

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

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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.

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