Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

UV radiation as a new tool to control microalgal bio-product yield and quality

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Industrial Biotechnology

at

Massey University, Palmerston North, New Zealand

Roland Schaap

2018

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Abstract

While ultraviolet (UV) radiation is most commonly known as an abiotic stress, various studies have shown targeted UV exposure increases bioproduct and biomass yields in microalgae. Microalgal cultivation processes face significant limitations in achievable bioproduct and biomass yields and thus improvements offered by targeted UV treatments during large-scale microalgae cultivation provide an opportunity for development of a novel UV treatment tool. Growing demand in microalgae (bio)products indicate there may be a substantial market for such UV treatment tools. No initiatives that explore the development of targeted UV treatments during large-scale microalgae cultivation have been found in the literature or in the industry. In collaboration with industrial partner BioLumic, a company specializing in applying targeted UV treatments in plants as a tool in agriculture, this PhD research examined if specific treatments of UV radiation (i.e. specific in UV waveband, irradiance and exposure duration) can reliably increase carotenoid accumulation in the microalga *Dunaliella salina* and if this new understanding can be feasibly used to develop an industrial system for UV treatment of microalgae.

The PhD research was conducted utilizing *D. salina* after evaluation in four commercially relevant microalgae species: *Arthrospira platensis, Chlorella vulgaris, Haematococcus pluvialis* and *D. salina*. A UV-A induced carotenoid accumulation response was identified in *D. salina* (strain UTEX 1644). Targeted UV-A treatments reliably induced carotenoid accumulation in this species, and the magnitude of the response depended on the UV-A wavelength, UV irradiance, UV exposure duration, and UV dose. The UV-A carotenoid accumulation response was induced within 6 hours and was largely complete in 96 hours (24 $h \cdot d^{-1}$ UV exposure). The highest UV-A dose tested induced the highest carotenoid accumulation rates and the highest total carotenoid concentrations after continuous UV exposure (24 $h \cdot d^{-1}$) at the highest UV-A irradiance tested (30 $W \cdot m^{-2}$). Total carotenoid concentration increases of up to 162% were thus observed after 72 hr of UV-A exposure. UV-A exposure was associated with slowed or stopped cell proliferation as well as increased *D. salina* cell size (up to 15%) and altered intracellular structural organization. Carotenoid accumulation ceased and cell proliferation increased when UV-A exposure was stopped, leading to a subsequent resumption of cell proliferation. UV-A induced carotenoid accumulation was improved 51% during UV-A exposure concomitant with non-UV carotenogenic stimuli (high PAR intensity and salinity) compared to UV-A exposure alone.

The observations from experiments carried out in the thesis served as inputs in a techno-economic analysis (TEA) model developed to assess feasibility of large-scale UV treatment. The TEA model was developed to allow assessment of the most critical areas for improving profitability of large-

scale UV treatment technology, rather than provide absolute economical outputs for revenue and profit. The TEA was based on two reference cultivation systems currently used for commercial *D. salina* cultivation. The TEA analysis considered four locations for the UV treatment system applied along the cultivation process: pre-cultivation stage (i.e. inoculum), main cultivation stage, post-cultivation stage (i.e. immediately prior to harvest) and during fluid transfer between stages. A dedicated post-cultivation UV treatment stage was shown to have a number of advantages over other treatment options.

A model cultivation system for the case-study of *D. salina* was developed assuming an annual β-carotene production of 1,000 kg. The developed TEA model cultivation system and TEA UV treatment system were able to identify a potential increase in profitability generated from the application targeted UV treatment during large-scale *D. salina* cultivation. The maximum increase in profitability was achieved using a broad wavelength UV treatment system (irradiance = 30 $W \cdot m^{-2}$, exposure duration = 24 $h \cdot d^{-1}$, surface area coverage = 100%) applied during an intensive cultivation post-cultivation system. A relatively small contribution of the UV treatment system to CAPEX and OPEX to overall β-carotene production cost (i.e. < 10%) combined with the large increase in β-carotene production (711 $kg \cdot y^{-1}$ and 895 $kg \cdot y^{-1}$ for fluorescent UV tube and UV LED systems, respectively) leads to potentially large increases in profitability. The TEA analysis identified the magnitude of the UV-A induced carotenoid accumulation response to be the most important factor to influence the potential profitability. Moreover, the TEA indicated the increases in profitability are strongly influenced by optical efficiency, electrical efficiency and maximum optical power. The profitability estimates from the current TEA indicate that UV treatment during commercial microalgae cultivation has potential and justifies further research.

To our knowledge the exploration of the fundamental UV photobiology in microalgae required to develop UV treatment regimes from discrete UV wavebands, complemented with a commercial microalgal-engineering insight, to produce UV treatment regimes and UV treatment technology for application during large-scale microalgae cultivation, has never been attempted. The multidisciplinary approach employed during this PhD research explored for the first time the development of a UV treatment system from laboratory observations to commercial cultivation. The current research described for the first time the UV response behaviour of *D. salina* (strain UTEX 1644) to varying UV waveband, UV irradiance, UV exposure durations as well as UV response interactions with PAR and salinity. The case-study of UV treatment during large-scale *D. salina* cultivation in this PhD research allowed recommendations to be made to the industrial partner BioLumic on potential areas of focus for continued research and development.

Acknowledgments

First and foremost, I would like to thank my supervisors Professor Benoit Guieysse, Associate Professor Jason Wargent and Professor Julian Heyes for their support and encouragement throughout this project. My time as a PhD student got off to a rocky start but I am thankful to my supervisors for their understanding and efforts during the unplanned end of one PhD project and the seamless transition into another. I am grateful to have had the opportunity to develop myself academically and professionally under their tutelage.

Thank you to BioLumic for offering me the opportunity to work on this project. Thank you to all the staff at BioLumic and in particular Warren Bebb and Hangfeng Ji at BioLumic for all their help. Also, I would like to thank Callaghan Innovation for providing the funding that made this research possible by awarding the R&D Fellowship Grant on not one, but two occasions.

My PhD would not have been the same had I not been surrounded by great people at Massey. Thank you first and foremost to all my colleagues in the office, in the lab and all around Massey with whom I have gotten to share my PhD journey. I'd like to thank everyone for the help they provided during my research in between the endless hours of banter, lunches, activities and beers we got to enjoy together. I'm glad I got to share so many good times with so many great people.

I would like to extend my gratitude to all the staff at the Riddet Institute for making my time at Massey a good one. Special thanks go out to all the staff at the Microlab for all their technical support and for always providing laughter and chatter during the endless hours in the lab. Thank you to Ann-Marie Jackson, Kylie Evans, John Edwards, John Sykes, Julia Good and Nereda Corbett for making the lab such an enjoyable place to be and tolerating me poking my nose in everywhere. Thank you to Glenda for all her work and for letting us stay in our office despite 'the state of it'.

As our three-year trip turned in to four (and counting) there has been a lot that we have had to miss out on back home. I would like to thank my family and friends in the Netherlands for all the support we have received and all the effort everyone has made to stay in frequent contact all these years. I am very grateful I got to share some of our life here in New Zealand with those who were able to make the time and effort to come and visit us on the other side of the world.

And last but definitely not least, I would like to thank my partner Mirjam de Oude who agreed to join me on a journey to the other side of the world, into the unknown, just so I could do my PhD research. She has been my support through thick and thin and I am proud of everything we have achieved here in New Zealand.

Table of contents

List of Figures

List of Tables

Abbreviations and terms

BWF	Biological weighing function
CAPEX	Capital expense
Car:Chl	Ratio 'total carotenoid concentration to total chlorophyll *(a+b)* concentration'
Cellular carotenoid/ chlorophyll *(a+b)* content	µg pigment·10^6 cells^{-1}
CPD	Cyclobutane-type pyrimidine dimer
DW	Dry weight
Extensive cultivation	Commercial microalgae cultivation process using open ponds
Carboxy-H_2DFFDA	5-(and-6)-carboxy-2',7'-difluorodihydrofluorescein diacetate
HRT	Hydraulic retention time (days)
Intensive cultivation	Commercial microalgae cultivation process using raceway ponds
MAA	Mycosporine-like amino acid
OD_{687}	Optical density at λ = 687 nm
ΔOD_{687}	Change in OD_{687} over time
OPEX	Operational expense
PAR	Photosynthetically Active Radiation (λ = 400 - 700 nm)
PAR-Only	Cultures exposed to PAR radiation only during experimental treatments
PFD	Photon Flux Density (µmol·m^{-2}·s^{-1})
PSII	Photosystem II
ROS	Reactive oxygen species
TEA	Techno-economic analysis
Total carotenoid/ chlorophyll *(a+b)* concentration	µg pigment·mL culture^{-1}
UV	Ultraviolet radiation (λ = 100 - 400 nm)
UV-A	Part of UV waveband with λ = 315 - 400 nm
UV-B	Part of UV waveband with λ = 280 - 315 nm

UV-C	Part of UV waveband with λ = 100 - 280 nm
λ_{max}	Emission peak