# Developing Next Generation Algae Bioplastic Technology

**by Shawn Price**

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the degree of

**Doctor of Philosophy**

under the supervision of Peter Ralph, Mathieu Pernice &
Unnikrishnan Kuzhiumparambil

University of Technology Sydney
Faculty of Science

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## CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Shawn Price, 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 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 the Australian Government Research Training Program.

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# Preface

## Thesis format

This thesis is written in the format of a thesis by compilation— a combination of published chapters and those unpublished but with the intention of publication in a peer reviewed scientific journal in the near future. Given that this thesis is presented as a series of ready to submit manuscripts, there is an element of repetition in the introduction of some chapters since they are each submitted as stand-alone manuscripts.

The first chapter is a literature review which introduces the field of cyanobacteria as a production platform for the bioplastic poly-hydroxy-butyrate (PHB). The second chapter explores random mutagenesis for the creation of novel cyanobacterial mutant strains with enhanced PHB productivities. The third chapter investigates the use of chemical modulators to elicit and inhibit PHB productivity in cyanobacteria. The fourth chapter explores the use of wastewater as a medium for cyanobacterial PHB production. The fifth chapter is a techno-economic assessment of the economic viability of industrial production of cyanobacterial PHB. The final chapter is a synthesis of the thesis with final perspectives on this exciting research area.

## Publications

At the time of thesis submission, Chapter 1 and 5 have been published in the peer reviewed *Journal of Environmental Chemical Engineering* (IF 5.909). Chapters 2, 3 and 4 are currently in review in different peer reviewed journals and are expected to be published in 2023.

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# Abstract

Plastics enable the modern world to function. They have applications in agriculture, medical biotechnology, consumer products, electronics, construction and much more. Unfortunately, their widespread use comes at the cost of the environment in the form of aquatic and terrestrial pollution. However, bioplastics made from renewable resources that can biodegrade in the environment offers a solution to this problem, but they are held back due to high substrate costs of sugar feedstock required for fermentation.

Cyanobacteria are microscopic photosynthetic organisms capable of converting atmospheric $CO_2$ into a widely used bioplastic, PHB (poly-hydroxy-butyrate). Although this feedstock is significantly cheaper compared to current bioplastic production, the industrial production of cyanobacterial PHB is still not economically viable due to lower PHB productivity rates and high cultivation equipment costs compared to fermentation. In order to progress towards economic viability, four areas were explored as separate data chapters in this thesis:

**Chapter 2: Creation of novel mutant strains through random chemical mutagenesis with superior cyanobacterial PHB productivity**

Targeted genetic engineering requires advanced technical manipulation and prior knowledge of which genes to target. However, random mutagenesis can create mutants with novel mutations which result in a desired phenotype that can be sequenced to learn about new PHB metabolic mechanisms. In this study, ethyl methane sulfonate (EMS) was used to create a mutant library which was screened using fluorescent activated cell sorting (FACS) to sort single cells with BODIPY 493/503 (a neutral lipid dye) into well plates. These mutants were then screened for growth rate before being tested for PHB productivity. Two mutant strains were created with enhanced PHB yields (29% and 26% higher than wild type), biomass densities (36% and 33% higher than wild type) and PHB volumetric densities (75% and 67% higher than wild type).

### Chapter 3: Identifying chemical enhancers and inhibitors of cyanobacterial PHB metabolism

Chemical modulators which affect cyanobacterial metabolism can be used to increase PHB production at industrial scales. The mechanisms of enhancers and inhibitors of PHB production can also be studied to identify genetic and regulatory information on PHB metabolism. Thus, 10 different compounds (including oxidants, antioxidants, phytohormones) were screened at 3 concentrations (0.1 µM, 1 µM and 10 µM) to identify compounds which boosted and reduced PHB production. Two treatments, 0.1 µM IAA and 1 µM methyl jasmonate were found to increase PHB yield (55% and 19% compared to control). Two treatments, 10 µM allopurinol and 10 µM ethynylestradiol, were found to decrease biomass density, PHB yield and PHB density.

### Chapter 4: Exploring municipal wastewater as a media for cyanobacterial PHB production

Using wastewater as a substrate not only reduces demand for fresh water, but also reduces production costs through not requiring synthetically made media. However, wastewater as a substrate introduces the possibility of culture contamination, presence of inhibitory pollutants and provided a unique nutrient profile. This study demonstrated the potential for primary domestic wastewater as a nutrient source of cyanobacterial biomass cultivation with no significant difference between biomass densities compared to the control culture. However, PHB yield was significantly inhibited (85% lower than control) which may have been linked to non-cyanobacterial biomass.

### Chapter 5: Techno-economic modelling to identify key financial drivers of cyanobacterial PHB profitability

This techno-economic modelling study breaks down the key capital and operating costs and identifies the major financial barriers to profitability. For a base case scenario, a 10,000 tonnes of PHB bioplastic resin per year facility in Australia was used with breakeven and sensitivity analysis to assess economic viability. The financial model was then used to explore potential paths to financial viability such as examining the effect of the scale of production volume, additional revenue from utilising a biorefinery approach to cyanobacterial biomass, use of holding or ripening tanks to reduce cultivation costs and geographic location of the hypothetical production facility. The base case revealed that the cost of production was $18.1k USD/tonne which is over four times the current market price of PHB. However, through the combination of several optimistic scenarios, the breakeven price could potentially reach $7.7k USD/tonne.