Development and implementation of high-throughput phenotyping tools for microalgae

by Harvey Bates

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Under the supervision of

Prof Peter Ralph

Dr. Milán Szabó

Dr. Alonso Zavaleta Fernandez de Cordova

University of Technology Sydney

Faculty of Science (Climate Change Cluster)

November 2020

Certificate of Original Authorship

I Harvey Bates, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science (Climate Change Cluster, C3) at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced 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 an Australian Government Research Training Program.

Signature:

Production Note:
Signature removed prior to publication.

Date: November 30, 2020

Acknowledgements

I would like to thank my supervisors, Prof Peter Ralph, Dr. Milán Szabó and Dr. Alonso Zavaleta for their support, guidance and encouragement over the past few years. Despite our time-zone and spatial differences it has been great gain your friendship and expertise. If it were not for your encouragement to peruse areas outside of my comfort zone this thesis could not have been formulated.

I would also like to thank the technical staff (including but not limited to Paul Brooks and Scott Allchin) at the Climate Change Cluster for their role in assisting me in the laboratory over the course of my candidature.

Finally, I would like to thank my family for their support during my studies.

Contribution of Authors

Each chapter of this thesis was overseen by Prof Peter Ralph, Dr. Milán Szabó and Dr. Alonso Zavaleta. This includes the formulation of concepts, experiments and reviews/editing of text.

Thesis Format

This thesis is compiled with published, submitted and in preparation manuscripts. An introductory chapter is used to explain concepts and review the current state of literature on the explored topics. Chapters two to four are experimental chapters where instrumentation is developed, tested and novel experiments are carried out. The thesis synthesis is used to explore the broad reach that this research applies to and present concepts that may help to drive future microalgal physiological research.

Nomenclature

Acronyms

ETR_{II}	Absolute electron transport rate of photosystem II
PAR_{II}	Photo absorption rate of photosystem II
PI_{abs}	Performance index (absorption)
R	Correlation coefficient
V_j	Relative variable fluorescence at the J-step
Z	Z-score
3D	Three dimensional
pmf	Proton motive force
A	Ampere
ADC	Analog-to-digital converter
ADP	Adenosine diphosphate
AL	Actinic light
ATP	Adenosine triphosphate
C	Capacitor or capacitance
CEF	Cyclic electron flow
DAQ	Data acquisition device
DC	Direct current
DC-DC	Direct current to direct current

Fd	Ferredoxin
FRR	Fast repetition rate
ID	Inner diameter
IDE	Integrated development environment
IV	Intravenous
LC	Light induction curve
LED	Light emitting diode
LEF	Linear electron flow
LHC	Light harvesting complex
LiPo	Lithium-ion polymer
MC-PAM	Multicolour pulse amplitude modulated fluorometer
$NADP^+$	Nicotinamide adenine dinucleotide phosphate reduced
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
ND	Neutral density
OD	Optical density or outer diameter
OD_{875}	Optical density determined at 875 nm
OEC	Oxygen evolving complex
P_{max}	Maximum photosynthetic rate as determined by a PI-Curve
PAM	Pulse Amplitude Modulation
PAR	Photosynthetically active radiation (400 - 700 nm)
PBR	Photobioreactor
PC	Plastocyanin or personal computer
PCB	Printed circuit board

PGI	Plotting graphical interface
Pi	Inorganic Phosphate
PI-Curve	Photosynthesis-irradiance curve
PLA	Polylactic acid
PQ	Plastoquinone
PQ Pool	Plastoquinone pool
PSI	Photosystem I
PSII	Photosystem II
PWM	Pulse width modulation
Q_A	Quinone A
Q_B	Quinone B
qE	Energy dependent quenching
R	Resistance
RB	Red blue
RCII	Reaction center of photosystem II
rETR	Relative electron transport rate
RGB	Red green blue
RPi	Raspberry pi computer
SMD	Surface mount device
SQL	Structural query language
T	Transistor
V	Volt or voltage
VDE	Violaxanthin de-epoxidase

VNC	Virtual network computing

Glossary

$\Delta\Psi$	Electrical potential
ΔpH	Change in pH (proton gradient)
$\phi PSII$	Effective quantum yield of photosystem II
$\phi PSIIQ_A$	Quantum efficiency at the J-step (Q_A only)
σ_λ	Wavelength-dependent functional cross section of photosystem II
$^1O_2$	Singlet oxygen
F_i	Fluorescence intensity at the I-step
F_j	Fluorescence intensity at the J-step
F_m	Maximum fluorescence intensity (equivalent to the P-step)
$F_m^{'}$	Maximum fluorescence intensity under actinic illumination
F_o	Minimum fluorescence intensity (equivalent to the O-step)
F_t	Fluorescence intensity immediately before saturation pulse under actinic illumination or the fluorescence intensity at some time-point
F_v	Variable fluorescence
F_v/F_m	Quantum efficiency of PSII if all reaction centres are open (ϕ_{Po} or ϕ_{po} or $Y(II)_{MAX}$)
FN_{1s}	Normalised fluorescence intensity at one second
FN_t	Normalised fluorescence intensity at a point in time
I_1-level	Maximum fluorescence intensity with an oxidised PQ pool
k	Doubling time of microalgal cells in solution

M_0	Closure rate of PSII
$O - I_1$ rise	Chlorophyll *a* fluorescence rise
V_t	Relative variable fluorescence
$Y(II)$	Effective quantum yield of photosystem II
Bilins	Phycobilisomes
CO_2	Carbon dioxide
FNR	ferredoxin-$NADP^+$ oxidoreductase
JIP-test	Technique for analysing OJIP transients
O_2	Dioxygen
OJIP	Chlorophyll *a* fluorescence technique (fast rise)
P680	Pigment center of photosystem II
$P680^+$	Excited state of the pigment center of photosystem II
P700	Pigment center of photosystem I
PQH_2	Plastoquinol

Constants

ETR_{Factor}	Incident PAR absorbed by photosynthetic antenna and the distribution of energy between PSII and PSI	0.42
L	Avogadro constant	$6.02214076 \times 10^{23}\, mol^{-1}$

LIST OF FIGURES

ABSTRACT

Microalgae are promising candidates for numerous biotechnological applications which may help to create sustainable industries of the future. These include the production of sustainable bio-fuels, bio-degradable plastics and food sources for aquaculture. Due to the scale of microalgal biodiversity there is a clear need for tools to assess microalgal phenotypes in an automated manner. However, currently available tools, such as incubators, are either not suitable for reproducible experiments, due to heterogeneous light exposures and lack of physiological measurements or are too expensive for a high-throughput assessment of genotypes, such as commercial photobioreactors. Therefore, this thesis aims to develop a platform upon which robust, open-source sensors and instruments can be created to propel microalgal research into the high-throughput realm.

This thesis presents a low-cost, open-source, multi-wavelength fast polyphasic rise (OJIP) chlorophyll *a* fluorometer (named *Open-JIP*). This device can assess the fine tuning of photosynthesis in microalgae in a non-invasive and rapid manner. The device is compared to commercial instrumentation to demonstrate its ability to resolve the polyphasic rise of chlorophyll *a* fluorescence and its usefulness as an indicator of stress in microalgae.

The developed fluorometer is then integrated into a 3D printed photobioreactor known as the *Phenobottle*. This instrument can assess photosynthesis in a real-time manner and gives the user the controls to automate environmental variables such as bubbling intensity, light quality/quantity and mixing of microalgal solutions. The devices reproducibility is assessed and a simple guide to its functions are presented.

Using these new devices, a novel probe of photosynthetic electron transport in the microalga *Chlorella vulgaris* is formulated (known as FN_{1s}). This measure-

ment allows for a real-time and non-invasive assessment of photosynthetic electron transport, in terms of the electron turnover rate of the electron transport chain in microalgae at a previously undocumented time resolution. Thus, changes in electron transport can now be observed in a matter of minutes rather than previous methods that took upwards of an hour, and no longer requires complex and expensive instrumentation. This may prove useful for researchers looking to optimise microalgal productivity by maximising photosynthetic productivity.

In the final chapter, the presented phenotyping platform is discussed for its potential applications in other areas of microalgal and terrestrial plant research. Additionally, a novel perspective on conducting microalgal physiological research is presented, which may fast-track biotechnological industries of the future.

CONTENTS