with relatively few technical innovations over time, open ponds still represent over 80% of all commercial microalgae production projects and produce large amounts of biomass at relatively low costs [25,30,31]. This is because their initial investment costs, even for large-scale plants, are rather low. They are generally constructed from concrete or dug into the earth and lined with plastic liners with a depth of 1–50 cm; to allow light penetration depths of 10–20 cm are typical [32,33]. Operational expenditures are comparatively low due to the simplicity of the technology, which means that little maintenance and repair is necessary. Nevertheless open ponds have several disadvantages. The biggest drawback is the low productivity compared to photobioreactors. This is the result of several factors: (i) Prolonged cycles of unequal light distribution ranging from photoinhibition at the surface to photolimitation at the ground, especially in dense cultures, lead to metabolic stress situations for the microalgae [25,30,31]. (ii) Open ponds lead to a high water loss due to evaporation,

**Table 6.1** General assets and drawback of microalgae mass cultivation systems.

Parameter	Open systems	Closed systems
Maturity	Established, multiple production plants	In development, mainly at pilot stage, only three production plants
Energy consumption (related to volume)	Low	Medium–high
Energy consumption (related to biomass produced)	High	Low
Process control (e.g., pH, temperature)	Low	High
Process stability	Low–medium	High
Growth rate	Low	High
Maximum biomass concentration	Low	High
Energy consumption for harvesting	High	Low
Water loss (evaporation)	High	Low
Capital expenditures	Low	Medium–high
Operational expenditures	Low	Medium–high
Scale-up	Easy	Difficult for most systems
Cleaning	Easy (mechanically)	Elaborate (mechanically and chemically)
Shear stress	Low	Low–medium

which increases the ionic strength of the media and thereby potentially stresses microalgal cultures [12,23,25]. The need to replenish water especially constitutes a problem in regions suffering from water scarcity. (iii) Temperature cannot be efficiently controlled, resulting in potentially sub-optimal regimes for a given algal species, [21,30,31], which, besides the poor mass transfer rate [25], (iv) limits carbon dioxide solubility and leads to its outgassing [21,25,30]. Overall, reduced productivity results in (v) an increased amount of cultivation area needed to achieve the intended amount of biomass when compared to photobioreactors [25]. (vi) A further limitation is the fact that only competitive microalgal species with distinct requirements can be cultivated and maintained over a long period under highly selective conditions. A few examples are *Chlorella* species withstanding high nutrient levels, *Dunaliella* species are able to proliferate in highly saline medium, and *Arthrospira* species (formerly *Spirulina*) are able to proliferate in highly alkaline medium [21,25,31]. Table 6.1 summarizes the assets and drawbacks of microalgae mass cultivation systems.

### 6.3.2

#### Open Ponds – Production Sites

During past decades, multiple microalgae production sites based on open pond technology were constructed. Table 6.2 summarizes the most prominent ones,

**Table 6.2** Microalgae production sites using open ponds (major players, excerpt).

Company	Country of cultivation	Species
Cyanotech, Inc.	USA	<i>Haematococcus pluvialis</i> , <i>Arthrospira platensis</i>
Earthrise Nutritionals, Inc.	USA	<i>Arthrospira platensis</i>
Parabel, Inc.	USA	Multiple
BASF AG/Cognis Nutrition and Health	Australia	<i>Dunaliella salina</i>
Parry Nutraceuticals	India	<i>Haematococcus pluvialis</i> , <i>Arthrospira platensis</i> , <i>Dunaliella salina</i>
Taiwan Chlorella Manufacturing Co. Ltd.	Taiwan	<i>Chlorella vulgaris</i>

giving the respective company, country of production, and species produced. Figure 6.1 shows an aerial image of the *Arthrospira platensis* farm of the Cyanotech Corporation based on Hawaii, USA.

### 6.3.3

#### Open Ponds – Performance

As is true for most cultivation systems, published data of open ponds are scattered over a broad range, with productivities up to  $0.3 \text{ g}_{\text{dry weight}} \text{ l}^{-1} \text{ d}^{-1}$  [34,35]. These were reached using integrated systems [36] or state the maximum productivity reached [34,35]. Average productivities are in the range of 0.05 to a maximum of  $0.2 \text{ g}_{\text{dry weight}} \text{ l}^{-1} \text{ d}^{-1}$  [37]. Final biomass concentrations are spread between 0.1 and



**Figure 6.1** Aerial photograph of the *Arthrospira platensis* farm of the Cyanotech Corporation based on Hawaii, USA. With kind permission of Jeane Vinson, Manager Cyanotech Sales.

1.6 g l<sup>-1</sup> [35], with the latter achieved using integrated systems [36]. Average standing biomass concentrations are usually in the range 0.1–0.5 g l<sup>-1</sup>. The data spread is due to the fact that published data resulted from cultivations using different experimental set-ups at different sites and comparison and normalization in terms of biomass production per relative light intensity, that is, light per cell, is hard to achieve for outdoor cultivations, especially when relevant data are not accessible. In addition, cultivations often aim for the production of distinct products that are mainly secondary metabolites. These are not only nonessential for culture proliferation but on the contrary slow down the growth rate by occupying space required for metabolism and by using up resources like biocatalysts and energy for their production rather than for cell growth [38].

Although at first glance the above stated values seem impressive the higher results arose from academic experiments with often only small-scale and well maintained open pond systems, many of them also well equipped with carbon dioxide sparging and other growth promoting devices not necessarily implementable for commercial large scale ponds of several hectares. Consequently, here productivity rarely exceeds 7 g<sub>dry weight</sub> m<sup>-2</sup> d<sup>-1</sup> [39,40] and, therefore, given a minimum water level of about 15 cm [37], 0.05 g<sub>dry weight</sub> l<sup>-1</sup> d<sup>-1</sup>. If a water level of about 25 cm is assumed, volumetric productivity decreases to as low as 0.03 g<sub>dry weight</sub> l<sup>-1</sup> d<sup>-1</sup>.

Only a few commercial plants are operating, therefore limiting the availability of data from large-scale microalgae cultures. The application of models to extrapolate algal yields obtained in small experimental ponds during a short period of time to large plants of one or more hectares and year-round production has never been examined and therefore reasonable values cannot be given for comparisons of cultivation systems [20].

#### 6.3.4

##### Open Ponds – Energy Consumption

Analogous to performance data, energy consumption data of open pond cultivation systems vary greatly. Here, too, comparison and normalization is difficult since the amount of available data is limited. Many publications did not evaluate the energy consumption by means of data collection but refer to other literature sources. In addition, various set-ups were used and crucial data are often missing for calculations. In general, energy consumption is rather a function of flow velocity than of pond depth, which in turn determines the standing biomass concentration, which has an impact on the energy demand for downstream processing. It is therefore legitimate to correlate the energy input to the occupied surface area (Table 6.3).

Again, at first glance the stated values seem impressive but, in fact, either result from more or less academic experiments or were extrapolated from such. For commercial ponds an energy consumption of 6 W m<sup>-2</sup> is generally considered as reasonable [41]. With the above-mentioned productivity and energy data it is now

**Table 6.3** Energy consumption of selected open pond systems at different velocities/depths.

Source	Energy consumption ( $\text{W m}^{-2}$ )	Velocity ( $\text{cm s}^{-1}$ )	Depth (cm)	Size ( $\text{m}^2$ )
[42]	4.6	15	30	80
	37	30	30	
[33]	0.07	25	20	100
	0.01	15	30	
	0.06	25	30	
	0.10	30	30	
[41]	6	30	30	100
[43]	0.7	25	20	1000
[44]	0.2	25	30	40 000

**Table 6.4** Energy consumption of commercial open pond systems.

Productivity ( $\text{g}_{\text{dryweight}} \text{m}^{-2} \text{d}^{-1}$ )	Energy consumption ( $\text{W m}^{-2}$ )	Energy consumption ( $\text{MJ kg}_{\text{dryweight}}^{-1}$ )
7	1	12.34
	2	24.69
	3	37.03
	4	49.37
	5	61.71
	6	74.06

possible to calculate the energy needed for the production of 1 kg microalgal dry matter (Table 6.4).

## 6.4 Photobioreactors – Technology Overview

Photobioreactors (PBRs) refer to a closed container, which strongly limits gas exchange with the environment. The containers are translucent and allow the supply of energy to the microalgae cultures by artificial and/or natural lighting [8]. Development started in the 1940s and vertical tubular reactors were the first to be described in the literature [45]. The design has been refined since then and further developed – particularly in the 1980s, when microalgae biotechnology research in closed systems intensified [24]. The intention was to overcome above-mentioned limitations of open pond systems. Photobioreactors are generally used to grow monoalgal cultures reproducibly at high productivity with an increased level of process control [35]. Photobioreactors allow the production of microalgal suspension with standing biomass concentrations of  $2\text{--}20 \text{ g l}^{-1}$ , depending on the

alga used and site of cultivation, and short harvesting intervals of 2–4 weeks (when not run in continuous mode). Both high productivity and high biomass concentration are due to the high surface to volume ratio and more straightforward process control in comparison to open pond systems, allowing the adjustment of favorable growth conditions (e.g., by heating/cooling). A PBR layout allows the production of various microalgal strains. Design requirements are equal light distribution with a minimum of non-illuminated surface area, fast convective mass transfer of chemicals, CO<sub>2</sub>, and O<sub>2</sub>, and prevention of biofouling. Biofouling on the surface area may result in reduced light permeability and undesired formation of secondary flora such as bacteria and fungi. Photobioreactors have been setup for indoor and outdoor cultivations. Indoor cultivations are performed using artificial lighting for research purposes or the production of high-value compounds. Outdoor cultivations are usually performed using natural lighting but can be combined with artificial lighting during times of insufficient light intensity. So far, sodium-vapor lamps are the light source of choice. LED lighting using specific spectra or even specific wavelengths are under continuous development and will, sooner or later, replace the energy intensive sodium-vapor lamps. The available designs of photobioreactors vary considerably. Depending on the scientific challenge, the climate, and organism used, a multitude of PBRs have been designed. For example, some PBRs work with transparent bags, tubes, or plates, which may be suspended or immersed in water. Others expose the microalgae suspension in a very thin layer (<1 cm) to the sun. Some are shaped like pyramids or Christmas trees. All of the above exist in multiple variations and a description of them all would go far beyond the scope of this chapter. Consequently, here the focus will be on the most prominent types for industrial cultivations, namely, tubular and flat-panel (or flat-plate) photobioreactors.

### 6.4.1 Photobioreactors – Tubular

Tubular photobioreactors have been operated in outdoor cultivations for many years. They are ordinarily made of glass or plastics. Culture streaming is typically generated by centrifugal pumps or, preferably, by airflow minimizing the energy input. As mentioned above, there is an endless variety of tubular arrays. Originated by Tamiya [46] these can be placed horizontally, vertically, inclined, coiled conically, coiled straight, placed as serpentine or manifold (parallel tubes connected with two manifolds at the ends for distribution and collection of the culture suspension), and so on. Tubular photobioreactors are well suited for microalgae mass production due to a high surface to volume ratio. A high ambient temperature can be controlled to a certain extent by evaporation of sprayed water. Spraying intervals can be controlled by temperature probes. Draining water is collected in reservoirs and can be re-used. The tube diameter is usually between 3 and 5 cm to ensure sufficient light availability. The biggest disadvantage of the tubular system is its poor mass transfer. For scale-up, meaning extension of the tubular pipeline system, the formation of chemical and gaseous gradients cannot be avoided. CO<sub>2</sub>

and other nutrients are supplied either through a collecting tank (gas exchanger) or at one manifold in front of the photosynthesis entity. Although carbon dioxide supply is typically guaranteed by addition to the air stream using a pH-stat system, sections of different pH and hence dissolved inorganic carbon species are formed depending on the position of the triggering pH probe. Microalgae growth in the photosynthesis entity results in the consumption of dissolved  $\text{CO}_2$  and  $\text{HCO}_3^-$  leading to an increase in basicity (decrease in available inorganic carbon and increase of  $\text{O}_2$  concentration). The length of the photosynthesis entity is, thereby, limited and overall productivity reduced. Scale-up can also be performed by increasing the diameter of the employed tubes. Nevertheless, this adds to the problem of photolimitation in the inner zones of the tubes whilst not addressing the common effect of photoinhibition at the outer culture layer [47]. In addition, the surface to volume ratio is decreased, leading to a reduction in volumetric productivity. Proper light distribution must be achieved by the incorporation of flow-leading devices (static mixers) into the tubes, guaranteeing continuous movement of the organisms to the illuminated tube surface and thereof away [48,49]. Notably, biofilm formation on the surface of tubes is common for this type of PBR, leading to downtimes for cleaning purposes [50].

#### 6.4.1.1 Tubular Photobioreactors – Production Sites

For reasons of high capital and operational expenditures there are, to the knowledge of the authors, only three companies commercially producing microalgae in tubular photobioreactors. One of them, Roquette Klötze GmbH & Co. KG, situated in Klötze, Germany, is producing *Chlorella* as food additive and health supplement. The plant is the largest ever realized (500 km tubing on an area of 1.2 ha) and started its operational business in 2000. The second company, Algatechnologies Ltd., situated in Eilat, Israel, is producing *Haematococcus* as source of the antioxidant astaxanthin (300 km tubing on an area of 4 ha). The company was founded in 1998. The third company, Salata GmbH, situated in Ritschenhausen, Germany, is producing *Nannochloropsis oculata* as base material for the cosmetics industry (25 km tubing). Figure 6.2 shows an example of a tubular photobioreactor system with a cultivation volume of 4 m<sup>3</sup>.

#### 6.4.1.2 Tubular Photobioreactors – Performance

As with open pond systems, published outdoor tubular PBR growth data are scattered over a broad range, with productivities up to 2.7 g<sub>dry weight</sub> l<sup>-1</sup> d<sup>-1</sup> (*Arthrospira platensis*) [21,35,50]. The high productivity was reached using a tubular undulating row photobioreactor (inner diameter 1 cm) with increased illuminated area (surface to volume ratio of 400 m<sup>-1</sup>) and a volume of 11 l. Experiments were carried out in July near Florence, Italy. The orientation of the reactor was north-south and a semi-continuous cultivation mode with a dilution rate of 0.3 d<sup>-1</sup> was applied. Values typically found in the literature lie in the range 0.2–0.8 g<sub>dry weight</sub> l<sup>-1</sup> d<sup>-1</sup>, especially when considering a set-up simulating a full-scale plant, which has a larger volume and prolonged cultivation duration. Average standing biomass concentrations usually lie in the range 1–8 g l<sup>-1</sup>.



**Figure 6.2** Photograph of the Photobioreactor PBR 4000 G, located in Nuthetal, Germany. With kind permission of IGV GmbH/IGV Biotech.

#### 6.4.1.3 Tubular Photobioreactors – Energy Consumption

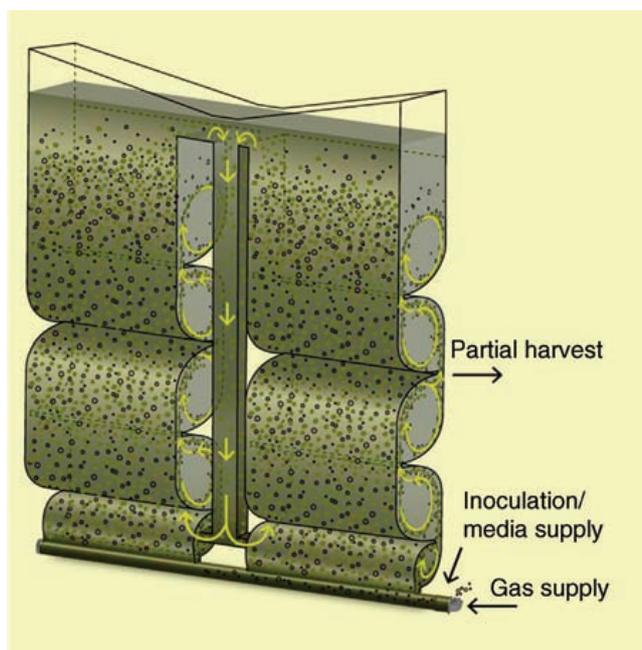
As with open ponds, the velocity in tubular photobioreactors is crucial for light distribution and convective mass transfer. The emergence of turbulent flow is characterized by the Reynolds number ( $Re$ ). Therefore, it is necessary to adjust the velocity to such that the Reynolds number is above 2300. Otherwise, the stream is laminar. An in-depth characterization of tubular photobioreactors was performed by Molina *et al.* [51]. A culture of the diatom *Phaeodactylum tricorutum* collapsed at a small velocity of  $0.17 \text{ m s}^{-1}$  ( $Re = 10000$ ). At this Reynolds number, the stream is in transition from laminar to turbulent. When increasing the velocity between  $0.35$  and  $0.5 \text{ m s}^{-1}$  stable growth could be identified. However, increased turbulence at higher velocities did not result in increased productivity. Similar observations were made by Carozzi [52] using *Arthrospira platensis*. Here an increase in productivity at the transition from laminar to turbulent stream could be observed. The maximum velocity in tubular photobioreactors is limited by the size of so-called microeddies, small vortices forming in turbulent streams. Their size can be calculated by the model of Kolmogoroff [53]. When the size of the microeddies is similar to that of cultivated organisms cytopathic effects may appear. The size of microeddies depends directly on the applied velocity and therefore with the energy input. Given *Phaeodactylum* with a size of around  $35 \mu\text{m}$ , the velocity should not exceed  $1.0 \text{ m s}^{-1}$ , resulting in a power consumption of around  $170 \text{ W m}^{-3}$ , depending on the used tube diameter (the larger the diameter, the higher the energy input for a given velocity). This relatively low power input is much smaller than the  $2000\text{--}6000 \text{ W m}^{-3}$  postulated by other authors [54]. On closer inspection this may be a result of a possibly false calculation of Hall *et al.* [55] performed on a small airlift-driven helical tubular bioreactor in which the volume-specific power input was not considered. When included, calculated energy

inputs vary between 70 and 200 W m<sup>-3</sup> depending on the aeration rate of the airlift drive. Pumps are necessary to further increase the velocity (e.g., above 0.5 m s<sup>-1</sup>) to generate the required pressure (otherwise the airlift drive must be designed with a height of at least 4 m). This principle was developed to market maturity by the working group of Professor Pulz (Lausitz University of Applied Sciences, Senftenberg, Germany; formerly, IGV GmbH, Nuthetal, Germany) and deployed in commercial plants. The installed power input (ca. 900 W m<sup>-3</sup>) allows velocities of up to 2.0 m s<sup>-1</sup>. With a power input this high, plants are usually run between 0.5 and 1.0 m s<sup>-1</sup> (power input between 100 and 500 W m<sup>-3</sup>). Given comparable high volumetric productivities at a velocity of 0.5 m s<sup>-1</sup> the power input is in the same range as that of flat-panel airlift photobioreactors. Nevertheless, direct comparison based on published data is impossible since reliable data from long-term cultivation on at least pilot scale are missing and statements on process stability, cleaning cycles, and so on are unavailable.

#### 6.4.2

##### Photobioreactors – Flat-Plate

Flat-plate photobioreactors have a long history similar to tubular photobioreactors. The first attempts of microalgae mass cultivations using this technology were made in 1953 by Milner [56] cultivating *Chlorella*. Since then, multiple designs have been developed, different alignments (cardinal direction) studied, and various propulsions evaluated. The positioning can be horizontal, vertical, or randomly inclined, similar to tubular systems. Systems equipped with motorized autotracking (light) technology can follow the course of the sun at the expense of energy input in order to increase daily light yield. Along plain cuboid panels other PBRs are constructed in an alveolar manner and, hence, are internally portioned, forming narrow channels called *alveoli* to direct culture flow. These are either open to only one side (e.g., alternating at the top and bottom) of the photobioreactor, resulting in a culture flow similar to that of tubular systems (with the problem of forming chemical and gaseous gradients), or open to both sides, allowing free flow of the culture suspension through the whole photobioreactor. Other FPAs (flat-plate airlift) photobioreactors lack this segmentation but are equipped with static mixers guaranteeing a continuous movement of the organisms through the different photic zones to allow light utilization at maximum efficiency (Figure 6.3). Furthermore, this unique reactor contains downcomer regions for vertical culture flow (loop principle). Coupling both horizontal and vertical culture flow results in an ideal intermixing of the culture compared to the microalgae's pace of growth [57,58]. The vessels vary in their layer thicknesses, ranging from a few millimeters (thin layer) to a couple of centimeters; a thickness of greater than 5 cm is rarely exceeded to ensure proper light penetration into the system. Initial flat-plate photobioreactors were run using centrifugal pumps. Recently, airlift drives have been preferred which allow aeration through integrated perforated tubes/membranes. Airlift drives are characterized by lower power consumption and constitute the gentlest way of mixing microalgae suspension with an absolute



**Figure 6.3** Schematic depiction of Subitec's Flat-Panel Airlift photobioreactor and the directed culture flow (green arrows) induced by static mixers.

minimum of shear stress. The continuous supply of compressed air enriched with carbon dioxide leads to a continuous supply of a carbon source at similar rates of oxygen discharge, eliminating the formation of inhibiting gaseous gradients. Aeration furthermore decreases the degree of biofilm formation on the plate's surfaces, which presently still constitutes a problem to be addressed further by PBR engineering. Flat-plate photobioreactors are well suited for microalgae mass production, featuring a high surface to volume ratio. Temperature may be controlled by the evaporation enthalpy of sprayed water which can be collected for reuse. Spraying intervals can be controlled by temperature probes. The scale-up of most flat-plate photobioreactors is more straightforward compared to tubular systems since the basic character, that is, the geometry (compartments, static mixers) and therefore the fluid dynamics of convective mass transfer within the reactor, is not altered; simply, more reactors are installed [59]. The individual reactors are linked through the plant's infrastructure (e.g., equipment for instrumentation, control, and automation, tubing for media, aeration, and harvest) facilitating the use of as few electrical loads (e.g., probes, pumps, compressors) as possible. Figure 6.4 shows an outdoor (greenhouse) pilot plant of the company Subitec GmbH (Stuttgart, Germany), based on 180l flat-panel airlift (FPAs) photobioreactors. The plant consists of three modules (rows of interconnected reactors) each containing four FPAs. The total working volume of the plant is around  $2.2 \text{ m}^3$ , depending on the aeration rate. Flue gas from a coal fired combined heat and power (CHP) unit is used as carbon source. The plant is located in Senftenberg, Germany, and run by



**Figure 6.4** Photograph of the microalgae production site (pilot) of Subitec GmbH, based in Senftenberg, Germany.

the regional energy supplier GMB GmbH, a subsidiary of the market listed Vattenfall Europe AG.

#### 6.4.2.1 Flat-Plate Photobioreactors – Production Sites

Presently, to the knowledge of the authors and for no obvious reason, no commercially operated production plant using flat-plate photobioreactor systems exists.

#### 6.4.2.2 Flat-Plate Photobioreactors – Performance

As for open pond and tubular photobioreactor systems, published outdoor flat-plate PBR growth data are spread over a broad range, with productivities up to  $4.3 \text{ g}_{\text{dry weight}} \text{ l}^{-1} \text{ d}^{-1}$  [60]. High productivity was reached using an inclined flat modular photobioreactor with a light path of 1.3 cm. The reactor with a volume of 6 l was placed with an inclination of  $30^\circ$ . Experiments were carried out in October. The location at which experiments were conducted is not explicitly named but with respect to residency of the authors the desert Negev, Israel, is most probable. Typical values found in the literature are in the range  $0.4\text{--}1.2 \text{ g}_{\text{dry weight}} \text{ l}^{-1} \text{ d}^{-1}$ . Average standing biomass concentrations usually are in the range  $1\text{--}17 \text{ g l}^{-1}$  [30,61–63]. Hence, both productivity and biomass concentration are higher compared with tubular systems, according to the literature.

#### 6.4.2.3 Flat-Plate Photobioreactors – Energy Consumption

The energy consumption of flat-plate photobioreactors driven by centrifugal pumps is similar to those of tubular systems using the same pumps for stream generation, being between  $100$  and  $500 \text{ W m}^{-3}$  depending on the plates setup (vertical or horizontal), light path, and height (hydrostatic pressure). For airlift driven flat-plate photobioreactors energy consumptions of as low as  $53 \text{ W m}^{-3}$  are

**Table 6.5** Energy consumption of the FPA system. Calculations based on 5913 MJ m<sup>-2</sup> a<sup>-1</sup> vertically (90°) received solar energy (Arabia), 4.5% solar conversion efficiency and 180IFPA-reactors with a row distance of 2.5 m. Calculations were performed for 150 W m<sup>-3</sup> (5.25 W m<sup>-2</sup>) and 60 W m<sup>-3</sup> (2.1 W m<sup>-2</sup>).

Productivity (g <sub>dryweight</sub> m <sup>-2</sup> d <sup>-1</sup> )	Energy consumption (MJ kg <sub>dryweight</sub> <sup>-1</sup> )	
	5.25 W m <sup>-2</sup>	2.1 W m <sup>-2</sup>
36.83	12.31	4.93
33.55	13.52	5.41
30.81	14.72	5.89
28.48	15.93	6.37
26.48	17.13	6.85
24.74	18.33	7.33
23.22	19.54	7.81

postulated [64]. Given the height of the photobioreactor (1.5 m) this energy input appears very low and reveals the physical limit. Overcoming the water column seems to be the only pressure drop considered in this scenario. Infrastructure such as piping systems, gas dispersion, sterilizing grade filters, and so forth, necessary for commercial scale production, were seemingly neglected. Subitec GmbH (Stuttgart, Germany) recorded an energy consumption of about 150 W m<sup>-3</sup> at a pilot plant located in Reutlingen, Germany with a cultivation volume of 4.3 m<sup>3</sup>. This value is well within the range of tubular systems and is under ongoing improvement, optimally resulting in around 60 W m<sup>-3</sup>.

With the above-mentioned productivity and energy data it is now possible to calculate the energy needed for the production of 1 kg microalgal dry matter (Table 6.5).

## 6.5

### Conclusion/Outlook

Microalgae have tremendous potential as renewable source for a wide variety of products. Given that today's major terrestrial crop plants have been screened for, cultivated, and bred for ten thousands years to maximize general biomass and/or product productivity it would be unfair to judge microalgal biotechnology by its current state of the art. Although this section of biotechnology is, by outsiders and due to media portrayal, regarded as a special and more straightforward part of agriculture "there are many wealthy farmers but as far as we know nobody who became a rich man by the cultivation of algae (or writing about it)" [20]. Several barriers await future development and it will be a challenging but certainly not impossible task to break down these obstacles. Whereas the present open pond technology is generally considered to be exhausted, a multitude of innovation possibilities are present for closed systems, both on the biological and the engineering side. Hereby, the main task is to reduce the costs for biomass and/or

substance production. From a biological point of view it can be stated that the high diversity of microalgal species is not exhausted at all. Most research on industrial scale cultivation focuses on known, well described species and attempts have been undertaken to produce these on various sites worldwide. Microalgae have evolved for the past millions of years and so, unsurprisingly, many species do not behave as desired during mass cultivation under factitious conditions in non-natural habitats. Onsite strains (or leastwise strains evolved under similar to onsite climatic conditions) should be isolated and screened for their potential to produce worthwhile and/or commercially relevant compounds. This is the major step to further increasing the output. Additionally, more sophisticated attempts can be undertaken. Tools for the genetic engineering of microalgae are constantly being refined and are even readily available for some species. They can be applied to increase the growth rate (maximize photosynthetic efficiency, adaptation to environmental fluctuations and stress), increase product formation rates, introduce secretion pathways for substance production (simplify product recovery), and/or introduction of mechanisms of (triggered) floc formation (simplify harvesting) [65]. Furthermore, there is room for improvement by focus on the biological system within the reactors. High-definition resolution online monitoring of relevant growth parameters, for example, biomass concentration (turbidity), dissolved inorganic carbon species, dissolved oxygen, and nutrients would be of benefit. These would allow evaluation of the physical-biological responses of the system towards diurnal and seasonal changes in, for example, light intensity and temperature and the assessment by an empirical approach. The resulting set of data can be used to adjust process parameters, resulting in highest possible yields. From an engineering point of view future attempts have to aim to reduce energy consumption and capital/operational expenditures. Energy consumption could be reduced by pulsed mixing (reduced utilization of main energy driver) of the culture suspension. In this respect, it is of utmost importance that the fluid dynamics (convective mass transfer) are not significantly disturbed, although mixing might be decreased drastically, especially during nighttime. Furthermore, in airlift systems, the decoupling of aeration for mixing and CO<sub>2</sub> supply may result in an improved mass transfer of CO<sub>2</sub> and less aeration needed in total. Focus of research and development on manufacturing materials of photobioreactors would promise cost reduction for large cultivation plants. Cultivation sites should utilize as many resources as possible (wastewater, exhaust gases from industrial processes, seawater). Last but not least, production plants need to be individually designed for specific product acquisition. In this regard, the integration of a closed system for the production of biomass and open systems for the triggered product formation seems reasonable, allowing exploitation of the advantages of both systems.

## References

- 1 Posten, C. (2012) Introduction – Discovering Microalgae as Source for Sustainable Biomass, in *Microalgal Biotechnology: Potential and Production* (eds

- C. Posten and C. Walter), De Gruyter, Berlin/Boston.
- 2 Anton, C. and Steinicke, H. (eds) (2012) *Bioenergy – Chances and Limits*, German National Academy of Sciences, Leopoldina, Halle.
  - 3 Lee, R.E. (ed.) (2008) *Phycology*, Cambridge University Press, New York.
  - 4 Pulz, O. and Gross, W. (2004) Valuable products from biotechnology of microalgae. *Appl. Microbiol. Biotechnol.*, **65** (6), 635–648.
  - 5 Keeling, P.J., Burger, G., Durnford, D.G., Lang, B.F., *et al.* (2005) The tree of eukaryotes. *Trends Ecol. Evol.*, **20** (12), 670–676.
  - 6 Norton, T.A., Melkonian, M., and Andersen, R.A. (1996) Algal biodiversity. *Phycologia*, **35** (4), 308–326.
  - 7 Andersen, R.A. (1992) Diversity of eukaryotic algae. *Biodivers Conserv.*, **1** (4), 267–292.
  - 8 Dalay, M.C. (2007) Introduction to The Algal Worlds, in *Algae and Cyanobacteria in Extreme Environments* (ed. J. Seckbach), Springer, Dordrecht.
  - 9 Friedl, T., Rybalka, N., and Kryvenda, A. (2012) Phylogeny and Systematics of Microalgae: An Overview, in *Microalgal Biotechnology: Potential and Production* (eds C. Posten and C. Walter), De Gruyter, Berlin/Boston.
  - 10 Muenkel, R., Schmid-Staiger, U., Werner, A., and Hirth, T. (2013) Optimization of outdoor cultivation in flat panel airlift reactors for lipid production by *Chlorella vulgaris*. *Biotechnol. Bioeng.*, **110** (11), 2882–2893.
  - 11 Olaizola, M. (2003) Commercial development of microalgal biotechnology: from the test tube to the marketplace. *Biomol. Eng.*, **20** (4–6), 459–466.
  - 12 Spolaore, P., Joannis-Cassan, C., Duran, E., and Isambert, A. (2006) Commercial applications of microalgae. *J. Biosci. Bioeng.*, **101** (2), 87–96.
  - 13 Sastre, R.R. (2012) Products from Microalgae, in *Microalgal Biotechnology: Integration and Economy* (eds C. Posten and C. Walter), De Gruyter, Berlin/Boston.
  - 14 Preisig, H.P. and Andersen, R.A. (2005) Historical Review of Algal Culturing Techniques, in *Algal Culturing Techniques* (ed. R.A. Andersen), Elsevier Academic Press, San Diego.
  - 15 Spoehr, H.A. and Milner, H.W. (1947) *Chlorella* as a source of food. *Yearb. Carnegie Inst. Wash.*, **46**, 47100–47103.
  - 16 Witsch, H. (1948) Beobachtungen zur Physiologie des Wachstums von *Chlorella* in Massenkulturen. *Biol. Zentralbl.*, **67**, 95–100.
  - 17 Tamiya, H. (1956) Growing *Chlorella* for food and feed. *Proc. World Symp. Appl. Sol. Energy*, 231–241.
  - 18 Burlew, J.S. (ed.) (1953) *Algal Culture: From Laboratory to Pilot Plant*, Carnegie Institution of Washington, Washington.
  - 19 Chen, F. and Johns, M.R. (1995) A strategy for high cell density culture of heterotrophic microalgae with inhibitory substrates. *J. Appl. Phycol.*, **7** (1), 43–46.
  - 20 Becker, E.W. (ed.) (1994) *Microalgal Biotechnology and Microbiology*, Cambridge University Press, New York.
  - 21 Zhu, J., Rong, J., and Zong, B. (2013) Factors in mass cultivation of microalgae for biodiesel. *Chin. J. Catal.*, **34** (1), 80–100.
  - 22 Mans, R.F., Tahir, A., Hua, L.M., Dayou, J. *et al.* (2012) Design of a pilot scale outdoor photobioreactor for mass cultivation of local microalga. *Int. J. Eng. Phys. Sci.*, 6348–6352.
  - 23 Dasgupta, C.N., Jose Gilbert, J., Lindblad, P., Heidorn, T., *et al.* (2010) Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. *Int. J. Hydrogen Energy*, **35** (19), 10218–10238.
  - 24 Carozzi, P. (2008) Closed photobioreactor assessments to grow, intensively, light dependent microorganisms: a twenty-year Italian outdoor investigation. *Open Biotechnol. J.*, 263–272.
  - 25 Singh, R.N. and Sharma, S. (2012) Development of suitable photobioreactor for algae production – a review. *Renew. Sustain. Energy Rev.*, **16** (4), 2347–2353.
  - 26 Borowitzka, M.A. (2005) Culturing Microalgae in Outdoor Ponds, in *Algal Culturing Techniques* (ed. R.A. Andersen), Elsevier Academic Press, San Diego.
  - 27 Oswald, W.J. (1988) Micro-algae and Waste-water Treatment, in *Micro-algal Biotechnology* (eds M.A. Borowitzka and L.J.

- Borowitzka), Cambridge University Press, Cambridge, pp. 305–328.
- 28 Abeliovich, A. (1986) Algae in Wastewater Oxidation Ponds, in *Handbook of Microalgal Mass Culture* (ed. A. Richmond), CRC Press, Boca Raton, FL.
- 29 Ketheesan, B. and Nirmalakhandan, N. (2012) Feasibility of microalgal cultivation in a pilot-scale airlift-driven raceway reactor. *Bioresour Technol.*, **108**, 196–202.
- 30 Ho, S., Chen, C., Lee, D., and Chang, J. (2011) Perspectives on microalgal CO<sub>2</sub>-emission mitigation systems – a review. *Biotechnol. Adv.*, **29** (2), 189–198.
- 31 Rawat, I., Ranjith Kumar, R., Mutanda, T., and Bux, F. (2013) Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. *Appl. Energy*, **103**, 444–467.
- 32 Boussiba, S., Sandbank, E., Shelef, E., Cohen, Z., and *et al.* (1988) Outdoor cultivation of the marine microalga *Isochrysis galbana* in open reactors. *Aquaculture*, **72**, 247–253.
- 33 Weissman, J.C., Goebel, R.P., and Benemann, J.R. (1988) Photobioreactor design: mixing, carbon utilization, and oxygen accumulation. *Biotechnol. Bioeng.*, **31**, 336–344.
- 34 Borowitzka, M.A. and Moheimani, N.R. (2013) Open Pond Culture Systems, in *Algae for Biofuels and Energy* (eds M.A. Borowitzka and N.R. Moheimani), Springer, Dordrecht.
- 35 Brennan, L. and Owende, P. (2010) Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sustain. Energy Rev.*, **14** (2), 557–577.
- 36 Pushparaj, B., Pelosi, E., Tredici, M.R., Pinzani, E. *et al.* (1997) As integrated culture system for outdoor production of microalgae and cyanobacteria. *J. Appl. Phycol.*, **9** (2), 113–119.
- 37 Tredici, M.R. (2004) Mass Production of Microalgae: Photobioreactors, in *Handbook of Microalgal Culture: Biotechnology and Applied Phycology* (ed. A. Richmond), Blackwell Science Ltd, Oxford.
- 38 Beardall, J. and Raven, J.A. (2013) Limits to Photosynthetic Growth in Dense Cultures: CO<sub>2</sub> Supply and Light, in *Algae for Biofuels and Energy* (eds M.A. Borowitzka and N.R. Moheimani), Springer, Dordrecht.
- 39 Benemann, J.R. (1994) Systems and Economic Analysis of Microalgae Ponds for Conversion of CO<sub>2</sub> to Biomass, 4th quarterly technical progress report, DE-FG22-93PC93204.
- 40 Vonshak, A. and Richmond, A. (1988) Mass production of the blue-green alga *Spirulina*: an overview. *Biomass*, **15** (4), 233–247.
- 41 Oilgae (2010) Comprehensive Oilgae Report Energy from Algae: Products, Market, Processes & Strategies. 706 pp.
- 42 Wagener, K. (1983) Mass cultures of marine algae for energy farming in coastal deserts. *Int. J. Biometeorol.*, **27** (3), 227–233.
- 43 Norsker, N.H., Barbosa, M.J., Vermue, M. H., and Wijffels, R.H. (2010) Microalgal production—a close look at the economics. *Biotechnol. Adv.*, **29**, 24–27
- 44 Lundquist, T.J., Woertz, I.C., Quinn, N.W. T., and Benemann, J.R. (2013) (06 September) A Realistic Technology and Engineering Assessment of Algae Biofuel Production [http://esd.lbl.gov/files/about/staff/nigelquinn/EBI\\_Algae\\_Biofuel\\_Report\\_2010.10.25.1616.pdf](http://esd.lbl.gov/files/about/staff/nigelquinn/EBI_Algae_Biofuel_Report_2010.10.25.1616.pdf).
- 45 Cook, P.M. (1950) Large-scale Culture of *Chlorella*, in *The Culture of Algae* (eds J. Brunel and G.W. Prescott), Charles F. Kettering Foundation, Dayton, OH.
- 46 Tamiya, H., Hase, E., Shibata, K., Mituya, A., Iwamura, T., Nihei, T. and Sasa, T. (1953) Kinetics of growth of *Chlorella*, with special reference to its dependence on quantity of available light and temperature, in *Algal Culture from Laboratory to Pilot Plant* (ed. J.S. Burlew), Carnegie Institution of Washington, Washington, DC, 204–234.
- 47 Vonshak, A. and Torzillo, G. (2004) Environmental Stress Physiology, in *Handbook of Microalgal Culture* (ed. A. Richmond), Blackwell Publishers, Oxford.
- 48 Ugwu, C.U., Ogbonna, J.C., and Tanaka, H. (2003) Design of static mixers for inclined tubular photobioreactors. *J. Appl. Phycol.*, **15** (2/3), 217–223.
- 49 Ugwu, C.U., Ogbonna, J.C., and Tanaka, H. (2005) Light/dark cyclic movement of algal culture (*Synechocystis aquatilis*) in outdoor inclined tubular photobioreactor equipped with static mixers for efficient production of biomass. *Biotechnol. Lett.*, **27** (2), 75–78.

- 50 Ugwu, C.U., Aoyagi, H., and Uchiyama, H. (2008) Photobioreactors for mass cultivation of algae. *Bioresour Technol.*, **99** (10), 4021–4028.
- 51 Molina, E., Fernandez, J., Acién, F.G., and Chisti, Y. (2001) Tubular photobioreactor design for algal cultures. *J. Biotechnol.*, **92** (2), 113–131.
- 52 Carlozzi, P. and Torzillo, G. (1996) Productivity of *Spirulina* in a strongly curved outdoor tubular photobioreactor. *Appl. Microbiol. Biotechnol.*, **45** (1–2), 18–23.
- 53 Kawase, Y. and Moo-Young, M. (1990) Mathematical models for design of bioreactors: applications of. *Chem. Eng. J.*, **43** (1), 19–21.
- 54 Norsker, N.-H., Barbosa, M.J., Vermue, M. H., and Wijffels, R.H. (2012) On energy balance and production costs in tubular and flat panel photobioreactors. *Technikfolgenabschätzung – Theor. Praxis*, **21** (1), 54–62.
- 55 Hall, D.O., Fernandez, F.G., Guerrero, E. C., Rao, K.K., *et al.* (2003) Outdoor helical tubular photobioreactors for microalgal production: modeling of fluid-dynamics and mass transfer and assessment of biomass productivity. *Biotechnol. Bioeng.*, **82** (1), 62–73.
- 56 Milner, H.W. (1953) Rocking Tray, in *Algal Culture from Laboratory to Pilot Plant* (ed. J.S. Burlew), Carnegie Institution of Washington, Washington, DC, 109–113.
- 57 Degen, J., Uebele, A., Retze, A., Schmid-Staiger, U., *et al.* (2001) A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. *J. Biotechnol.*, **92** (2), 89–94.
- 58 Bergmann, P., Ripplinger, P., Beyer, L., and Trösch, W. (2013) Disposable flat panel airlift photobioreactors. *Chem-Ing-Tech*, **85** (1–2), 202–205.
- 59 Kunjapur, A.M. and Eldridge, R.B. (2010) Photobioreactor design for commercial biofuel production from microalgae. *Ind. Eng. Chem. Res.*, **49** (8), 3516–3526.
- 60 Hu, Q., Guterman, H., and Richmond, A. (1996) A flat inclined modular photobioreactor for outdoor mass cultivation of photoautotrophs. *Biotechnol. Bioeng.*, **51** (1), 51–60.
- 61 Carvalho, A.P., Meireles, L.A., and Malcata, F.X. (2006) Microalgal reactors: a review of enclosed system designs and performances. *Biotechnol. Prog.*, **22** (6), 1490–1506.
- 62 Janssen, M., Tramper, J., Mur, L.R., and Wijffels, R.H. (2003) Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects. *Biotechnol. Bioeng.*, **81** (2), 193–210.
- 63 Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., *et al.* (2009) Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.*, **102** (1), 100–112.
- 64 Sierra, E., Acién, F.G., Fernández, J.M., García, J.L. *et al.* (2008) Characterization of a flat plate photobioreactor for the production of microalgae. *Chem. Eng. J.*, **138** (1–3), 136–147.
- 65 Vuttipongchaikij, S. (2012) Genetic manipulation of microalgae for improvement of biodiesel production. *Thai. J. Genet.*, **5** (2), 130–148.

## 4.2 Repeated Fed-batch Cultivation of Thermosynechococcus elongatus BP-1

Published as Original Research Article:

Bergmann, Peter and Trösch, Walter (2016). “Repeated fed-batch cultivation of *Thermosynechococcus elongatus* BP-1 in flat-panel airlift photobioreactors with static mixers for improved light utilization: Influence of nitrate, carbon supply and photobioreactor design”. *Algal Research* 17, pp. 79–86.

Five-Year Impact Factor: 4.621 (Elsevier, 2017)

The publication describes and quantifies the benefits that arise from the utilization of culture-flow directing installations, e.g. static mixers, within PBRs during the cultivation of phototrophic microorganisms. These are especially effective during outdoor cultivation in order to minimize growth inhibitive effects, predominantly photolimitation.

Various preliminary experiments were performed in order to reduce any limitations ( $\text{NO}_3^-$ ,  $\text{CO}_2$ ,  $\text{HCO}_3^-$ ) beyond the sphere of influence of PBR design.

Cultivations utilizing PBRs with and without static mixers are compared and when using the former, an increase in volumetric productivity and final biomass concentration by a factor of 3.4 and 2.0 was detected, respectively. The maximum productivity attained  $2.9 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ d}^{-1}$ , the highest to be reported for the cyanobacterium *T. elongatus* BP1.



## Repeated fed-batch cultivation of *Thermosynechococcus elongatus* BP-1 in flat-panel airlift photobioreactors with static mixers for improved light utilization: Influence of nitrate, carbon supply and photobioreactor design



Peter Bergmann<sup>a,\*</sup>, Walter Trösch<sup>b</sup>

<sup>a</sup> Subitec GmbH, Julius-Hölder-Str. 36, 70597 Stuttgart, Germany

<sup>b</sup> University of Hohenheim, Institute of Food Science and Biotechnology, Garbenstraße 25, 70599 Stuttgart, Germany

### ARTICLE INFO

#### Article history:

Received 13 January 2016

Received in revised form 11 March 2016

Accepted 30 March 2016

Available online 7 May 2016

#### Keywords:

Cyanobacterium

Thermophile

Flat-panel airlift photobioreactor

Static mixers

Light utilization

Productivity

### ABSTRACT

Microalgae mass cultivation is limited by light availability due to effects of absorption and reflection. These effects can only partially be coped with by increasing the photon-flux density. Further inhibitive effects, especially during outdoor cultivations, are described by photoinhibition and photosaturation. Therefore, moving the cells towards the light source and away from it in a distinct mode by convective mass transfer is the method of choice to cope with these inhibitive effects. Hereby, the utilization of culture-flow directing installations, e.g. static mixers, within photobioreactors can be of great benefit. The thermophilic model organism *Thermosynechococcus elongatus* BP-1 was cultivated in flat-panel airlift photobioreactors with and without static mixers in order to show their positive influence on growth kinetics. Optimal nitrate and carbon concentrations were  $2000 \text{ mg L}^{-1} \text{ NO}_3^-$ ,  $0.04 \text{ g L}^{-1} \text{ Na}_2\text{CO}_3$  combined with 6.3% v/v  $\text{CO}_2$ . It was shown that photobioreactors with static mixers increase volumetric productivity by a factor of 3.4 and final biomass concentration by a factor of 2.0. A maximum productivity of  $2.9 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ d}^{-1}$  was demonstrated, to the knowledge of the authors the highest to be reported for the cyanobacterium *T. elongatus* BP-1.

© 2016 Elsevier B.V. All rights reserved.

### 1. Introduction

It is frequently postulated that the cultivation of photosynthetic microorganisms for the production of biofuels and bioproducts is superior compared to the farming of terrestrial crops [1,2]. This is due to several facts. Besides the circumstance that photosynthetic microorganisms show high growth rates leading to high aerial productivity their biological material is free of lignocellulose, which eases downstream processing, and reduces waste streams [2]. Furthermore, biomass composition may be deliberately modified by the application of cell stress, e.g. by nutrient depletion, salinity or light intensity, in order to shift the cell's metabolism towards e.g. lipid or pigment accumulation [3,4]. Cultivated in closed systems, utilization of water and nutrients is much more efficient when compared to conventional agriculture [2]. Furthermore, carbon dioxide can be introduced directly into the system and therefore concentrated resulting in enhanced productivity. Hereby, microalgae and cyanobacteria may even tolerate very high carbon dioxide concentrations as found in e.g. flue gas (typically 15–20%  $\text{CO}_2$ ) and tolerances of up to 100% were reported [5]. Finally yet importantly, photosynthetic microorganisms may be

cultured on non-arable land in arid regions, therefore their cultivation does not compete with the food and feed production. Although advantageous on a first glance, these regions are also hostile for photosynthetic microorganisms since photobioreactors (PBRs) easily heat up to 55 °C during daytime [6,7]. This is not only true for arid and at least temporarily hot regions such as deserts but in general for sunny territories like e.g. Southern Europe. Photobioreactors act as thermal collectors focusing additional heat on them especially in dense cultures with high specific heat capacity. These circumstances require energy and/or water consuming cooling measures since temperatures of only 2–4 °C above optimum are often detrimental if not even lethal for many species [6] reducing overall process economy [1]. An alternative approach is represented by the cultivation of thermophilic species capable of withstanding elevated temperatures. Thermophiles are defined as microorganisms which optimal growth temperature is within the range of 45–80 °C [8]. Another advantage of using thermophilic microorganism is the minimized risk of culture contamination [9,10]. In general, extremophilic microalgae draw increasing interest in biotechnology [11] but their cultivation on a scale >1 L in photobioreactors is hardly reported. The organism used in the present study, *Thermosynechococcus elongatus* BP-1, has an optimal growth temperature of 55 °C. It was isolated from a hot spring [12] and its genetic information is readily available [13]. The aim of this study was

\* Corresponding author.

E-mail address: [p.bergmann@subitec.com](mailto:p.bergmann@subitec.com) (P. Bergmann).

to investigate the growth of *T. elongatus* in a 6 L flat-panel airlift photobioreactor equipped with static mixers (10 L control PBR without mixers) focusing on supply of nitrate, carbonate and carbon dioxide as well as PBR design. The former examinations served to exclude or at least limit effects beyond the sphere of influence of PBR design whereas the latter served to access the impact of reactor geometry on light utilization. For economic reasons during industrial applications, nutrient levels must be kept to a minimum whilst allowing for non-limiting as well as non-inhibiting growth that may be observed nutrient-specifically depending on the species used [14]. Besides the supply of adequate media (macro- and micro-nutrients) and the adjustment and retention of proper temperature (enzyme activity) and pH (enzyme activity, cell permeability [15] and availability of dissolved inorganic carbon (DIC) species) regimes, light is the most crucial factor in microalgal mass production. As solar photon-flux density (PFD) undergoes natural diurnal and seasonal variations between 0 and  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the PAR (photosynthetically active radiation) region, both phenomena, photolimitation (e.g. dense culture and/or dim light) [16] and photoinhibition (e.g. low density cultures and/or excess light) [17,18] can minimize productivity and even irreversibly damage algal cultures [19]. Light is exponentially attenuated within photobioreactors due to self-shading of cells and reflection effects (water column, material, bubbles) [20], again limiting its availability. Light utilization efficiency can be improved by reducing the optical paths of photobioreactors, which in turn does not address photoinhibition. Furthermore, light paths cannot be decreased as desired for economic reasons since material costs per production volume increase drastically. On the other hand, convective mass transfer ("ideal" mixing) can be used to offset limiting and inhibitive effects by directed allocation of biocatalysts within the culture volume. During photosynthesis, photon capture occurs much faster than actual carbon fixation during which electron transport might be saturated and further impinging photons therefore wasted. By applying a continuously directed flow of the culture suspension induced by a firm PBR geometry, e.g. by the incorporation of static mixers, the culture's residence time within different photic reactor zones can be adjusted. With short residence time in photic zones and prolonged residence time in aphotic zones, carbon capture can catch up leading to more efficient photon utilization [21]. Furthermore, horizontally (static mixers in riser compartments) and vertically (downcomer compartments, loop principle) directed mixing within bioreactors represents the key to realizing adequate mixing times avoiding cellular stress resulting from chemical gradients (N, P, C<sub>i</sub>) within the photobioreactor.

## 2. Material and methods

### 2.1. Organism and medium

#### 2.1.1. Organism

*T. elongatus* BP-1 was kindly supplied by the Humboldt Universität zu Berlin, Berlin, Germany. Directly upon receipt, the culture was diluted in sterile 100 mL Erlenmeyer flasks (culture volume of 50 mL) using autoclaved Blue-Green medium (BG 11) (see 2.1.3) at standard concentration so as to achieve an optical density of 0.1 at 750 nm ( $\text{OD}_{750}$ ). Flasks were incubated using an illuminated incubator (Multitron Pro, Infors AG, Bottmingen, Switzerland) set to 55 °C and 120 rpm at a circular motion diameter of 50 mm. Light was continuously supplied at a PFD of  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the PAR region using the integrated daylight (cool white) fluorescent tubes. Culture scale-up by means of increasing the amount of Erlenmeyer flasks was performed weekly until a final volume of 0.5 L was transferred to culture maintenance (see 2.1.2) serving as inoculum for the experiments.

#### 2.1.2. Culture maintenance

Culture maintenance was performed so as to guarantee continuous inoculum availability for the initiation of experiments. Maintenance

was conducted using autoclaved 5 L borosilicate glass bottles equipped with a magnetic agitator (stirrer) and placed on a magnetic power unit with heating function (type D-6010, neoLab Migge Laborbedarf-Vertriebs GmbH, Heidelberg, Germany). Heating capacity of the unit was set so as to achieve a culture temperature of  $55 \pm 2$  °C within the bottle. Agitation was set to 300 rpm. The culture was aerated by air ( $60 \text{ L h}^{-1}$ ) supplemented with technical carbon dioxide ( $\text{CO}_2$ ) (2% v/v) passing a sterilizing grade filter with a pore size of 0.2  $\mu\text{m}$  (Midisart 2000 type 17805, Sartorius AG, Goettingen, Germany). Exhaust air was allowed to leave the system by means of an identical filter. Light was continuously supplied at a PFD of  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the PAR region using daylight (cool white) fluorescent tubes (type Lumilux 18 W/840, Osram GmbH, Munich, Germany). Culture maintenance was performed by weekly addition of 1 L of autoclaved BG 11 (see 2.1.3) at threefold the standard concentration adapting the culture to the medium used during flat-panel airlift (FPA) photobioreactor (PBR) cultivations. Once the culture reached its final volume of 5 L, the culture was diluted to 0.5 L by transferring it into a new flask for discarding and successive medium addition was performed. Both, medium addition and culture dilution were accomplished using sterile couplings with shut-off function (CPC type HFC35, Infiltec GmbH, Speyer am Rhein, Germany) and a peristaltic pump (Pumpdrive 5006, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany).

#### 2.1.3. Medium

Performed cultivations used the inorganic BG 11 prepared in deionized water and modified from Rippka and Herdmann [22] applying the micronutrient solution from Kuhl and Lorenzen [23] in accordance to the medium recipe stated by the Culture Collection of Algae at Goettingen University (SAG). All chemicals used were of analytical grade. At standard concentration, medium contained (in  $\text{g L}^{-1}$ )  $\text{CaCl}_2$ , 0.027;  $\text{C}_6\text{H}_8\text{O}_7$ , 0.006;  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ , 0.075;  $\text{Na}_2\text{CO}_3$ , 0.02;  $\text{Na}_2\text{EDTA}$ , 0.001 and micronutrients (in  $\text{mg L}^{-1}$ )  $(\text{NH}_4)_6\text{MO}_7\text{O}_{24} \cdot 4 \text{H}_2\text{O}$ , 0.0125;  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ , 0.0025;  $\text{H}_3\text{BO}_3$ , 0.0061;  $\text{MnSO}_4 \cdot 7 \text{H}_2\text{O}$ , 0.169;  $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ , 0.287,  $\text{C}_6\text{H}_5\text{FeO}_7 \cdot \text{H}_2\text{O}$ , 11.75 unless specified differently. Medium pH was 6.5. Nitrogen and phosphorus were supplied by  $\text{NaNO}_3$  and phosphate buffer containing  $\text{K}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$  (pH 6.75 at 20 °C room temperature), respectively. Both components were added to the medium by sterile filtration (pore size of 0.2  $\mu\text{m}$ ; Minisart type 16534, Sartorius AG, Goettingen, Germany) past steam autoclavation (121 °C, 20 min) during up-scale and culture maintenance. During PBR cultivation, nitrogen and phosphorus were directly injected into the culture by sterile filtration. Throughout culture up-scale in Erlenmeyer flasks medium was used at standard concentration containing (in  $\text{mg L}^{-1}$ )  $\text{NO}_3$ , 500 and  $\text{PO}_4$ , 200. During culture maintenance and PBR cultivations medium was used at threefold the standard concentration to allow rapid cell proliferation to high densities. Medium contained (in  $\text{mg L}^{-1}$ )  $\text{NO}_3$ , 2000 and  $\text{PO}_4$ , 200 unless specified differently.

## 2.2. Analytics

### 2.2.1. Optical density

$\text{OD}_{750}$  was determined using a tabletop spectrophotometer (type DR 3900, Hach Lange GmbH, Berlin, Germany). Measurements were performed in triplicates against deionized water. Samples were diluted with deionized water so as to result in an  $\text{OD}_{750}$  between 0.1 and 0.4 during measurements.

### 2.2.2. Dry weight

Samples of 10 mL were centrifuged at 4816 g for 5 min (centrifuge type Heraeus Megafuge 40R, rotor 75003607, Thermo Fisher Scientific Inc., Waltham, USA) and the supernatant discarded. Two successive washing steps consisting of pellet resuspension in 10 mL deionized water, repeated centrifugation and supernatant discarding were performed. Finally, the resulting pellet was resuspended in 5 mL deionized water transferred to desiccated and pre-weighed (precision scale type

ABS 220–4, KERN & SOHN GmbH, Balingen, Germany) aluminum bowls and dried until weight constancy at 105 °C. Samples were allowed to cool down in a desiccator for at least 15 min and repeatedly weighed. Known sample volume and difference in weight allowed for the calculation of dry weight (DW) per volume. Correlation between DW and  $OD_{750}$  was performed using the linear fitting function of the software OriginPro (v9.1, OriginLab Corp., Northampton, USA) (see Eq. 1).

$$DW \left[ g L^{-1} \right] = 0.277 * OD_{750} \left( R^2 = 0.999, N = 9 \right) \quad (1)$$

### 2.2.3. Photon-flux density

PFD was determined in the PAR region using a mobile quantum sensor (type LI-190, LI-COR, Inc., Nebraska, USA) and light meter (type: LI-250A, LI-COR, Inc., Nebraska, USA). PFD was measured at 10 points evenly distributed over the FPA-PBR's surface and averaged. PFD was set to  $780 \mu mol m^{-2} s^{-1}$  throughout the experiments.

### 2.2.4. Nutrient concentration

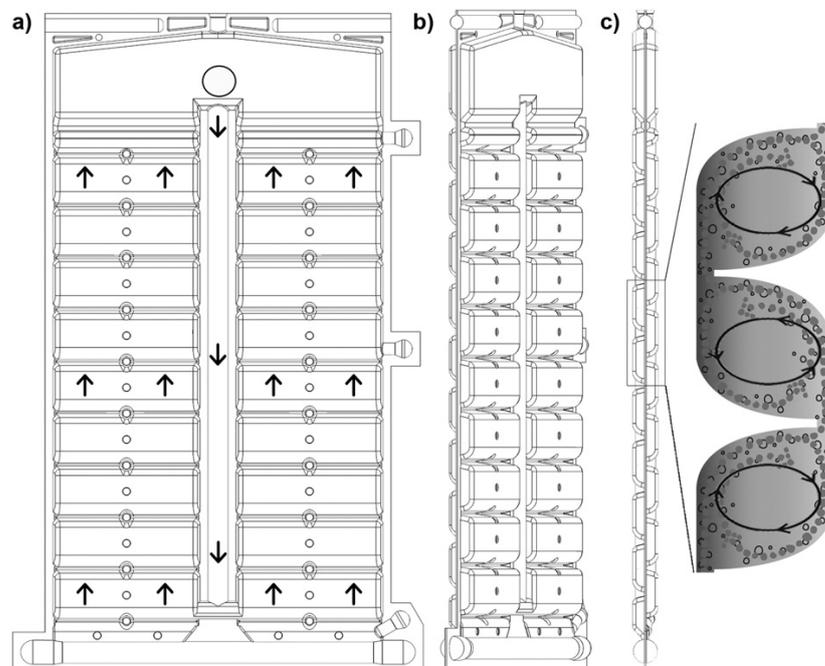
Concentrations of nitrogen and phosphorus were monitored throughout the FPA-PBR experiments on a daily basis and re-fed if necessary. Samples were centrifuged at 4816 g for 5 min (centrifuge type Heraeus Megafuge 40R, rotor 75003607, Thermo Fisher Scientific Inc., Waltham, USA), the supernatant filtered (pore size of 0.2  $\mu m$ ; Minisart type 16534, Sartorius AG, Goettingen, Germany) and adequately diluted in deionized water so as to adjust concentrations to the test's measuring range and water down interfering ions. Tests were performed following the respective manuals. Phosphorus and nitrogen were measured by using quantitative colorimetric cuvette tests (type LCK049 and LCK339, respectively, Hach Lange GmbH, Berlin, Germany). Cuvette tests were analyzed using a tabletop spectrophotometer (type DR 3900, Hach Lange GmbH, Berlin, Germany).

## 2.3. Photobioreactor design and operation

### 2.3.1. Geometry and functionality

The PBR operated during the experiments represents a FPA-PBR with integrated flow directing static mixers (horizontal culture flow) and downcomer domains (vertical culture flow, loop principle) (see Fig. 1). The reactor was initially developed, patented [24,25] and described [26,27,28] by the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB (IGB), Stuttgart, Germany, and is now commercialized by Subitec GmbH, Stuttgart, Germany. The most distinctive feature of the FPA-PBR is the incorporated static mixers allowing for a controlled movement of microalgal cells from the illuminated surface area to the dimly lit interior of the PBR. Hereby, negative effects of photoinhibition and photolimitation should be reduced by intermittent exposure towards the excess light at the PBR's surface versus the insufficiently illuminated interior. Frequency of cycling can be adjusted by the aeration rate applied.

During the experiments, FPA-PBRs made of polymethyl methacrylate (PMMA) were used in order to withstand applied temperatures. Cultivation volume was 6 L. The light path was 3 cm. The control PBR (PBR<sub>control</sub>) was identical to the FPA-PBR except the lack of static mixer (plain flat surface), resulting in a cultivation volume of 10 L. Reactor periphery (silicone tubing for inoculation, medium supply, harvest as well as foam trap with exhaust air filter) was autoclaved (121 °C, 15 min) prior to connection to the FPA-PBR. The FPA-PBR was disinfected prior to inoculation using H<sub>2</sub>O<sub>2</sub> (3% v/v) followed by three washing steps with deionized water. Inoculation was performed applying inoculum generated during culture maintenance (see Section 2.1.2) using sterile couplings with shut-off function (CPC type HFC35, Infiltec GmbH, Speyer am Rhein, Germany) and a peristaltic pump (Pumpdrive 5006, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Medium, administered by a peristaltic pump, passed a sterilizing grade filter with a pore size of 0.2  $\mu m$  (Sartobran P MidiCap type 5235307H8–00, Sartorius AG, Goettingen, Germany). Aeration was applied via a perforated (1 mm perforation diameter) silicone



**Fig. 1.** Flat-panel airlift photobioreactor (FPA-PBR) with flow directing static mixers. a) Front view: arrows indicate culture flow in riser (aerated) and downcomer domains; the circle above the downcomer indicates site of sampling and feeding. b) Angled view: depicting risers, downcomer and static mixers. c) Lateral view: arrows indicate culture flow induced by static mixers.



**Table 1**

Overview of experiments stating experimental motivations and essential parameters; pH states the minimum and maximum pH-value measured at the beginning and end of each experiment, respectively (NM – not measured).

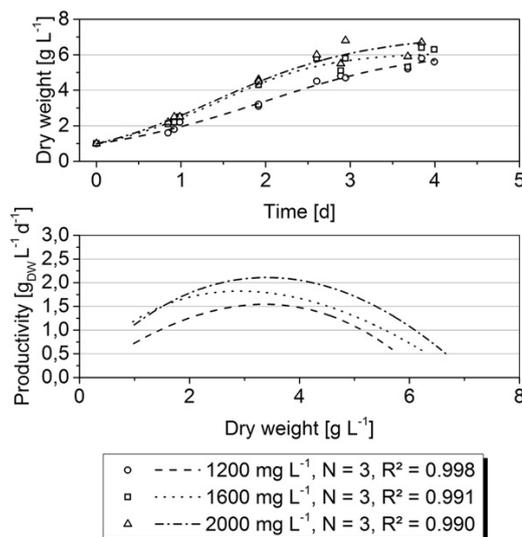
Experiment	Motivation	NO <sub>3</sub> <sup>-</sup> conc. (mg L <sup>-1</sup> )	Na <sub>2</sub> CO <sub>3</sub> conc. (g L <sup>-1</sup> )	CO <sub>2</sub> cont. (% v/v)	pH
Nitrate concentration	Rule out nitrogen limitation	1200	0.02	3	NM
		1600	0.02		NM
		2000	0.02		7.6–8.3
CO <sub>2</sub> content	Rule out dissolved inorganic carbon limitation	2000	0.02	0	7.9–10.1
		2000	0.02	1.6	7.0–7.9
		2000	0.02	6.3	6.9–7.7
		2000	0.02	9.1	7.0–8.0
Carbonate concentration	Rule out dissolved inorganic carbon limitation	2000	0.00	6.3	7.0–8.0
		2000	0.02		7.3–8.1
		2000	0.04		7.1–7.9
		2000	0.04	6.3	7.2–8.1
Photobioreactor design	Evaluate impact of photobioreactor design	2000	0.04	6.3	7.2–8.1

beyond the sphere of influence of photobioreactor design. Table 1 provides an overview of the experiments performed. Experimental motivations and essential parameters are stated.

### 3. Results and discussion

#### 3.1. Nitrate concentration

As represented in Fig. 3, even relatively high initial concentrations of NO<sub>3</sub><sup>-</sup> (1200 and 1600 mg L<sup>-1</sup>) were limiting the growth of *T. elongatus* BP-1 during the cultivation in the FPA-PBR. In fact, during those cultivations nitrate concentration in the medium repeatedly dropped to zero from one day to the other, despite the fact that nitrate was measured and re-fed on a daily basis throughout the experiments. Nitrate limitation was prevented by applying a daily concentration of 2000 mg L<sup>-1</sup>. By doing so, maximum P<sub>vol.</sub> was increased from 1.5 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup> to 2.1 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup> using 1200 mg L<sup>-1</sup> and 2000 mg L<sup>-1</sup> nitrate, respectively. Cultivations performed using 2000 mg L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> allowed for the calculation of the nominal N content of the biomass by correlating consumed N and biomass increase resulting in 10.5% w/w (N = 12). In order to consume the overall nitrate supplied, productivity should have been in the range of 2.6 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup>



**Fig. 3.** Growth kinetics including the Richards growth fit-function (top) and productivity course calculated as the first derivative of the Richards growth fit-function (bottom) of *T. elongatus* at different nitrate concentrations during repeated fed-batch cultures. Light was continuously supplied by HPS lamps at a PFD of 780 μmol m<sup>-2</sup> s<sup>-1</sup>. CO<sub>2</sub> was continuously supplied at a fraction of 3% v/v. N states the number of repeated fed-batches performed.

and 4.3 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup> using 1200 mg L<sup>-1</sup> and 2000 mg L<sup>-1</sup> nitrate, respectively. Differences in practical and theoretical productivity may arise from intracellular N-storages that escaped the methodology applied, artificially increasing the theoretical productivity. Furthermore, some nitrogen may have left the system by means of waterlogged air bubbles considering the temperature and aeration rate applied. In addition, biomass production may be slightly underestimated due to biomass discharge by flotation. Despite P<sub>vol.</sub>, also the final biomass concentration increased with increasing nitrate concentration which is due to the fact, that growth was limited beforehand. Hereby, biomass concentration after four days of growth increased from 5.6 g<sub>DW</sub> to 6.7 g<sub>DW</sub> using 2000 mg L<sup>-1</sup> nitrate despite the fact the self-shading was increased at the same time. Further experiments were therefore performed using a nitrate concentration of 2000 mg L<sup>-1</sup>.

#### 3.2. Phosphate concentration

Phosphate concentration was measured throughout the experimental period and did, at no time, limit microalgal growth. It has always been present in concentrations above 100 mg L<sup>-1</sup>.

#### 3.3. Carbon dioxide content

As represented in Fig. 4, carbon dioxide concentration may limit the growth of *T. elongatus* BP-1 during the cultivation in the FPA-PBR. Of the four concentrations tested, zero growth was observed at a carbon dioxide concentration of 0% v/v. When using CO<sub>2</sub> at a concentration of 1.6% v/v growth was detected but slightly limited compared to higher concentrations tested (e.g. 6.3% v/v). Anyway, limitation by carbon dioxide availability was only low compared to that arising from nitrate concentration (see 3.1). Maximum P<sub>vol.</sub> was increased from 2.4 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup> to 2.7 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup> using 1.6% v/v and 6.3% v/v, respectively. Further increase of carbon dioxide content to 9.1% v/v showed no effect. Furthermore, final biomass concentration after five days of growth was identical for all concentrations tested and resulted in 6.4 g<sub>DW</sub> L<sup>-1</sup>.

On one hand it is to be expected that elevated CO<sub>2</sub> concentrations of a maximum of 9.1% v/v show only limited effects on productivity. This is due to the short residence time of the air/carbon dioxide bubbles within the reactor and the culture temperature of 55 °C that drastically reduces solubility. Hereby, CO<sub>2</sub> transfer rates are not only limited by its partial pressure within the bubbles but also the factors mentioned previously. Therefore, a 5-fold increase in carbon dioxide concentration (1.6 to 9.1% v/v) only results in 12% increased maximum productivity. Nevertheless, the present data shows that using a CO<sub>2</sub> fraction of 1.6% v/v supplies the culture with excess dissolved inorganic carbon to sustain growth within the framework of the experimental set-up. Growth was only slightly limited by DIC availability for a short period of time during which the interplay of biomass concentration, turbulence and light availability allowed for maximum productivity (see 3.5). This is also shown by the respective minimum and maximum pH values measured

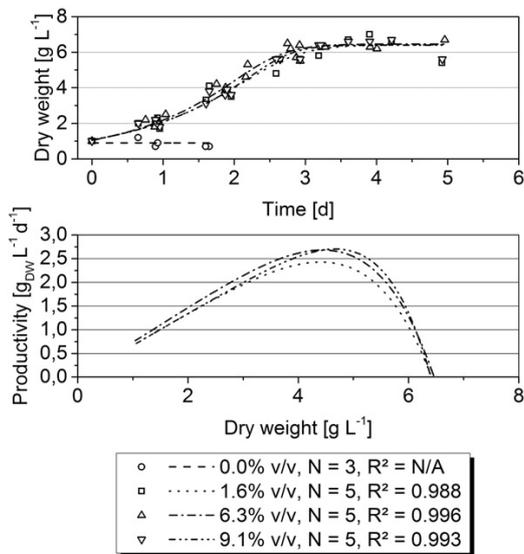


Fig. 4. Growth kinetics including the Richards growth fit-function (top) and productivity course calculated as the first derivative of the Richards growth fit-function (bottom) of *T. elongatus* at different CO<sub>2</sub> fractions during repeated fed-batch cultures. Light was continuously supplied by HPS lamps at a PFD of 780  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . N states the number of repeated fed-batches performed.

(see Table 1). These were highly similar throughout the experiments performed between 1.6% (v/v) and 9.1% (v/v) indicating an excess of carbon dioxide provided to the system re-feeding the carbonate buffer system as a result of H<sub>2</sub>CO<sub>3</sub> and HCO<sub>3</sub><sup>-</sup> consumed by cyanobacterial growth. In fact, at the pH levels given, >80% of dissolved inorganic carbon is represented by HCO<sub>3</sub><sup>-</sup> (pK<sub>s</sub> H<sub>2</sub>CO<sub>3</sub>, 6.5; pK<sub>s</sub> HCO<sub>3</sub><sup>-</sup>, 10.4) at pH 7.0 and almost all at pH 8.0. Nevertheless, growth has been limited by the buffering capacity, thus the prevailing concentration of HCO<sub>3</sub><sup>-</sup> in the medium. This assumption is supported by the fact that carbon

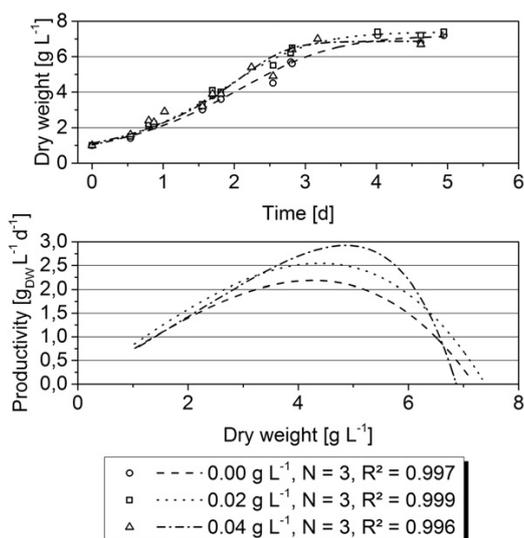


Fig. 5. Growth kinetics including the Richards growth fit-function (top) and productivity course calculated as the first derivative of the Richards growth fit-function (bottom) of *T. elongatus* at different carbonate concentrations during repeated fed-batch cultures. Light was continuously supplied by HPS lamps at a PFD of 780  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . CO<sub>2</sub> was continuously supplied at a fraction of 6.3% v/v. N states the number of repeated fed-batches performed.

dissolved beforehand (e.g. carbonates) shows a more direct influence on productivity (see 3.4). Further experiments were performed using a carbon dioxide content of 6.3% v/v.

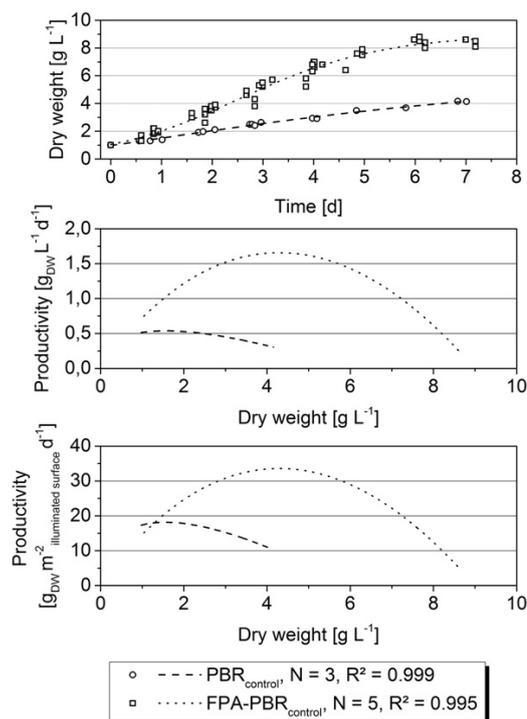
### 3.4. Carbonate concentration

As represented in Fig. 5, carbonate concentration may limit the growth of *T. elongatus* BP-1 during the cultivation in the FPA-PBR. Although substantial growth was also detected whilst initial carbonate was completely eliminated from the medium, addition of it significantly increased the growth performance. Maximum P<sub>vol</sub> was increased from 2.2 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup> to 2.9 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup> using 0.00 g L<sup>-1</sup> and 0.04 g L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub>, respectively. The latter being the highest productivity measured during the course of the experiments, further experiments were performed using a Na<sub>2</sub>CO<sub>3</sub> concentration of 0.04 g L<sup>-1</sup>.

The increase in maximum productivity can be explained by the increased availability of carbon in the medium due to the addition of Na<sub>2</sub>CO<sub>3</sub>. Applying Henry's Law for CO<sub>2</sub> using the Henry-constant of  $3.3 \cdot 10^{-4} \text{ mol m}^{-3} \text{ Pa}^{-1}$  (0.0334 mol L<sup>-1</sup> atm<sup>-1</sup>) and the Henry-coefficient of 2400 K [31], the maximum concentration of C dissolved in water at 55 °C is 12.13 mg L<sup>-1</sup> when aerated with carbon dioxide at 6.3% v/v and without the addition of any further inorganic carbon source. At average pH values of 7.6, generally prevailing after a cultivation duration of 2 days representing the period of maximum productivity (i.e. biomass concentration around 4 g L<sup>-1</sup>, see Section 3.5) >95% of the DIC species are represented by HCO<sub>3</sub><sup>-</sup> and therefore readily available for cyanobacterial growth. The addition of 0.02 or 0.04 g L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> adds 2.27 or 4.53 mg L<sup>-1</sup> C, respectively. Thereby, the concentration of C is increased 1.19- or 1.37-fold, respectively. This addition is directly correlated to increased maximum productivity during the period mentioned beforehand as shown in Fig. 5. Maximum productivity increased by a factor of 1.18 when 0.02 g L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> were added (2.2 vs. 2.6 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup>). It further increased by a factor of 1.32 when 0.04 g L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> were added (2.2 vs. 2.9 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup>). The increase in productivity is a result of the increased medium buffering capacity by raising the concentration of HCO<sub>3</sub><sup>-</sup>. Thereby, more carbon dioxide provided to the system can be re-fed into the carbonate buffer system increasing DIC availability and therewith biomass productivity. Similar findings were reported by Su et al. [32] for *Thermosynechococcus* sp. who showed that highest cell mass productivity was reached under the highest DIC level tested. The maximum biomass concentration was not influenced by the addition of Na<sub>2</sub>CO<sub>3</sub> since carbonate concentration was not monitored throughout the experiments and highly probable decreased rapidly during the course of the cultivations, thus decreasing the buffering capacity.

### 3.5. Photobioreactor design

Having excluded or at least reduced limiting effects beyond the sphere of influence of photobioreactor design, results arising from experiments performed in both reactor geometries are represented in Fig. 6. Also light path, material and cultivation parameters were identical; growth performance of *T. elongatus* BP-1 significantly varied in the reactor geometries. Both, volumetric productivity and final biomass concentration are influenced by the geometry of the photobioreactor. Maximum volumetric P<sub>vol</sub> was 3.4-fold higher in the FPA-PBR compared to the PBR<sub>control</sub> (1.7 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup> and 0.5 g<sub>DW</sub> L<sup>-1</sup> d<sup>-1</sup>, respectively). Including the difference in volume of the two geometries resulting from the incorporation of the static mixers, maximum areal productivity, based on illuminated surface area, was 1.8-fold higher in the FPA-PBR compared to the PBR<sub>control</sub> (33.6 g<sub>DW</sub> m<sup>-2</sup> d<sup>-1</sup> and 18.2 g<sub>DW</sub> m<sup>-2</sup> d<sup>-1</sup>, respectively). Although there are some fluctuations, biomass concentration at which maximum productivity was achieved was around 3.7-fold higher in the FPA-PBR compared to the PBR<sub>control</sub> (4–5 g<sub>DW</sub> L<sup>-1</sup> and 1.5 g<sub>DW</sub> L<sup>-1</sup>, respectively). Slight fluctuations may be explained by tolerances in handling techniques and analytical



**Fig. 6.** Growth kinetics including the Richards growth fit-function (top) and volumetric (center) as well as areal (bottom) productivity course calculated as the first derivative of the Richards growth fit-function of *T. elongatus* in different photobioreactor designs. PBR<sub>control</sub><sup>1</sup>: flat-panel with plain flat surface. FPA-PBR: flat-panel with static mixers. Light was continuously supplied by HPS lamps at a PFD of 780  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . CO<sub>2</sub> was continuously supplied at a fraction of 6.3% v/v. N states the number of repeated fed-batches performed.

instrumentation. So far, the authors have no explanation for the strong but reproducible fluctuation within the experiment on nitrate concentration (see 3.1). Since the biomass concentration at which maximum productivity is achieved is a function of light availability, thus applied PFD and aeration rate, a similar optimal biomass concentration would have been expected due to the fact that both parameters were kept constant. Final biomass concentration after seven days of growth was 8.6  $\text{g}_{\text{DW}} \text{L}^{-1}$  in the FPA-PBR compared to 4.2 in the PBR<sub>control</sub>.

Differences in growth performance between the two photobioreactor geometries drastically exceeded those determined by Degen et al. [26] who compared the initially developed FPA-PBR with a bubble column of the same dimension. Using *Chlorella vulgaris* SAG 211–12, maximum volumetric productivity was only 1.7-fold higher in the photobioreactor equipped with static mixers. The final biomass concentrations varied by a factor of 1.2 after a cultivation period of seven days. Although a different species was used during the experiments it is believed that the present significant differences in growth performance result from further refinement of the reactor's geometry e.g. using rounded instead of rectangular culture chambers in the riser regions and centralizing the downcomer compartment. It can be seen, that improvement of existing photobioreactor designs regarding the induction of directed turbulence may have a tremendous influence on culture performance and can improve economics of microalgae biotechnology. In fact, energy input (aeration) during the experiments described were identical but performance based on maximum areal productivity increased by a factor 1.8 whilst final biomass concentration increased by a factor of 2. Since all process parameters, including optical light path, were kept constant, the enhanced performance must be attributed to the incorporation of static mixers. By doing so, specific light availability (e.g. light per cell)

for the microbial community as a whole is improved. Cells are frequently translocated from the PBR's surface, characterized by access light availability beyond the level of saturation, to the dark interior of the culture. Hereby, their exposition time towards the light source is reduced. Inhibitory effects, thus the light-dependent downregulation of quantum-yield, are therefore reduced. On a cellular level, less energy must be spent for photoprotective mechanisms such as heat dissipation (non-photochemical quenching) or PSII repair. On a community level, more photons can effectively be used for assimilatory photochemistry, thus carbon fixation and biomass production. Compared to randomly mixed cultures, average exposition time of cells within aphotic PBR zones is reduced as well. Light limitation and respiratory losses are therefore reduced. Comprehensively considered, PBR performance is enhanced by the incorporation of static mixers due to the reduction of negative effects resulting from photolimitation and photoinhibition.

#### 4. Conclusion and outlook

The present paper represents the first publication on the FPA-PBR describing the cultivation of prokaryotic photosynthetic microorganisms. It also represents the first publication on a thermophilic strain. Stable and reproducible cultivation of *T. elongatus* BP-1 was performed in flat-panel airlift photobioreactors with and without static mixers. Maximum productivity and final biomass concentration was increased by increasing both, nitrate concentration and carbon dioxide supply. Further increase in maximum productivity resulted from a different supply of carbon as sodium carbonate. Given a maximum productivity of 2.9  $\text{g}_{\text{DW}} \text{L}^{-1} \text{d}^{-1}$  (see 3.4) and a final biomass concentration of 8.6  $\text{g}_{\text{DW}} \text{L}^{-1}$  after seven days of cultivation (see 3.5), the FPA-PBR performed well compared to cultivations completed in other laboratories. Leu et al. [33] reported a maximum productivity of around 0.6  $\text{g}_{\text{DW}} \text{L}^{-1} \text{d}^{-1}$  for *T. elongatus* TA-1 resulting in a final biomass concentration of 3.6  $\text{g}_{\text{DW}} \text{L}^{-1}$  after 12 days of cultivation. A productivity of 1.7  $\text{g}_{\text{DW}} \text{L}^{-1} \text{d}^{-1}$  was reported for *Thermosynechococcus* sp. TCL-1 by Su et al. [32] during continuous cultivation using a tubular bioreactor of 1 L in volume. A maximum productivity of 1.7  $\text{g}_{\text{DW}} \text{L}^{-1} \text{d}^{-1}$  was found for the same strain by Su et al. [34] using a flat plate photobioreactor with a light path of 3 cm and a volume of 2 L. Compared to the data presented in the present study, the high productivity (compared to 0.5  $\text{g}_{\text{DW}} \text{L}^{-1} \text{d}^{-1}$ ) may be explained by either the strain, the higher PFD (1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), the higher dissolved inorganic carbon concentration (DIC) or a combination thereof. Further examinations will address the influence of light intensity and aeration rate on the productivity and achievable biomass concentration of *T. elongatus* BP-1 cultivated in the FPA-PBR equipped with intrinsic static mixers (FPA-ISM).

#### References

- [1] Y. Chisti, Biodiesel from microalgae, *Biotechnol. Adv.* 25 (3) (2007) 294–306.
- [2] G.C. Dismukes, D. Carrieri, N. Bennette, G.M. Ananyev, M.C. Posewitz, Aquatic phototrophs: efficient alternatives to land-based crops for biofuels, *Curr. Opin. Biotechnol.* 19 (3) (2008) 235–240.
- [3] P. Singh, S. Kumari, A. Guldhe, R. Misra, I. Rawat, F. Bux, Trends and novel strategies for enhancing lipid accumulation and quality in microalgae, *Renew. Sust. Energ. Rev.* 55 (2016) 1–16.
- [4] M. Orosa, E. Torres, P. Fidalgo, J. Abalde, Production and analysis of secondary carotenoids in green algae, *J. Appl. Phycol.* 12 (3/5) (2000) 553–556.
- [5] J. Seckbach, H. Gross, M.B. Nathan, Growth and photosynthesis of *Cyanidium caldarium* cultured under pure CO<sub>2</sub>, *Isr. J. Bot.* 20 (1971) 84–90.
- [6] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, *Renew. Sust. Energ. Rev.* 14 (1) (2010) 217–232.
- [7] B. Uyar, N. Kapucu, Passive temperature control of an outdoor photobioreactor by phase change materials, *J. Chem. Technol. Biotechnol.* 90 (5) (2015) 915–920.
- [8] M.T. Madigan, J.M. Martinko, J. Parker, *Brock Biology of Microorganisms*, 9/e ed. Prentice-Hall Inc., New Jersey, 2000.
- [9] J.G. Day, S.P. Slocombe, M.S. Stanley, Overcoming biological constraints to enable the exploitation of microalgae for biofuels, *Bioresour. Technol.* 109 (2012) 245–251.
- [10] G. Antranikian, *Extremophiles and biotechnology*, Encyclopedia of Life Sciences (ELS), John Wiley & Sons, Ltd, Chichester, 2009.

- [11] P. Varshney, P. Mikulic, A. Vonshak, J. Beardall, P.P. Wangikar, Extremophilic microalgae and their potential contribution in biotechnology, *Bioresour. Technol.* 184 (2015) 363–372.
- [12] T. Yamaoka, K. Satoh, S. Katoh, Photosynthetic activities of a thermophilic blue-green alga, *Plant Cell Physiol.* 19 (6) (1978) 943–954.
- [13] Y. Nakamura, T. Kaneko, S. Sato, M. Ikeuchi, H. Katoh, S. Sasamoto, A. Watanabe, M. Iriguchi, K. Kawashima, T. Kimura, Y. Kishida, C. Kiyokawa, M. Kohara, M. Matsumoto, A. Matsuno, N. Nakazaki, S. Shimpō, M. Sugimoto, C. Takeuchi, M. Yamada, S. Tabata, Complete genome structure of the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1, *DNA Res.* 9 (4) (2002) 123–130.
- [14] K.A. Radzun, J. Wolf, G. Jakob, E. Zhang, E. Stephens, I. Ross, B. Hankamer, Automated nutrient screening system enables high-throughput optimisation of microalgae production conditions, *Biotechnol. Biofuels* 8 (65) (2015) 1–17.
- [15] M. Lavoie, S. Le Faucheur, A. Boullemant, C. Fortin, P.G.C. Campbell, The influence of pH on algal cell membrane permeability and its implications for the uptake of lipophilic metal complexes, *J. Phycol.* 48 (2) (2012) 293–302.
- [16] C. Sorokin, R.W. Krauss, The effects of light intensity on the growth rates of green algae, *Plant Physiol.* 33 (2) (1958) 109–113.
- [17] A. Vonshak, R. Guy, Photoadaptation, photoinhibition and productivity in the blue-green alga, *Spirulina platensis* grown outdoors, *Plant Cell Environ.* 15 (5) (1992) 613–616.
- [18] M. Cuaresma, M. Janssen, C. Vilchez, R.H. Wijffels, Horizontal or vertical photobioreactors? How to improve microalgae photosynthetic efficiency, *Bioresour. Technol.* 102 (8) (2011) 5129–5137.
- [19] A.P. Carvalho, S.O. Silva, J.M. Baptista, F.X. Malcata, Light requirements in microalgal photobioreactors: an overview of biophotonic aspects, *Appl. Microbiol. Biotechnol.* 89 (5) (2011) 1275–1288.
- [20] C. Lee, Calculation of light penetration depth in photobioreactors, *Biotechnol. Bioprocess Eng.* 4 (1) (1999) 78–81.
- [21] E. Sforza, D. Simonato, G.M. Giacometti, A. Bertucco, T. Morosinotto, Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors, *PLoS One* 7 (6) (2012) 1–10.
- [22] R. Rippka, H. Herdman, Pasteur culture collection of cyanobacterial strains in axenic culture, *Catalogue and Taxonomic Handbook*, Institut Pasteur, Paris, 1992.
- [23] A. Kuhl, H. Lorenzen, Handling and culturing of *Chlorella*, in: D.M. Prescott (Ed.), *Methods in Cell Physiology*, Academic Press, New York and London, 1964.
- [24] W. Trösch, U. Schmid-Staiger, A. Zastrow, A. Retze, F. Brucker, Photobioreactor With Improved Supply of Light by Surface Enlargement, Wavelength Shifter Bars or Light Transport, Vol. no. EP 1169428 B12000.
- [25] W. Trösch, Bio-Reactor for the Cultivation of Micro-Organisms and Method for the Production Thereof, Vol. no. EP 1326959 B12001.
- [26] J. Degen, A. Uebele, A. Retze, U. Schmid-Staiger, W. Trösch, A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect, *J. Biotechnol.* 92 (2) (2001) 89–94.
- [27] A. Meiser, U. Schmid-Staiger, W. Trösch, Optimization of eicosapentaenoic acid production by *Phaeodactylum tricoratum* in the flat panel airlift (FPA) reactor, *J. Appl. Phycol.* 16 (3) (2004) 215–225.
- [28] R. Münkler, U. Schmid-Staiger, A. Werner, T. Hirth, Optimization of outdoor cultivation in flat panel airlift reactors for lipid production by *Chlorella vulgaris*, *Biotechnol. Bioeng.* 110 (11) (2013) 2882–2893.
- [29] G.A.F. Seber, C.J. Wild, *Nonlinear Regression*, John Wiley & Sons, Inc., New York, 1989.
- [30] C.P.D. Birch, A new generalized logistic sigmoid growth equation compared with the Richards growth equation, *Ann. Bot.* 83 (6) (1999) 713–723.
- [31] R. Sander, Compilation of Henry's law constants (version 4.0) for water as solvent, *Atmos. Chem. Phys.* 15 (2015) 4399–4981.
- [32] C.M. Su, H.T. Hsueh, H.H. Chen, H. Chu, Effects of dissolved inorganic carbon and nutrient levels on carbon fixation and properties of *Thermosynechococcus* sp. in a continuous system, *Chemosphere* 88 (2012) 706–711.
- [33] J.-. Leu, T.-. Lin, M.J.P. Selvamani, H.-. Chen, J.-. Liang, K.-. Pan, Characterization of a novel thermophilic cyanobacterial strain from Taian hot springs in Taiwan for high CO<sub>2</sub> mitigation and C-phycoyanin extraction, *Process Biochem.* 48 (1) (2013) 41–48.
- [34] C.M. Su, H.T. Hsueh, T.Y. Li, L.C. Huang, Y.L. Chu, C.M. Tseng, H. Chu, Effects of light availability on the biomass production, CO<sub>2</sub> fixation, and bioethanol production potential of *Thermosynechococcus* CL-1, *Bioresour. Technol.* 145 (2013) 162–165.

## 4.3 Operating Strategy to Reduce the Energy Consumption of a FPA-PBR

Published as Original Research Article:

Bergmann, Peter and Trösch, Walter (2018). “Empirical operating strategy to reduce the light-specific energy consumption of a flat-panel airlift photobioreactor with intrinsic static mixers cultivating *Thermosynechococcus elongatus* BP-1. *Algal Research* 29, pp. 354–360.

Five-Year Impact Factor: 4.621 (Elsevier, 2017)

The publication describes and quantifies an operating strategy to decrease the specific energy requirement when cultivating phototrophic microorganisms in distinct FPA- PBRs, thus the energy consumed per unit of biomass produced. The strategy is based on adapting the superficial gas velocity in dependence on the prevailing biomass concentration and PFD, thus utilizing only valid and readily available measurement and control technology. This is especially important for outdoor cultivation as the superficial gas velocity is the one and only culture parameter that may be adjusted on time scales identical to those of PFD fluctuation. Therefore, applying the proposed strategy is most efficient at locations with only stochastic light supply, e.g. in temperate latitudes.

Cultivations performed at multiple PFDs and superficial gas velocities utilizing FPA- PBRs are compared and it was found that the superficial gas velocity during sub-saturating PFD and/or low biomass concentration may be reduced to cut OPEX on mixing, whilst an increase during supra-saturating PFD and/or high biomass concentration enhances productivity and final biomass yield. By doing so, taking day courses of PFD originating from an either sunny or cloudy summer day in Zeitz, Germany, as the basis, productivity may be increased by 24% and energy consumption with respect to mixing as well as CO<sub>2</sub>-demand cut by 37%.

The findings of the work were registered for patent approval (see section 5.1).



Contents lists available at ScienceDirect

Algal Research

journal homepage: [www.elsevier.com/locate/algal](http://www.elsevier.com/locate/algal)

## Empirical operating strategy to reduce the light-specific energy consumption of a flat-panel airlift photobioreactor with intrinsic static mixers cultivating *Thermosynechococcus elongatus* BP-1<sup>☆</sup>

Peter Bergmann<sup>a,\*</sup>, Walter Trösch<sup>b</sup><sup>a</sup> Subitec GmbH, Julius-Hölder-Str. 36, 70597 Stuttgart, Germany<sup>b</sup> University of Hohenheim, Institute of Food Science and Biotechnology, Garbenstraße 25, 70599 Stuttgart, Germany

### ARTICLE INFO

#### Keywords:

Flat-panel airlift photobioreactor  
Intrinsic static mixers  
Light utilization  
Specific energy consumption  
Automation  
Outdoor

### ABSTRACT

During photoautotrophic cultivations using optimized synthetic media, light is the limiting “substrate” of high-density cultures, especially throughout outdoor cultivations prohibiting adjustment of photon-flux density (PFD). Convective mass transfer (turbulence) is the method of choice to cope with that limiting effect. Then again, turbulence comes at costs through the energy required for its generation. In this context and based on laboratory data generated with *Thermosynechococcus elongatus* BP-1, an empiric operating strategy for flat-panel airlift photobioreactors with intrinsic static mixers is suggested. Repeated cultivations were performed from sub- to supra-saturating PFD ( $180$  to  $780 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at aeration rates ranging from  $0.11$  to  $0.83$  vvm assessing the cultures' productivities along the courses of biomass concentrations. The results indicate that, owing to the culture-flow directing mixers, there is a strong interrelation between the effects of PFD, biomass concentration and aeration rate (thus energy input) applied. Hereby, the positive impact of directed turbulence on the cultures' performance with respect to productivity and yield was greatest at high biomass concentration ( $> 5 \text{ g}_{\text{DW}} \text{L}^{-1}$ ) and PFDs with effects becoming less dominant while the latter two were reduced. Based on the quantitative findings and utilizing the easily monitored parameters PFD and biomass concentration, a computational model may be defined automatically controlling the easily adjustable parameter aeration rate. Without a negative influence on cultures' performance, this will allow for both, increase of culture performance at times of intense light and high biomass concentration as well as reduction of operational expenditures (OPEX) at times of dim light or low biomass concentration. Compared to the standard, continuous aeration regime, this may lead to a reduction of energy required for the generation of turbulence of 37%, especially when considering outdoor cultivations in temperate climate zones.

### 1. Introduction

Worldwide, microalgae are investigated as potential candidates to positively impact global challenges such as the supply of food [1], feed [2], bulk chemicals [3] and even biofuels [4] since decades [5]. Nevertheless, for the time being microalgae derived and commercially available products are, besides the utilization of *Chlorella* and *Arthrospira* (*Spirulina*) biomass as nutraceuticals, restricted to only a few high added-value products (HAVs) [6]. Besides regulatory obstacles for the introduction of novel HAVs such as antimicrobial agents [7], introduction and market penetration of these products is restricted by the currently high production costs of microalgal biomass, either associated

to downstream operations, i.e. de-watering of low-density cultures, or actual operational expenditures of cultivation in closed photobioreactors (PBRs) [8]. In low-cost open systems, numbers of microalgae species to be cultivated is limited by their ability to withstand extreme environments, i.e. high salinity or pH value, while decreasing the number of potential contaminants [9]. Although measures of pest control are under continuous examination [10], their economically viable implementation on industrial scale is yet to be proven for mesophilic strains. Therefore, PBRs are considered that, besides the reduced risk of contamination, allow for streamlined process control, ultimately resulting in maximized growth rates and biomass concentrations [9]. Hereby, PBR performance and thus growth rate is

<sup>☆</sup> Previous submission to Algal Research:

Bergmann, P. and Trösch, W. (2016): Repeated fed-batch cultivation of *Thermosynechococcus elongatus* BP-1 in flat-panel airlift photobioreactors with static mixers for improved light utilization: Influence of nitrate, carbon supply and photobioreactor design. *Algal Research* (17), pp. 79–86.

\* Corresponding author.

E-mail address: [p.bergmann@subitec.com](mailto:p.bergmann@subitec.com) (P. Bergmann).

<https://doi.org/10.1016/j.algal.2017.10.014>

Received 22 March 2017; Received in revised form 21 October 2017; Accepted 21 October 2017

Available online 22 November 2017

2211-9264/© 2017 Elsevier B.V. All rights reserved.

determined by the interplay of hydrodynamics and light distribution [11], inevitably confronting a light gradient through the PBR resulting from mutual shading of the cells in dependence of the prevailing PFD and cell density. Fluid turbulence is known to contribute to algal proliferation as it keeps cells in suspension, eliminates thermal stratification and allows for homogeneous nutrient distribution [12]. In addition, turbulence results in constant reallocation of photosynthetic cells through the PBR's photic zones, from the dimly lit or rather completely dark interior to the illuminated PBR surface often characterized by excessive light availability. By inducing these light/dark (L/D) cycles, microalgal growth is supported [13]. Hereby, productivity increases along with increasing frequency of the L/D cycle [14]. Novel PBR geometries therefore aim towards the induction of frequent L/D cycles by the incorporation of static mixers [15,16,17] and their implementation was shown to have significant positive effects on growth rate and final biomass concentration [18]. Although these installations efficiently increase solar conversion efficiency by at least partially overcoming the inhibitive effects predominant in only randomly mixed cultures (photoinhibition and photolimitation), the generation of turbulence remains one of the main costs drivers in microalgal biotechnology contributing to overall biomass production costs. This is especially true when using closed PBR systems [19] for which production costs from around 5 € kg<sup>-1</sup> [19], over 69 € kg<sup>-1</sup> [20] to even US\$ 400 kg<sup>-1</sup> [21] are reported in literature. On the contrary, production costs well below 1 € kg<sup>-1</sup> dry weight are aspired for bulk application of microalgal biomass [22]. Therefore, any attempt targeting the reduction of operational expenditures is relevant.

The present study aimed towards generating an insight into the complex interplay between hydrodynamics and light distribution when cultivating the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1 in a flat-panel airlift photobioreactor with intrinsic static mixers (FPA-PBR) [18]. Ultimately, the study aimed towards the development of the groundwork for an advanced control-command strategy enabling an automated adjustment of the aeration rate (turbulence) in dependence of the prevailing PFD and biomass concentration, thus reducing the light-specific energy input during microalgal cultivation. Light is the most crucial factor in microalgal mass production. As solar PFD undergoes natural diurnal and seasonal variations between 0 and 2000 μmol m<sup>-2</sup> s<sup>-1</sup> in the PAR (photosynthetically active radiation) region, both phenomena, photolimitation (e.g. dense culture and/or dim light) [23] and photoinhibition (e.g. low density cultures and/or excess light) [24,25] can minimize productivity and even irreversibly damage algal cultures [26]. It is therefore necessary to adapt cultures' hydrodynamics with respect to these changing conditions to maximize productivity and/or minimize energy demand for culture mixing. Intermittent aeration over the nighttime has already been studied and showed that a significant decrease of 45% in aeration (and thus energy demand) is possible without influencing algal growth [27]. Here, a pursuing strategy for FPA-PBR operation over the course of the day is suggested.

## 2. Material and methods

### 2.1. Organism and medium

#### 2.1.1. Organism

*Thermosynechococcus elongatus* BP-1 was kindly supplied by the Humboldt Universität zu Berlin, Berlin, Germany. Directly upon receipt, cultures were up-scaled and transferred to culture maintenance serving as inocula for experiments as described in [18].

#### 2.1.2. Medium

Performed cultivations used the inorganic BG 11 prepared in deionized water and modified from Rippka and Herdmann [28] applying the micronutrient solution from Kuhl and Lorenzen [29] in accordance to the medium recipe stated by the Culture Collection of Algae at

Goettingen University (SAG). During culture maintenance and PBR cultivations, medium was used at threefold the standard concentration allowing for rapid cell proliferation to high densities. Based on previous findings [18], medium was supplemented to contain (in mg L<sup>-1</sup>) NO<sub>3</sub>, 2000; PO<sub>4</sub>, 200. and Na<sub>2</sub>CO<sub>3</sub>, 40. All chemicals used were of analytical grade.

## 2.2. Analytics

### 2.2.1. Optical density

Optical density (OD<sub>750</sub>) was determined using a tabletop spectrophotometer (type DR 3900, Hach Lange GmbH, Berlin, Germany). Measurements were performed in triplicates against deionized water. Samples were diluted with deionized water to result in an OD<sub>750</sub> between 0.1 and 0.4 during measurements.

### 2.2.2. Dry weight

Dry weight (DW) was determined by identifying the difference in weight of pre-weight aluminium bowls following the addition of cyanobacterial suspension and successive drying to constant weight at 105 °C as described previously [18]. Correlation between DW and OD<sub>750</sub> was performed using the linear fitting function of the software OriginPro (v9.1, OriginLab Corp., Northampton, USA) (see Eq. 1).

$$DW [g L^{-1}] = 0.277 \cdot OD_{750} \quad (R^2 = 0.999, N = 9) \quad (1)$$

### 2.2.3. Light Source and photon-flux density

High pressure sodium-vapor (HPS) lamps were used. Photon-flux density (PFD) was determined in the PAR region using a mobile quantum sensor (type LI-190, LI-COR, Inc., Nebraska, USA) and light meter (type: LI-250A, LI-COR, Inc., Nebraska, USA). PFD was measured at 10 points evenly distributed over the FPA-PBR's surface and averaged. PFD was adjusted according to the scientific question under examination.

### 2.2.4. Nutrient concentration

Concentrations of nitrogen and phosphorus were monitored daily throughout the FPA-PBR experiments and re-fed if necessary. Therefore, samples were centrifuged and respective concentration measured in the resulting supernatant using quantitative colorimetric cuvette tests as previously described [18]. Nutrients were re-fed by sterile filtration.

## 2.3. Photobioreactor design and operation

### 2.3.1. Geometry and functionality

The PBR operated during the experiments represents a FPA-PBR with integrated flow directing static mixers (horizontal culture flow) and downcomer domains (vertical culture flow, loop principle) (see Fig. 1). The reactor was initially developed, patented [30,31] and described [32,33] by the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB (IGB), Stuttgart, Germany, and is now commercialized by Subitec GmbH, Stuttgart, Germany. The most distinctive feature of the FPA-PBR are the incorporated static mixers allowing for a controlled movement of microalgal cells from the illuminated surface area to the dimly lit interior of the PBR. Hereby, negative effects of photoinhibition and photolimitation are reduced by intermittent exposure towards the excess light at the PBR's surface versus the insufficiently illuminated interior. Frequency of cycling can be adjusted by the aeration rate applied. The design of the reactor and utilized peripherals as well as procedures of initiating experiments are extensively described by Bergmann *et al.* [18]. The present work focused on the interaction of the PFD and aeration rate applied and their mutual influence on cyanobacterial growth kinetics (productivity and biomass concentration). Therefore, PFD and aeration rate were varied over the course of the experiments between 180 and 780 μmol m<sup>-2</sup> s<sup>-1</sup> and 40

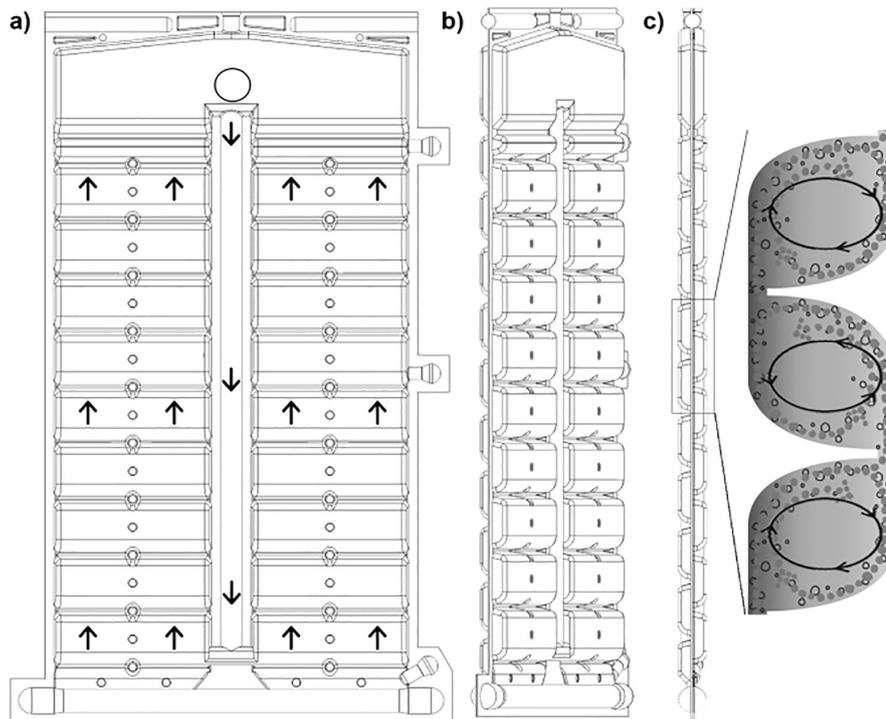


Fig. 1. Flat-panel airlift photobioreactor (FPA-PBR) with flow directing static mixers. a) Front view: arrows indicate culture flow in riser (aerated) and downcomer domains; the circle above the downcomer indicates site of sampling and feeding. b) Angled view: depicting risers, downcomer and static mixers. c) Lateral view: arrows indicate culture flow induced by static mixers.

to  $300 \text{ L h}^{-1}$  (0.11 to  $0.83 \text{ vvm}$ ), respectively. Hereby,  $\text{CO}_2$  concentration was kept constant at  $6.3\% \text{ v/v}$ .

### 2.3.2. Mode of operation

The FPA-PBRs were operated in repeated fed-batch mode. Following the inoculation, experiments were performed with fed-batches run at least in duplicates. One fed-batch lasted between 4 and 7 days. Nitrogen and phosphorus were constantly monitored and re-fed throughout the experiments. Following the completion of a fed-batch, aeration rates and PFDs were adjusted if necessary and cultures diluted to a DW of around  $1.0 \text{ g L}^{-1}$  and cultures' performances repeatedly monitored. Prior to measurements, filling levels of the FPA-PBRs were corrected using fresh medium counteracting evaporative losses and water discharge by saturated exhaust gas.

### 2.3.3. Experimental replication and statistical treatment

As previously stated, given experiments were performed at least in duplicates. The exact number of replicates is stated for each experiment. To cope with fluctuations in growth deriving from the biological system as well as technical limits (e.g. slight fluctuations of temperature and  $\text{CO}_2$  supply) data was evaluated by overlaying the available growth data for given experiments. This procedure also allowed for the presentation of an otherwise vast data pool gained in multiple photobioreactors resulting from randomized experiments. Following data overlaying, growth was fitted to result in a single representative growth curve for each experiment. For this purpose, the sigmoidal "Richards" logistic function type 2 [34] was chosen as previously described by Bergmann et al. [18].

## 3. Results and discussion

### 3.1. Productivity versus irradiance

To select adequate PFDs for further experiments that reflect the three lighting conditions prevalent with respect to the PBR's surface

(sub-saturating, saturating and supra-saturating PFD) the preliminary experiment served to establish a modified photosynthesis-irradiance curve. For this, experimentally determined productivity data resulting from the linear phases of growth (slopes of linear regressions between day one and three, see Fig. 3) in experiments performed at standard aeration rate ( $0.5 \text{ vvm}$ ) and different PFDs were used. This procedure resulted in a productivity-irradiance curve, which is system-specific in dependence on the strain used as well as the aeration rate and inoculation density applied. Figure two (Fig. 2) represents the emerged

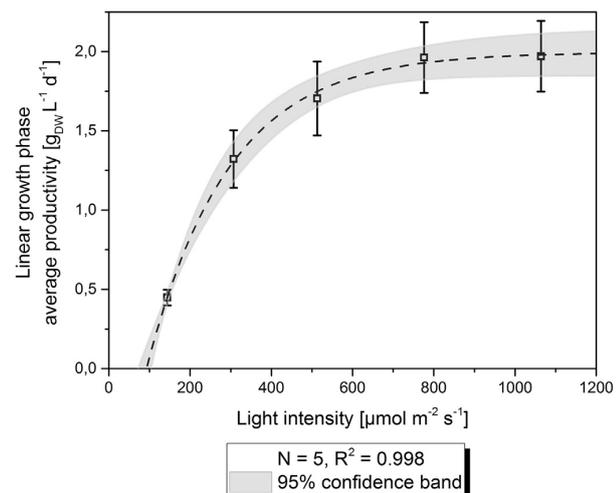


Fig. 2. Modified photosynthesis-irradiance curve (based on empirical productivity data) of *T. elongatus* cultivated in FPA-PBRs with an aeration rate of  $180 \text{ L h}^{-1}$  ( $0.5 \text{ vvm}$ ). Light was continuously supplied by HPS lamps.  $\text{CO}_2$  was continuously supplied at a fraction of  $6.3\% \text{ v/v}$ . N states the number of repeated fed-batches performed for each point of measurement.

saturation kinetic in which the light compensation point ( $I_C$ ) and the irradiance of saturation ( $I_K$ ) are located at PFDs of around  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. In the given set-up, photosynthesis was limited by the number of impinging photons at PFDs below  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Therefore, compared to the standard, continuous aeration regime, culture turbulence and thus energy input may be minimized as photon abundance represents the process' bottleneck. On the contrary, above  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  photons were not efficiently used for assimilatory photochemistry and thus wasted. Translated to outdoor cultivations at full sunlight ( $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), this corresponds to a wastage rate of  $> 75\%$ . In order to counteract that, culture turbulence may be increased allowing for increased quantum conversion and thus productivity as a larger number of cells is directly translocated towards the light sources in a given unit of time compensating the small light doses available for single cells [35]. Hereby, the utilization of culture flow directing installations permits for the utilization of longer light paths increasing process economics. Further experiments were performed to quantify potential benefits of light-dependent adaptations of the aeration rate by (i) increasing productivity during times of supra-saturating light conditions and (ii) reducing the energy input during times sub-saturating light conditions.

### 3.2. Productivity versus aeration rate

In order to quantify potential benefits of light-dependent adaptations of the aeration, cultivation experiments were performed utilizing aeration rates varying from  $40 \text{ L h}^{-1}$  (0.11 vvm) to  $300 \text{ L h}^{-1}$  (0.83 vvm) at sub-saturating ( $180 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), saturating ( $405 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and supra-saturating ( $780 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) PFD. As shown in figure three (Fig. 3), there is a strong connection between applied PFD and productivity as well as final biomass concentration which was to be expected. At a given aeration rate, (maximum) productivity and final biomass concentration increase with increasing PFD. In example, maximum productivity at an aeration rate of  $180 \text{ L h}^{-1}$  (0.5 vvm) almost tripled from  $0.6$  to  $1.7 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ d}^{-1}$  when increasing the PFD from  $180$  to  $780 \mu\text{mol m}^{-2} \text{s}^{-1}$ . At the same aeration rates and PFDs applied, final biomass concentration after 7 days of growth more than doubled from  $4.2$  to  $8.7 \text{ g}_{\text{DW}} \text{ L}^{-1}$ , respectively.

Hereby, the reason for the non-linear correlation between applied PFD and/or final biomass concentration results from the fact that light is exponentially attenuated within photobioreactors [36]. Therefore, average culture illumination ( $I_{AV}$ ) does not increase linearly with applied surface illumination ( $I_C$ ) resulting in biomass steady-states (during chemostat cultures) following light limitation [37]. The adjustment of the applied aeration rate can further promote growth at supra-saturating PFDs. Hereby, and with respect to process economics, it is a necessity to induce a directed culture flow (e.g. by utilizing flow directing installations such as static mixers) as promoting effects are dependent on a continuously sustained allocation of the cells towards the outer culture volume flooded with light. Although similar effects may be observed utilizing random mixing only, their manifestation is by far less dominant when applying identical volumetric gas flow rates and thus does not account for the increased energy use. When increasing the aeration rate from  $0.5$  to  $0.83$  vvm ( $300 \text{ L h}^{-1}$ ) at supra-saturating light conditions, maximum productivity further increased from  $1.7$  to  $2.0 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ d}^{-1}$ . The same is in tendency true for the final biomass concentration after a given unit of time although during these very experiments, biomass concentration after 7 days of growth was similar which highly probably represents an artifact of data processing as the underlying trend may be extracted from the course of the aeration rates applied. When utilizing randomly mixed photobioreactors of similar dimensions as described in [18], no increase in maximum productivity ( $0.6 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ d}^{-1}$ ) or final biomass concentration ( $4.9 \text{ g}_{\text{DW}} \text{ L}^{-1}$ ) was observed between an aeration  $0.5$  to  $0.83$  vvm (data not shown). It is to be noted that, interestingly, the promoting effect of

increased aeration is dependent on the prevailing biomass concentration. As shown in figure three (Fig. 3) growth kinetics at different aeration rates did not vary significantly until a culture density of  $3 \text{ g}_{\text{DW}} \text{ L}^{-1}$  was reached. Only then, growth started to slow down with the lowest aeration rates ( $0.11$  and  $0.17$  vvm) used. The same was observed at  $4 \text{ g}_{\text{DW}} \text{ L}^{-1}$  for  $0.5$  and  $0.67$  vvm and at  $5 \text{ g}_{\text{DW}} \text{ L}^{-1}$  for  $0.83$  vvm, respectively. Therefore, increased aeration may serve to increase the biomass concentration at which severe light limitation manifests in the transition from exponential to linear growth. By knowing so and being able to easily monitor prevailing PFDs and biomass concentrations (turbidity) online, the aeration rate may be set in dependence on the parameters mentioned before, in order to achieve maximum growth rates at simultaneously least possible energy expenses. In the same context, the aeration rate may not only be adjusted at supra-saturating PFD but also during times of saturating PFD at which a volumetric flow velocity  $> 0.5$  vvm corresponds to wasted energy as no growth promoting effects occur. At sub-saturating PFD, aeration rate may be kept to a minimum in order to keep cells in suspension (see Fig. 3).

## 4. Conclusion and outlook

The described findings present an insight into the complex interplay of photon-flux density, biomass concentration and aeration rate and their influence on the growth kinetics of phototrophic microorganisms, exemplarily represented by the cyanobacterium *T. elongatus* BP1. Based on the findings, the implementation of an empiric operating strategy for flat-panel airlift photobioreactors is suggested, that considers the prevailing photon-flux density as well as biomass concentration as control parameters to automatically adjust the system's turbulence during outdoor cultivations by setting the aeration rate. Hereby, the aeration rate represents the one and only control parameter that may be used to compensate for fluctuating specific light availabilities (light per unit of biomass) during outdoor cultivations. By doing so, process economics may be improved by enhanced productivity during times of supra-saturating light conditions and high biomass concentration. Even more importantly, energy input may be decreased during times of dim light and low biomass concentrations, without cutting on culture productivity and/or yield. Reduction in aeration rate be achieved by reducing the absolute volumetric gas flow rate, as conducted within the studies, or by implementing intermittent aeration as already described for nighttime operations of flat-panel airlift photobioreactors [27]. Hereby, cultures' productivity is not negatively influenced as during times of sub-saturating light conditions, microalgal proliferation is limited by the number of impinging photons and may not be accelerated by elevated aeration rates (see Fig. 3 a)). Therefore, aeration rates may be reduced in order to decrease operational expenditures (OPEX). During times of excess light on the contrary, microalgal proliferation benefits from increased turbulence and associated light availability, ultimately promoting algal growth (see Fig. 3 c)). The application of the proposed empirical operating strategy will therefore result in (i) similar growth kinetics at reduced energy input and (ii) enhanced growth kinetic and similar energy input.

In order to quantify the results when translated to exemplary outdoor PFD data (Fig. 4) on a clear vs. cloudy day, comparisons at an illustrative and easily achieved prevailing culture density of  $4 \text{ g}_{\text{DW}} \text{ L}^{-1}$  were performed. Hereby, three aeration set points ( $0.11$ ,  $0.5$  and  $0.83$  vvm) were chosen reflecting the minimum, medium (standard) and maximum gas flow rates examined in this study. The three PFD ranges examined in this study ( $< 180$ ,  $180$ – $405$  and  $> 405 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) were chosen as light thresholds at which the aeration rate is adjusted. During these PFD ranges, volumetric productivity at  $4 \text{ g}_{\text{DW}} \text{ L}^{-1}$  and a given moment in time depends on the aeration rate applied. These are extractable from Fig. 3 and summarized in Table 1. Productivity at a PFD below  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  was assumed to be zero, as this reflects the light compensation point of the given set-up

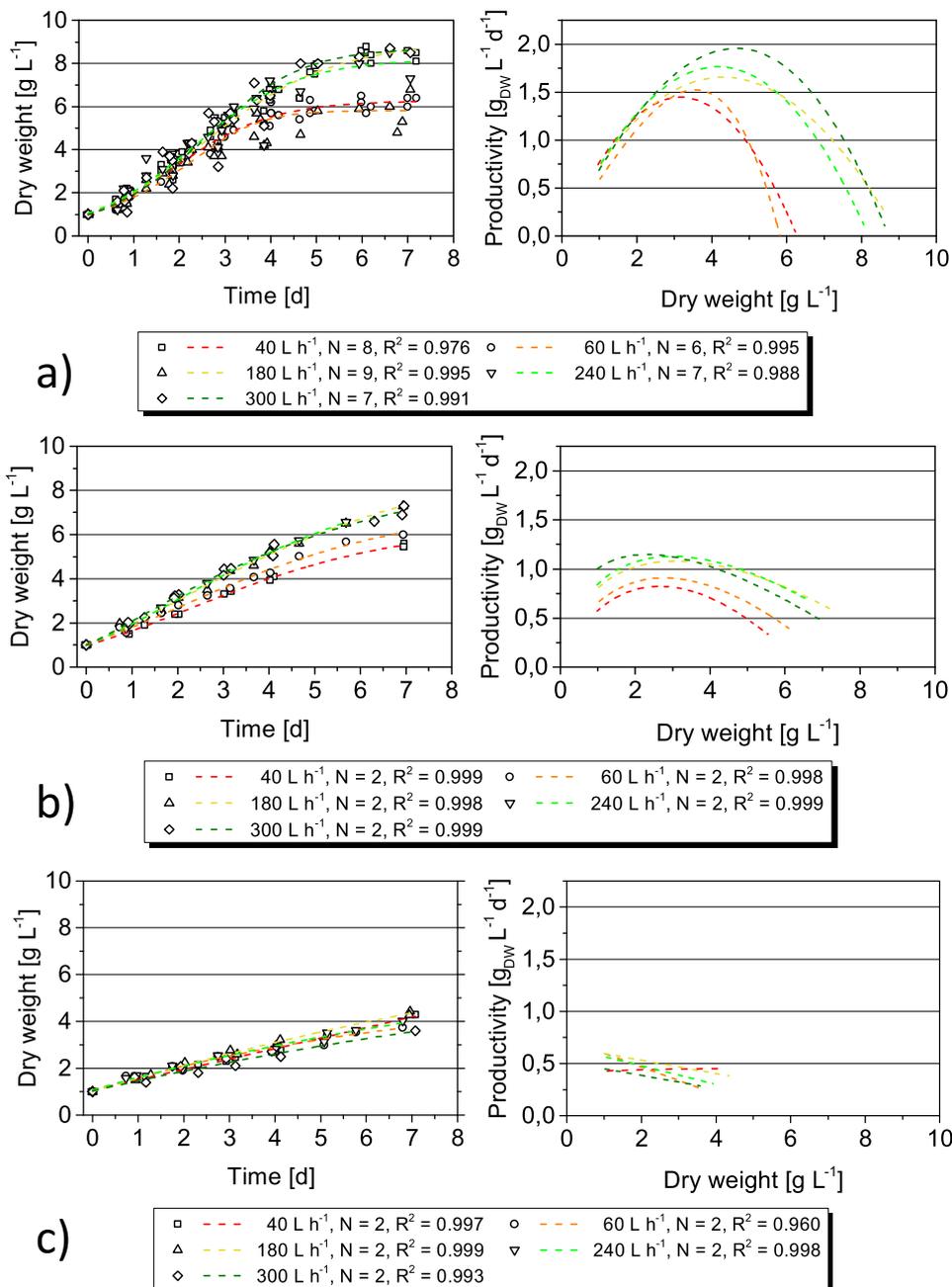


Fig. 3. Growth kinetics of *T. elongatus* at a PFD of a)  $780 \mu\text{mol m}^{-2} \text{s}^{-1}$ , b)  $405 \mu\text{mol m}^{-2} \text{s}^{-1}$  and c)  $180 \mu\text{mol m}^{-2} \text{s}^{-1}$  applying various aeration rates during repeated fed-batch cultures. Richards growth fit-functions (left) and productivity courses calculated as the first derivative of the Richards growth fit-function (right) are included. Light was continuously supplied by HPS lamps.  $\text{CO}_2$  was continuously supplied at a fraction of 6.3% v/v. N states the number of repeated fed-batches performed.

(Fig. 2).

Comparisons were performed by relating the integrals of the curves of Fig. 4, assuming the integrals of the aeration rate and volumetric productivity at standard aeration on a sunny day to be 100%.

By relating the volumetric productivity and energy input for PBR mixing utilizing the standard aeration regime vs. the adapted aeration regime it can be seen, that in this exemplary case the proposed operating strategy utilizing three aeration set points performs well (Table 2). During the sunny day, not only productivity is increase by

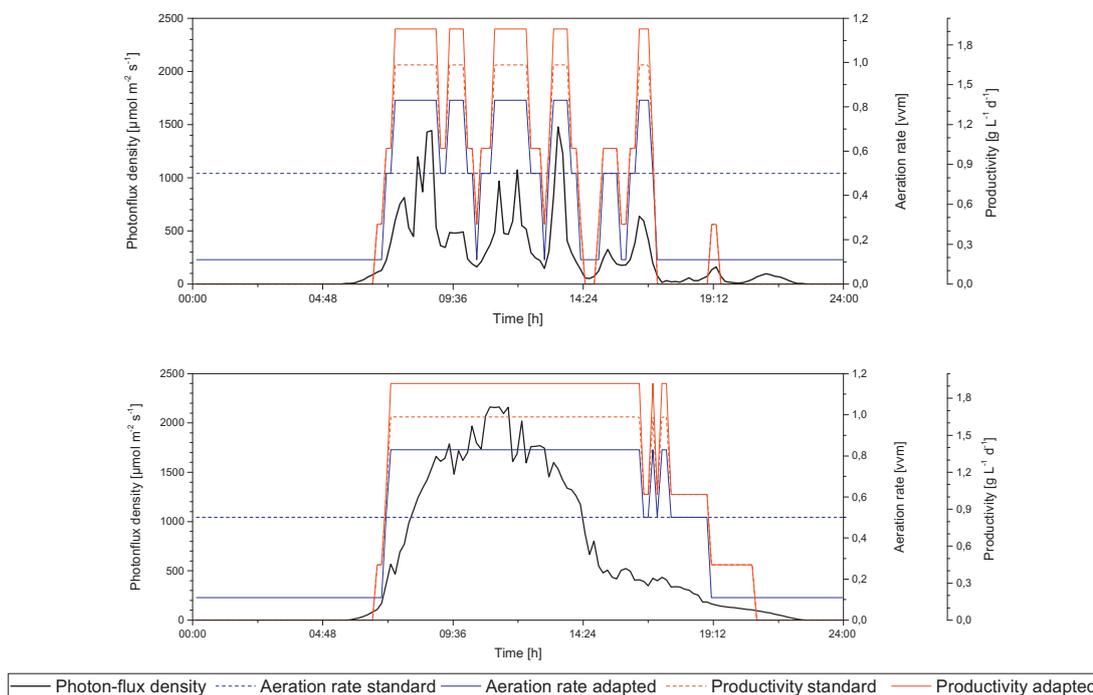
24% but at the same time, energy input is reduced by 11%. Although the aeration rate is increased during times of high light, this is easily compensated by reduced aeration during morning, evening and night hours. Besides the energy input,  $\text{CO}_2$  requirement is reduced at the same time as it was supplied at a fixed concentration of 6.3% (v/v), thus directly correlated to the aeration rate applied. During the cloudy day, productivity is slightly increased but even more importantly, energy consumption for PBR mixing and  $\text{CO}_2$  requirement are reduced by 37%.

Although the findings are believed to be very similar as the online

### 4.3 Operating Strategy to Reduce the Energy Consumption of a FPA-PBR

P. Bergmann, W. Trösch

Algal Research 29 (2018) 354–360



**Fig. 4.** Exemplary PFD (vertical) day courses of two successive summer days in Zeitz, Germany, measured in June 2015. Bottom: predominantly clear sky. Top: partly cloudy. Blue lines depict the applied standard aeration rate vs. the adapted aeration rate using set points of 0.11, 0.5 and 0.83 vvm at PFD thresholds of < 180, 180–405 and > 405  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Red lines depict respective volumetric productivities at given moments in time assuming a prevailing biomass concentration of 4  $\text{g}_{\text{DW}} \text{L}^{-1}$ . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**  
Volumetric productivity at 4  $\text{g}_{\text{DW}} \text{L}^{-1}$  in dependence on the prevailing PFD and aeration rate applied.

Productivity [ $\text{g}_{\text{DW}} \text{L}^{-1} \text{d}^{-1}$ ]			
Photonflux-density [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	Gas flow rate [vvm]		
	0,11	0,5	0,83
< 100	0	0	0
100–180	0,45	0,45	0,45
181–405	0,71	1,02	1,02
> 405	1,34	1,65	1,92

**Table 2**  
Relative volumetric productivity and energy input for PBR mixing utilizing the standard aeration regime vs. the adapted aeration regime; values of standard aeration regime during a sunny are assumed 100%.

		Sunny	Cloudy
Volumetric productivity	Standard aeration	100	64
	Adapted aeration	124	71
Energy input for mixing	Standard aeration	100	100
	Adapted aeration	89	63

measurement of PFD and DW as well as the precise control of the gas flow velocity represent readily available and valid measurement and control technology executable on similar time scales (real time adjustment of aeration rate to stochastic relative light intensities), future work will validate them on further photosynthetic microorganisms under actual production conditions outdoors. In addition, an advanced control-command system will be established allowing for a model-based infinitely variable adjustment of the aeration rate, ultimately

fine-tuning the described findings maximizing process efficiency. To the knowledge of the authors, this is the first proposition of an operating strategy aiming towards the reduction of the specific operational expenditures of flat-panel airlift photobioreactors by allowing maximum productivity at lowest specific energy inputs during phototrophic outdoor cultivations with stochastic light supply.

#### Acknowledgements

No external funding was received for the work performed.

#### Declaration of author contributions

Peter Bergmann was responsible for experimental conception and design, data acquisition, analysis and interpretation, and drafting of the manuscript. Walter Trösch critically revised the manuscript.

No conflicts, informed consent, human or animal rights applicable.

#### References

- [1] R.A. Kay, L.L. Barton, Microalgae as food and supplement, *Crit. Rev. Food Sci. Nutr.* 30 (6) (1991) 555–573.
- [2] E.W. Becker (Ed.), *Microalgae Biotechnology and Microbiology*, Cambridge University Press, New York, 1994.
- [3] R.H. Wijffels, M.J. Barbosa, M.H.M. Eppink, Microalgae for the production of bulk chemicals and biofuels, *Biofuels Bioprod. Biorefin.* 4 (3) (2010) 287–295.
- [4] L. Brennan, P. Owende, Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products, *Renew. Sust. Energ. Rev.* 14 (2) (2010) 557–577.
- [5] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, Commercial applications of microalgae, *J. Biosci. Bioeng.* 101 (2) (2006) 87–96.
- [6] S. Leu, S. Boussiba, Advances in the production of high-value products by microalgae, *Ind. Biotechnol.* 10 (3) (2014) 169–183.
- [7] M. Gantar, Z. Svircev, Microalgae and cyanobacteria: FOOD for Thought(1), *J. Phycol.* 44 (2) (2008) 260–268.
- [8] Y. Lee, Microalgal mass culture systems and methods: their limitation and potential,

- J. Appl. Phycol. 13 (4) (2001) 307–315.
- [9] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, *Renew. Sust. Energ. Rev.* 14 (1) (2010) 217–232.
- [10] R.C. McBride, S. Lopez, C. Meenach, M. Burnett, P.A. Lee, F. Nohilly, C. Behnke, Contamination management in low cost open algae ponds for biofuels production, *Ind. Biotechnol.* 10 (3) (2014) 221–227.
- [11] H. Luo, A. Kemoun, M.H. Al-Dahhan, J.M.F. Sevilla, J.L.G. Sánchez, F.G. Camacho, E.M. Grima, Analysis of photobioreactors for culturing high-value microalgae and cyanobacteria via an advanced diagnostic technique: CARPT, *Chem. Eng. Sci.* 58 (12) (2003) 2519–2527.
- [12] C.U. Ugwu, H. Aoyagi, H. Uchiyama, Photobioreactors for mass cultivation of algae, *Bioresour. Technol.* 99 (10) (2008) 4021–4028.
- [13] H. Qiang, A. Richmond, Productivity and photosynthetic efficiency of *Spirulina platensis* as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor, *J. Appl. Phycol.* 8 (2) (1996) 139–145.
- [14] C.U. Ugwu, J.C. Ogonna, H. Tanaka, Light/dark cyclic movement of algal culture (*Synechocystis aquatilis*) in outdoor inclined tubular photobioreactor equipped with static mixers for efficient production of biomass, *Biotechnol. Lett.* 27 (2) (2005) 75–78.
- [15] C.U. Ugwu, J.C. Ogonna, H. Tanaka, Design of static mixers for inclined tubular photobioreactors, *J. Appl. Phycol.* 15 (2/3) (2003) 217–223.
- [16] Q.H. Zhang, X. Wu, S.Z. Xue, Z.H. Wang, C.H. Yan, W. Cong, Hydrodynamic characteristics and microalgae cultivation in a novel flat-plate photobioreactor, *Biotechnol. Prog.* 29 (1) (2013) 127–134.
- [17] J. Degen, A. Uebele, A. Retze, U. Schmid-Staiger, W. Trösch, A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect, *J. Biotechnol.* 92 (2) (2001) 89–94.
- [18] P. Bergmann, W. Trösch, Repeated fed-batch cultivation of *Thermosynechococcus elongatus* BP-1 in flat-panel airlift photobioreactors with static mixers for improved light utilization: Influence of nitrate, carbon supply and photobioreactor design, *Algal Res.* 17 (2016) 79–86.
- [19] N.H. Norsker, M.J. Barbosa, M.H. Vermue, R.H. Wijffels, Microalgal production—a close look at the economics, *Biotechnol. Adv.* 29 (1) (2010) 24–27.
- [20] F.G. Acién, J.M. Fernandez, J.J. Magan, E. Molina, Production cost of a real microalgae production plant and strategies to reduce it, *Biotechnol. Adv.* 30 (6) (2012) 1344–1353.
- [21] P. Coutteau, P. Sorgeloos, The use of algal substitutes and the requirement for live algae in the hatchery and nursery of bivalve molluscs: an international survey, *J. Shellfish Res.* 11 (1992) 467–476.
- [22] R.H. Wijffels, M.J. Barbosa, An outlook on microalgal biofuels, *Science* 329 (5993) (2010) 796–799.
- [23] C. Sorokin, R.W. Krauss, The effects of light intensity on the growth rates of green algae, *Plant Physiol.* 33 (2) (1958) 109–113.
- [24] A. Vonshak, R. Guy, Photoadaptation, photoinhibition and productivity in the blue-green alga, *Spirulina platensis* grown outdoors, *Plant Cell Environ.* 15 (5) (1992) 613–616.
- [25] M. Cuaresma, M. Janssen, C. Vilchez, R.H. Wijffels, Horizontal or vertical photobioreactors? How to improve microalgae photosynthetic efficiency, *Bioresour. Technol.* 102 (8) (2011) 5129–5137.
- [26] A.P. Carvalho, S.O. Silva, J.M. Baptista, F.X. Malcata, Light requirements in microalgal photobioreactors: an overview of biophotonic aspects, *Appl. Microbiol. Biotechnol.* 89 (5) (2011) 1275–1288.
- [27] E. Eustance, J.T. Wray, S. Badvipour, M.R. Sommerfeld, The effects of limiting nighttime aeration on productivity and lipid accumulation in *Scenedesmus dimorphus*, *Algal Res.* 10 (2015) 33–40.
- [28] R. Rippka, H. Herdman, Pasteur Culture Collection of Cyanobacterial Strains in Axenic Culture. Catalogue and Taxonomic Handbook, Institut Pasteur, Paris, 1992.
- [29] A. Kuhl, H. Lorenzen, Handling and culturing of *Chlorella*, in: D.M. Prescott (Ed.), *Methods in Cell Physiology*, Academic Press, New York and London, 1964.
- [30] W. Trösch, U. Schmid-Staiger, A. Zastrow, A. Retze, F. Brucker, Photobioreactor With Improved Supply of Light by Surface Enlargement, Wavelength Shifter Bars or Light Transport, (2000) (no. EP 1169428 B1).
- [31] W. Trösch, Bio-reactor for the Cultivation of Micro-organisms and Method for the Production Thereof, (2001) (no. EP 1326959 B1).
- [32] A. Meiser, U. Schmid-Staiger, W. Trösch, Optimization of eicosapentaenoic acid production by *Phaeodactylum tricornutum* in the flat panel airlift (FPA) reactor, *J. Appl. Phycol.* 16 (3) (2004) 215–225.
- [33] R. Münkler, U. Schmid-Staiger, A. Werner, T. Hirth, Optimization of outdoor cultivation in flat panel airlift reactors for lipid production by *Chlorella vulgaris*, *Biotechnol. Bioeng.* 110 (11) (2013) 2882–2893.
- [34] G.A.F. Seber, C.J. Wild, *Nonlinear Regression*, John Wiley & Sons, Inc., New York, 1989.
- [35] A. Richmond, Principles for attaining maximal microalgal productivity in photobioreactors: an overview, *Hydrobiologia* 512 (1) (2004) 33–37.
- [36] C. Lee, Calculation of light penetration depth in photobioreactors, *Biotechnol. Bioprocess Eng.* 4 (1) (1999) 78–81.
- [37] E.G. Evers, A model for light-limited continuous cultures: growth, shading, and maintenance, *Biotechnol. Bioeng.* 38 (3) (1991) 254–259.

# CHAPTER 5

---

## Patent Application

---

### 5.1 Bioreactor

Bergmann, P., Beyer, L., Trösch, W., Ruppel, V., Ripplinger, P. (2017). "Bioreactor". *UK Patent Application*, GB 2542817 A.

Date of Filing : 30.09.2015  
Date of Publication : 05.04.2017

This patent application relates to a method of operating a bioreactor in order to reduce OPEX. Hereby, the key element is the adjustment of the superficial gas velocity, either continuously by e.g. mass flow controllers (MFCs) or intermittently e.g. by using solenoid valves, without comprising growth kinetics. By doing so, energy demand for the generation of turbulence and thus OPEX is reduced.



## CHAPTER 6

---

### Final Discussion and Remarks

---

The local utilization of microalgae for nutritional purposes is ancient history. Dating back thousands of years, these photosynthetic microorganisms have been consumed in China, Chad and Mexico to combat famine or upgrade the nutritional value of daily high-fiber meals. Along the past 80 years, a small but global industry developed dedicated to the industrial scale cultivation of microalgae creating value from their biomass. Here, the global cultivation of photosynthetic prokaryotic cyanobacteria and eukaryotic microalgae predominantly aims towards the production of high added-value compounds such as pigments, essential fatty acids as well as functional proteins and polysaccharides with applications mainly in the field of dietary supplements and cosmetics. During the decades, significant progress was made in understanding microalgal biology allowing for their economic exploitation. Major factors influencing the cultivation process are temperature, pH, nutrients including the availability of dissolved inorganic carbon (DIC) and most importantly, light. Here, not only its mere presence but its availability to every single cell is of importance.

As light may not be supplied in excess to the inside of dense microalgal cultures, cells must be enabled to efficiently capture photons by constantly exposing them towards the light source. Therefore, agitation of the culture suspension not only avoids sedimentation and thermal stratification but also guarantees nutrient availability and increases gas-liquid mass transfer and light conversion efficiency. Nevertheless, mixing does come at costs and economically viable regimes must be selected depending on the species used and product desired. Although generally promoting photon conversion efficiency and thus product output, excessive agitation may result in shear stress and potentially complete loss of culture.

Therefore, means beyond a plain increase of the culture suspension's agitation, through e.g. mechanical pumps or air blowers, must be put to practice in order to allow for biocompatible and cost-effective microalgal production. This is especially true since current production costs are yet one of the major drawbacks of microalgal biotechnology, whereby the process-energy required for culture mixing contributes to a significant extent (see Section 3.3).

Maximum growth rate (e.g. volumetric productivity) is achieved at a photon-flux density (PFD) corresponding to the point of saturating light availability. Nevertheless, the key to economic viability of commercial microalgae production is given by maximum yield (e.g. final biomass concentration) which is obtained when the prevailing PFD level is above saturation but below inhibition, as growth is proportional to light energy conversion. This is easily achieved during continuous indoor cultivations by adapting the detention time of the culture in dependence on the PFD applied and alga cultivated. Outdoors, on the other hand, this approach is unconvertible due to fluctuating light conditions.

Taking into consideration that microalgae proliferation is a function of light availability, thus hydrodynamics and light conditions, the development of strategies maximizing photon conversion efficiency at equitable energy input must be driven forward. Therefore, as summarized in Section 3.5, the experimental work performed in the course of the present thesis aimed towards generating a deep insight and understanding of the complex interplay between hydrodynamics and specific light availability when cultivating phototrophic microorganisms in flat-panel airlift loop photobioreactors (FPA-PBRs), ultimately resulting in an improved mode of operation decreasing operational expenditures (OPEX). A unique statistical treatment of experimental data was developed in order to cope with fluctuations in growth deriving from the biological system as well as technical limits. In addition, the treatment allowed for a quantitative assessment of the expected volumetric productivity in dependence on the prevailing biomass concentration and PFD.

In order to attain the overarching objective, first investigations provided insights into the efficiency of installations generating a directed horizontal flow of the culture suspension within FPA-PBRs (see Section 4.2). Although the optimization of hydrodynamics utilizing static-mixers is neither a novelty nor a unique feature of the deployed technology (Huang *et al.*, 2014; Ugwu *et al.*, 2005; Ugwu *et al.*, 2002; Ugwu *et al.*, 2003), this was the first work to report data on commercially utilized and mass-produced PBRs. Both, volumetric productivity

---

and final biomass yield outperformed data originating from other studies although it needs to be taken into account that the organisms in question differed. It was duly presented that directed turbulence has a significant influence on culture performance and can improve economics of microalgae biotechnology through reducing light limitation and respiratory losses. In fact, at an identical energy input, maximum productivity increased by a factor 3.7 and final biomass yield doubled. Therefore, assuming a proportion of the mixing-energy spent on total biomass production costs of 23% (see Section 3.3), these may be reduced to 11.5% implementing changes in the hardware deployed.

Subsequent investigations addressed striven outdoor cultivations towards the production of bulk (see Section 4.3). These cultivations are prone to fluctuating light conditions whereby light, depending on the prevailing biomass concentration and PFD, may be limiting or inhibiting photosynthesis and thus reduce product yield. Hereby, depending on prevalent weather conditions, fluctuations may occur within time intervals being as short as milliseconds, technologically prohibiting the adaptation of the culture density to the actual PFD. Therefore, other technological means are required to increase process performance whilst minimizing energy demand. Operating strategies were developed in order to reduce the energy spent on mixing by applying discontinuous aeration (Leupold *et al.*, 2013) or by reducing agitation during nighttime (Eustance *et al.*, 2015). Whilst energy savings were accompanied with reduced growth when applying the former strategy, the latter only reduced the energy input during nighttime characterized by static darkness. The operating strategy developed within the present study represents a novelty beyond the state-of-the-art as an on-line adaptation of the energy input, e.g. the superficial gas velocity, in dependence on the prevailing biomass concentration and PFD is proposed. Hereby, only readily available and valid measurement and control technology is used, capable of adjusting process parameters timely and on demand. When mathematically translated to exemplary outdoor diurnal cycles of the PFD, productivity is increased by 24%, while reducing not only energy input but also CO<sub>2</sub>-demand by 11%. On cloudy days, productivity is only slightly increased but energy input and CO<sub>2</sub>-demand reduced by 37%.

Future work should now validate the proposed strategy under actual production conditions outdoors and translate findings to further photosynthetic microorganisms. In addition, an advanced control-command system should be established allowing for a model based in-

finitely variable adjustment of the aeration rate, ultimately fine-tuning the described findings maximizing process efficiency.

Conclusively, within the framework of the detailed development it was achieved to not only evolve a solution for outdoor application, but also for microalgal cultivations taking place indoors by applying an intermittent aeration (see section 5.1). In both cases, reduction of OPEX is not only attributed to minimizing the specific energy demand required for culture mixing, but also to reducing the applied CO<sub>2</sub>-feed to the same extend. This is especially relevant for indoor cultivations targeting the production of added-value products such as pharmaceuticals or cosmetic ingredients, thus prohibiting the utilization of industrial exhaust emissions as a source of carbon dioxide whilst being dependent on rather expensive technical CO<sub>2</sub>.

During outdoor cultivation utilizing solar light as energy source, electrical power spent on culture mixing through e.g. compressors or pumps is one of the major cost drivers of microalgal biotechnology. Being able to cut these by 37% will take the industry one step further along the path towards a sustainable supply of bulk in the philosophy of a *Blue Economy*.

# CHAPTER 7

---

## Bibliography

---

- Acién, F. G., Fernández, J. M., Magán, J. J., and Molina, E. (2012). “Production cost of a real microalgae production plant and strategies to reduce it”. *Biotechnol. Adv.* 30(6), pp. 1344–1353.
- Béchet, Q., Muñoz, R., Shilton, A., and Guieysse, B. (2013). “Outdoor cultivation of temperature-tolerant *Chlorella sorokiniana* in a column photobioreactor under low power-input”. *Biotechnol. Bioeng.* 110(1), pp. 118–126.
- Blanken, W., Cuaresma, M., Wijffels, R. H., and Janssen, M. (2013). “Cultivation of microalgae on artificial light comes at a cost”. *Algal Research* 2(4), pp. 333–340.
- Borowitzka, M. A. (1997). “Microalgae for aquaculture: opportunities and constraints”. *J. Appl. Phycol.* 9(5), pp. 393–401.
- (2016). “Systematics, Taxonomy and Species Names: Do They Matter?” In: *The Physiology of Microalgae*. Ed. by M. A. Borowitzka, J. Beardall, and J. A. Raven. Cham: Springer International Publishing, pp. 655–681.
- Clement, G., Giddey, C., and Menzi, R. (1967). “Amino acid composition and nutritive value of the alga *Spirulina maxima*”. *J. Sci. Food Agric.* 18(11), pp. 497–501.
- Credence Research (2017). *Credence Research Homepage*. URL: <https://www.cred-enceresearch.com/report/algae-products-market>. Accessed: 2018-06-19.
- Cuaresma, M., Janssen, M., Vílchez, C., and Wijffels, R. H. (2009). “Productivity of *Chlorella sorokiniana* in a short light-path (SLP) panel photobioreactor under high irradiance”. *Biotechnol. Bioeng.* 104(2), pp. 352–359.

- Doucha, J. and Lívanský, K. (2006). "Productivity, CO<sub>2</sub>/O<sub>2</sub> exchange and hydraulics in outdoor open high density microalgal (*Chlorella* sp.) photobioreactors operated in a Middle and Southern European climate". *J. Appl. Phycol.* 18(6), pp. 811–826.
- Dvořák, P., Poulíčková, A., Hašler, P., Belli, M., Casamatta, D. A., and Papini, A. (2015). "Species concepts and speciation factors in cyanobacteria, with connection to the problems of diversity and classification". *Biodiversity and Conservation* 24(4), pp. 739–757.
- Eberly, J. O and Ely, R. L. (2012). "Photosynthetic accumulation of carbon storage compounds under CO<sub>2</sub> enrichment by the thermophilic cyanobacterium *Thermosynechococcus elongatus*". *J. Ind. Microbiol. Biotechnol.* 39(6), pp. 843–850.
- Elsevier (2017). *Elsevier's Algal Research Journal Homepage*. URL: <https://www.journals.elsevier.com/algal-research>. Accessed: 2017-08-16.
- Eustance, E., Wray, J. T., Badvipour, S., and Sommerfeld, M. R. (2015). "The effects of limiting nighttime aeration on productivity and lipid accumulation in *Scenedesmus dimorphus*". *Algal Research* 10, pp. 33–40.
- Fasaei, F., Bitter, J. H., Slegers, P. M., and Boxtel, A. J. B. van (2018). "Techno-economic evaluation of microalgae harvesting and dewatering systems". *Algal Research* 31, pp. 347–362.
- Flores, E. and Herrero, A. (2014). *Introduction to the cyanobacteria*. Ed. by E. Flores and A. Herrero. Norfolk: Caister Academic Press, pp. IX–X.
- Freeman, B. C. and Bhowan, A. S. (2011). "Assessment of the technology readiness of post-combustion CO<sub>2</sub> capture technologies". *Energy Procedia* 4, pp. 1791–1796.
- Fujisawa, T., Narikawa, R., Maeda, S., Watanabe, S., Kanesaki, Y., Kobayashi, K., Nomata, J., Hanaoka, M., Watanabe, M., Ehira, S., *et al.* (2016). "CyanoBase: a large-scale update on its 20th anniversary". *Nucleic Acids Res.* 45(D1), pp. D551–D554.
- García, J. L., Vicente, M., and Galan, B. (2017). "Microalgae, old sustainable food and fashion nutraceuticals". *Microb. Biotechnol.* 10(5), pp. 1017–1024.
- Giordano, M., Beardall, J., and Raven, J. A. (2005). "CO<sub>2</sub> concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution". *Annu. Rev. Plant Biol.* 56, pp. 99–131.
- Graham, L.E. and Lee, W. (2000). *Algae*. Upper Saddle River: Prentice Hall.

- 
- Gris, B., Sforza, E., Morosinotto, T., Bertucco, A., and La Rocca, N. (2017). "Influence of light and temperature on growth and high-value molecules productivity from *Cyanobacterium aponinum*". *J. Appl. Phycol.* 29(4), pp. 1781–1790.
- Grossman, A. R., Schaefer, M. R., Chiang, G. G., and Collier, J. L. (1993). "The phycobilisome, a light-harvesting complex responsive to environmental conditions." *Microbiological Reviews* 57(3), pp. 725–749.
- Gudin, C. and Thepenier, C. (1986). "Bioconversion of solar energy into organic chemicals by microalgae". *Advances in Biotechnological Processes*.
- Guiry, M. D. (2012). "How many species of algae are there?" *J. Phycol.* 48(5), pp. 1057–1063.
- Guiry, M. D. and Guiry, G. M. (2018). *AlgaeBase. World-wide electronic publication, National University of Ireland, Galway*. URL: <http://www.algaebase.org>. Accessed: 2018-06-15.
- Habib, M. A. B., Huntington, T., and Hasan, M. R. (2008). *A review on culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish*. Rome: Food and Agriculture Organization of the United Nations.
- Hagemann, M. and Hess, W. R. (2018). "Systems and synthetic biology for the biotechnological application of cyanobacteria". *Curr. Opin. Biotechnol.* 49, pp. 94–99.
- Hamilton, T. L., Bryant, D. A., and Macalady, J. L. (2016). "The role of biology in planetary evolution: cyanobacterial primary production in low-oxygen Proterozoic oceans". *Environ. Microbiol.* 18(2), pp. 325–340.
- Hanagata N. and Takeuchi, T., Fukuju, Y., Barnes, D.J., and Karube, I. (1992). "Tolerance of microalgae to high CO<sub>2</sub> and high temperature". *Phytochemistry* 31(10), pp. 3345–3348.
- Hoiczuk, E. (2000). "Gliding motility in cyanobacteria: observations and possible explanations". *Arch. Microbiol.* 174(1-2), pp. 11–17.
- Hori, K., Okamoto, J., Tanji, Y., and Unno, H. (2003). "Formation, sedimentation and germination properties of *Anabaena akinetes*". *iochem. Eng. J.* 14(1), pp. 67–73.
- Huang, J., Li, Y., Wan, M., Yan, Y., Feng, F., Qu, X., Wang, J., Shen, G., Li, W., Fan, J., *et al.* (2014). "Novel flat-plate photobioreactors for microalgae cultivation with special mixers to promote mixing along the light gradient". *Bioresour. Technol.* 159, pp. 8–16.
- Huang, Q., Jiang, F., Wang, L., and Yang, C. (2017). "Design of photobioreactors for mass cultivation of photosynthetic organisms". *Engineering* 3(3), pp. 318–329.
-

- Kaneko, T., Nakamura, Y., Wolk, C. P., Kuritz, T., Sasamoto, S., Watanabe, A., Iriguchi, M., Ishikawa, A., Kawashima, K., Kimura, T., *et al.* (2001). "Complete genomic sequence of the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120". *DNA Research* 8(5), pp. 205–213.
- Kern, J., Loll, B., Lüneberg, C., DiFiore, D., Biesiadka, J., Irrgang, K.-D., and Zouni, A. (2005). "Purification, characterisation and crystallisation of photosystem II from *Thermosynechococcus elongatus* cultivated in a new type of photobioreactor". *Biochimica Et Biophysica Acta (BBA)-Bioenergetics* 1706(1-2), pp. 147–157.
- Kim, M. (2017). "Phototaxis of Cyanobacteria under Complex Light Environments". *mBio* 8(2), e00498–17.
- Knoche, W. (1980). "Chemical Reactions of CO<sub>2</sub> in Water". In: *Biophysics and Physiology of Carbon Dioxide*. Ed. by C. Bauer, G. Gros, and H. Bartels. Berlin, Heidelberg: Springer, pp. 3–11.
- Kölsch, A., Hejazi, M., Stieger, K. R., Feifel, S. C., Kern, J. F., Müh, F., Lisdat, F., Lokstein, H., and Zouni, A. (2018). "Insights into the binding behavior of native and non-native cytochromes to photosystem I from *Thermosynechococcus elongatus*". *J. Biol. Chem.* 293(23), pp. 9090–9100.
- Lee, R. E. (2008). *Phycology*. New York: Cambridge University Press.
- Lem, N. W. and Glick, B. R. (1985). "Biotechnological uses of cyanobacteria". *Biotechnol. Adv.* 3(2), pp. 195–208.
- Leupold, M., Hindersin, S., Kerner, M., and Hanelt, D. (2013). "The effect of discontinuous airlift mixing in outdoor flat panel photobioreactors on growth of *Scenedesmus obliquus*". *Bioprocess. Biosyst. Eng.* 36(11), pp. 1653–1663.
- Liang, F., Englund, E., Lindberg, P., and Lindblad, P. (2018). "Engineered cyanobacteria with enhanced growth show increased ethanol production and higher biofuel to biomass ratio". *etab. Eng.* 46, pp. 51–59.
- Mao, R. and Guo, S. (2018). "Performance of the mixed LED light quality on the growth and energy efficiency of *Arthrospira platensis*". *Appl. Microbiol. Biotechnol.* 102(12), pp. 5245–5254.
- Mata, T. M., Martins, A. A., and Caetano, N. S. (2010). "Microalgae for biodiesel production and other applications: a review". *Renewable Sustainable Energy Rev.* 14(1), pp. 217–232.

- 
- Nakamura, Y., Kaneko, T., Sato, S., Ikeuchi, M., Katoh, H., Sasamoto, S., Watanabe, A., Iriguchi, M., Kawashima, K., Kimura, T., *et al.* (2002). "Complete genome structure of the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1". *DNA Research* 9(4), pp. 123–130.
- Norsker, N.-H., Barbosa, M. J., Vermuë, M. H., and Wijffels, R. H. (2011). "Microalgal production—a close look at the economics". *Biotechnol. Adv.* 29(1), pp. 24–27.
- Nowruzzi, B., Haghghat, S., Fahimi, H., and Mohammadi, E. (2018). "Nostoc cyanobacteria species: a new and rich source of novel bioactive compounds with pharmaceutical potential". *Journal of Pharmaceutical Health Services Research* 9(1), pp. 5–12.
- Oren, A (2014). "Cyanobacteria: biology, ecology and evolution". In: *Cyanobacteria: An Economic Perspective*. Ed. by K. S. Naveen, K. R. Ashwani, and J. S. Lucas. Oxford: Wiley-Blackwell, pp. 1–20.
- Ovando, C. A., Carvalho, J. C., G., Vinícius M. P., Jacques, P., Soccol, V. T., and Soccol, C. R. (2018). "Functional properties and health benefits of bioactive peptides derived from *Spirulina*: A review". *Food Reviews International* 34(1), pp. 34–51.
- Panjjar, N., Mishra S. and Yadav, A. N., and Verma, P. (2017). "Functional Foods from Cyanobacteria: An Emerging Source for Functional Food Products of Pharmaceutical Importance". *Microbial Functional Foods and Nutraceuticals*, pp. 21–37.
- Plaza, I., Garcia, J. L, and Villarroel, M. (2018). "Effect of spirulina (*Arthrospira platensis*) supplementation on tilapia (*Oreochromis niloticus*) growth and stress responsiveness under hypoxia". *Spanish Journal of Agricultural Research* 16(1), p. 0606.
- Pulz, O. and Gross, W. (2004). "Valuable products from biotechnology of microalgae". *Appl. Microbiol. Biotechnol.* 65(6), pp. 635–648.
- Richmond, A. (2004). "Principles for attaining maximal microalgal productivity in photobioreactors: an overview". In: *Asian Pacific Phycology in the 21st Century: Prospects and Challenges*. Dordrecht: Springer, pp. 33–37.
- Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., and Stanier, R. Y. (1979). "Generic assignments, strain histories and properties of pure cultures of cyanobacteria". *Microbiology* 111(1), pp. 1–61.
- Ruiz, Jesús, Olivieri, Giuseppe, Vree, Jeroen de, Bosma, Rouke, Willems, Philippe, Reith, J Hans, Eppink, Michel HM, Kleinegris, Dorinde MM, Wijffels, René H, and Barbosa, Maria J
-

- (2016). "Towards industrial products from microalgae". *Energy & Environmental Science* 9(10), pp. 3036–3043.
- Schulze, P. S. C., Barreira, L. A., Pereira, H. G. C., Perales, J. A., and Varela, J. C. S. (2014). "Light emitting diodes (LEDs) applied to microalgal production". *Trends in biotechnology* 32(8), pp. 422–430.
- Selim, S., Hussein, E., and Abou-Elkhair, R. (2018). "Effect of *Spirulina platensis* as a feed additive on laying performance, egg quality and hepatoprotective activity of laying hens". *European Poultry Science* 82.
- Seyidoglu, N., Inan, S., and Aydin, C. (2017). "A prominent superfood: *Spirulina platensis*". In: *Superfood and Functional Food-The Development of Superfoods and Their Roles as Medicine*. Ed. by N. Shiomi and V. Waisundara. InTech.
- Shay, L. K., Hunt, H. R., and Wegner, G. H. (1987). "High-productivity fermentation process for cultivating industrial microorganisms". *J. Ind. Microbiol.* 2(2), pp. 79–85.
- Tredici, M. R., Rodolfi, L., Biondi, N., Bassi, N., and Sampietro, G. (2016). "Techno-economic analysis of microalgal biomass production in a 1-ha Green Wall Panel (GWP®) plant". *Algal Research* 19, pp. 253–263.
- Trentacoste, E. M., Martinez, A. M., and Zenk, T. (2015). "The place of algae in agriculture: policies for algal biomass production". *Photosynth. Res.* 123(3), pp. 305–315.
- Ugwu, C., Ogbonna, J., and Tanaka, H. (2002). "Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers". *Appl. Microbiol. Biotechnol.* 58(5), p. 600.
- Ugwu, C. U., Ogbonna, J. C., and Tanaka, H. (2003). "Design of static mixers for inclined tubular photobioreactors". *J. Appl. Phycol.* 15(2), pp. 217–223.
- (2005). "Characterization of light utilization and biomass yields of *Chlorella sorokiniana* in inclined outdoor tubular photobioreactors equipped with static mixers". *Process Biochem.* 40(11), pp. 3406–3411.
- Varshney P. and Mikulic, P., Vonshak, A., and Beardall J. and Wangikar, P. P. (2015). "Extremophilic micro-algae and their potential contribution in biotechnology". *Bioresour. Technol.* 184, pp. 363–372.
- Whitton, B. A. and Potts, M. (2012). "Introduction to the cyanobacteria". In: *Ecology of Cyanobacteria II*. Ed. by B. Whitton. Dordrecht: Springer, pp. 1–13.

- 
- Wijffels, R. H. and Barbosa, M. J. (2010a). "An outlook on microalgal biofuels". *Science* 329(5993), pp. 796–799.
- Wijffels, R. H., Barbosa, M. J., and Eppink, M. H. M. (2010b). "Microalgae for the production of bulk chemicals and biofuels". *Biofuels, Bioprod. Biorefin.* 4(3), pp. 287–295.
- Yamaoka, T., Satoh, K., and Katoh, S. (1978). "Photosynthetic activities of a thermophilic blue-green alga". *Plant and Cell Physiology* 19(6), pp. 943–954.