Effect of culture conditions on recombinant protein production in *Chlamydomonas reinhardtii*

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Certificate of original authorship

I, Navpreet Kaur Walia declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney. This research is supported by an Australian Government Research Training Program. 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.

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Preface

This thesis has been prepared for submission as a thesis by compilation, however the thesis contains a publishable work. As a result, there is a degree of repetition across chapters, particularly within the introductions and materials and methods sections of each chapter. Supplementary information for each chapter appears in the supplementary data and have been re-numbered accordingly. The referencing format used in this thesis is nature referencing style.

This thesis is dedicated to my loving parents

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Abstract

Microalgae are increasingly being used in recombinant protein production for a number of reasons including low cultivation costs, and the presence of post-translational modification mechanisms. There has been a lot of research on optimization of culture conditions in other expression systems like mammalian cell lines and plants to improve the yield of recombinant proteins. However, not much has been done on optimizing culture conditions to improve the yield of recombinant protein production from microalgae so far. The focus of this thesis was to investigate how different culture conditions affect recombinant protein production from nuclear transgenes in *C. reinhardtii,* with the ultimate goal to find suitable optimized culture strategies to increase production without significant negative side effect on the growth and health of algal cultures. More particularly, this thesis investigated the effect of a culturing strategy using two different growth phases (i.e. biphasic growth) on recombinant protein production in *C. reinhardtii*. The recombinant protein used in this study, interferon alpha 2 A (rIFN α2A) was chosen because of its high commercial value, anti-viral and anti-cancer properties.

Overall, my results suggest that biphasic growth (temperature regime and carbon source) can have significant effect on recombinant protein production in *C. reinhardtii* and should definitively be considered to improve application of microalgae as a commercially viable platform for recombinant protein production.

Publications:

- Chapter 2 has been recently submitted to Biotechnology & Bioengineering and the manuscript is under review now: Navpreet Kaur Walia, Audrey S. Commault, Michele Fabris, Lorenzo Barolo, Jack Adriaans, Peter J. Ralph, Mathieu Pernice. Biphasic temperature regime increases the yield of a therapeutic recombinant protein in the green alga *Chlamydomonas reinhardtii*

- Chapter 3-4 will be submitted in the near future to peer-reviewed scientific journals.