UTS UNIVERSITY OF TECHNOLOGY SYDNEY

Design of a scalable, single-use photo-bioreactor for the growth of algae in axenic conditions

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PhD by research

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Certificate of original authorship

I, Julian R. Kofler, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctorate of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by the Australian Government Research Training Program.

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Julian R. Kofler
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Thesis format

This thesis is comprised of an introduction chapter (Chapter 1), three data chapters (Chapter 2 to 4) and a conclusion chapter (Chapter 5). A detailed project overview is given subsequently after Chapter 1. At time of thesis submission, Chapter 1 (review portion) and 2 are in final draft for publication.

List of publications

The method that forms the basis for Chapter 2 has been submitted:

Alonso Zavafer, Harvey Bates, Leen Labeeuw, **Julian R. Kofler**, Peter J. Ralph, (2021). Normalized chlorophyll fluorescence imaging: a method to determine irradiance and photosynthetically active radiation in phytoplankton cultures. *Algal Research, (in review)*

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List of Abbreviations

AUS	Australia
BR	Bioreactor
C3G	C3 glomerulopathy
CAD	Computer aided design
CAPEX	Capital expenditure
CFD	Computational fluid dynamics
CHO	Chinese Hamster Ovary
D/L	Dark to light
D50	Penetration depth of 50% light attenuation in microalgae culture
D90	Penetration depth of 90% light attenuation in microalgae culture
DCFI	Direct Chlorophyll Fluorescence Imaging
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EPO	Erythropoetin
ETR	Electron transport rate
EU	European Union
FDA	Food and Drug Administration
FFT	Fast Fourier transformation
GEM	Genetically engineered microalgae
GESD	Generalized extreme studentized deviate
GM	Genetically modified
GMO	Genetically modified organism
GMP	Good manufacturing practice
GRAS	Generally recognized as Safe
HCL	Hydrochloric acid
ISAAA	International Service for the Acquisition of Agri-Biotech
ISPE	International Society for Pharmaceutical Engineering
LED	Light emitting diode
NaOH	Sodium hydroxide
NCFI	Normalized Chlorophyll Fluorescence Imaging
OGTR	Office of the Gene Technology Regulator
OPEX	Operating expenses
PAM	Pulse amplitude modulation
PAR	Photosynthetically Active Radiation
PBR	Photo-bioreactor
PCB	Printed circuit board
PE	Polyethylene
PPFD	Photosynthetic photon flux density
PSII	photosystem II
PTM	Post-translational modification
qPCR	quantitative Polymerase Chain Reaction
ROI	Regions of Interest
SD	Standard deviation
USDA	U.S. Department of Agriculture
WFI	water for injection

Abstract

Microalgal cultivation systems for biopharmaceutical production are currently limited and current biopharmaceutical bioreactors are not optimized in terms of efficient light and substrate supply for algae. This project aims to address this gap, by establishing a process to convert and optimize a bioreactor system which is already established in the biopharmaceutical sector into a photo-bioreactor (PBR) system, facilitating axenic microalgae growth at an industrial scale in a regulated environment. The system to be converted is an industrially used single-use bioreactor, for which an optimization platform was designed including both physical and digital components. The physical part consisted of a 200 L PBR and a scaled down 20 L PBR, both mimicking physical characteristics of the industrial bioreactor, thereby enabling the rapid testing of new illumination systems. Different methods, such as gassing-in method (mass transfer), pH- and dye-method (mixing time) and optical particle tracing (hydrodynamic flow) were utilized to characterise the system and validate the down-scaling process, which revealed similar cultivation features compared to the industrial bioreactor. The predominant focus of the optimization platform was the supply of light: as such, accurate and precise data of the light attenuation were needed. A novel, practical, and easily applicable optical method using modified cameras for measuring the light distribution of complex light sources was developed to address this – Direct Chlorophyll Fluorescence Imaging (DCFI). DCFI was applied to *Phaeodactylum tricronutum* and *Chlorella vulgaris* cultures at different cell concentrations for a variety of LED wavelengths, yielding precise light maps of the light distribution into the culture. These light maps and the particle tracing data were combined in a computer aided design (CAD) process which enabled the calculation of the best configuration of the artificial light system (LEDs) according to the optimal light experience for the microalgae cells. The CAD forms the digital component of the optimization platform and completes the system. The optimization platform and the underlying methodology builds the foundation for a streamlined approach to convert existing bioreactor systems or to optimize alternative PBR systems. As such, this technology can help in establishing microalgae as a cultivation system in the biopharmaceutical sector.