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Raceway-based production of microalgae for possible use in making biodiesel

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

Oils from microalgae are of interest as a potential feedstock for producing renewable transport fuels including gasoline, diesel, biodiesel and jet fuel. For producing feedstock oils, an alga must be capable of being grown easily in readily available seawater and have a high productivity of biomass and oil. This study explored the biomass and lipid production potential of the microalga *Chlorella vulgaris* in seawater media, as a potential producer of feedstock oils. The alga was grown photoautotrophically under various conditions in ~2 L Duran bottles and a pilot scale (~138 L) raceway system. Initially, eight species of microalgae of different classes were assessed under nutrient sufficient growth conditions for the production of biomass and lipids in ~2 L Duran bottles. Two of the promising species (*C. vulgaris* and *Nannochloropsis salina*) were then further evaluated extensively under various conditions (i.e. salinity stress, different levels of nitrogen in growth media, continuous light and light-dark cycling). Based on these assessments *C. vulgaris* stood out as the best alga for further detailed study. *C. vulgaris* was evaluated for biomass production and lipid production. The consumption rates of major nutrients (N and P) were quantified. Biomass was characterized for elemental composition and energy content at the end of the growth cycle. A maximum lipid productivity of ~31 mg L$^{-1}$ d$^{-1}$ was attained in Duran bottle batch culture under nitrogen starvation in continuous light with a lipid content in the biomass of ~66% (dry weight). This appears to be the highest lipid content reported for *C. vulgaris* grown in seawater and demonstrates an excellent ability of this alga to accumulate high levels of oil. Under a 12:12 h light-dark cycle, the lipid content and productivity in Duran bottle batch culture were decreased by 13% and 41%, respectively, relative to the case for continuous illumination. Energy content of the biomass produced in Duran bottle batch culture exceeded 30 kJ g$^{-1}$ both in continuous light and the 12: 12 h light-dark cycle.
Batch and continuous culture kinetics of *C. vulgaris* in the raceway system were assessed. The alga was subjected to various light regimes and nitrogen starvation conditions. Although the N starvation enhanced the lipid accumulation by 42% relative to nutrient sufficient growth in batch culture, the highest biomass and oil productivities were attained under nutrient sufficient conditions in continuous mode of cultivation. Under nutrient sufficiency in continuous culture with a constant illumination of 91 \( \mu \text{mol-m}^{-2}\text{s}^{-1} \), the productivities of biomass and lipid in the raceway were >61 mg L\(^{-1}\) d\(^{-1}\) and >8 mg L\(^{-1}\) d\(^{-1}\), respectively.

This work represents the first detailed study of *C. vulgaris* in a raceway pond in full strength seawater media. Previous studies of this alga were almost always carried out in freshwater media.
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# Table of Contents

Abstract ........................................................................................................................................... iii
Acknowledgments ............................................................................................................................. v
Table of Contents .............................................................................................................................. vii
LIST OF FIGURES ............................................................................................................................ xiii
LIST OF TABLES .............................................................................................................................. xxvi
Abbreviations ..................................................................................................................................... xxvii

Chapter 1 ........................................................................................................................................ 1
INTRODUCTION ................................................................................................................................. 1

Chapter 2 ........................................................................................................................................ 5
LITERATURE REVIEW ......................................................................................................................... 5
  2.1 Microalgae .................................................................................................................................. 5
  2.2 Microalgae as a fuel source ......................................................................................................... 8
  2.3 Algal cultivation ............................................................................................................................. 9
    2.3.1 Photosynthesis and algal production ..................................................................................... 10
    2.3.2 Growth parameters and limitation to biomass production .................................................. 12
      2.3.2.1 Light ............................................................................................................................... 12
      2.3.2.2 Temperature ..................................................................................................................... 14
      2.3.2.3 Nutrients ......................................................................................................................... 15
        2.3.2.3a Macronutrients ............................................................................................................ 15
        2.3.2.3b Micronutrients and trace elements ............................................................................. 17
      2.3.2.4 Salinity ............................................................................................................................ 17
      2.3.2.5 Mixing ............................................................................................................................. 18
      2.3.2.6 Biotic factors (contamination) ....................................................................................... 18
    2.4 Lipids in microalgae .................................................................................................................... 19

vii
2.4.1 Lipid accumulation ................................................................. 22
  2.4.1.1 Effect of nutrients starvation ............................................. 23
  2.4.1.2 Light stress ................................................................. 25
  2.4.1.3 Temperature stress ....................................................... 25
  2.4.1.4 Salinity stress ............................................................ 26
2.5 Strain selection ......................................................................... 26
  2.5.1 Chlorella vulgaris .............................................................. 27
2.6 Microalgal production systems .................................................. 34
  2.6.1 Raceway and its operation ................................................... 35
2.2 Contributions of this study ....................................................... 44

Chapter 3 ..................................................................................... 47
MATERIALS AND METHODS .......................................................... 47
  3.1 Introduction ............................................................................ 47
  3.2 Microalgal strains, sources, maintenance and cultivation .............. 48
    3.2.1 Growth media ................................................................. 49
      3.2.1.1 Preparation of BG 11 medium ..................................... 50
      3.2.1.2 Vitamins solution ...................................................... 52
      3.2.1.3 Silicate solution (for diatom culture only) .................... 53
      3.2.1.4 Artificial seawater .................................................. 53
    3.2.2 Duran bottle batch culture ............................................... 54
      3.2.2.1 Harvesting of biomass from Duran bottles .................. 55
    3.2.3 Raceway pond culture system .......................................... 56
      3.2.3.1 Calibration of the impeller speed ............................... 66
      3.2.3.2 Relationship between the impeller speed and liquid flow velocity 66
      3.2.3.3 Raceway batch culture ............................................ 67
      3.2.3.4 Raceway continuous culture ................................... 68
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.2.3 Salinity tolerance of C. fusiformis</td>
<td>100</td>
</tr>
<tr>
<td>4.2.3 Effect of nutrients concentration</td>
<td>104</td>
</tr>
<tr>
<td>4.2.3.1 Effect of initial phosphate concentration</td>
<td>104</td>
</tr>
<tr>
<td>4.2.3.1a C. vulgaris</td>
<td>104</td>
</tr>
<tr>
<td>4.2.3.1b N. salina</td>
<td>107</td>
</tr>
<tr>
<td>4.2.3.2 Effect of initial nitrate concentration</td>
<td>110</td>
</tr>
<tr>
<td>4.2.3.2a C. vulgaris</td>
<td>110</td>
</tr>
<tr>
<td>4.2.3.2b N. salina</td>
<td>119</td>
</tr>
<tr>
<td>4.2.3.2a.a Biomass coloration</td>
<td>124</td>
</tr>
<tr>
<td>4.3 Biomass production in the raceway</td>
<td>130</td>
</tr>
<tr>
<td>4.3.1 Raceway batch culture</td>
<td>130</td>
</tr>
<tr>
<td>4.3.1.1 Standard raceway batch culture (normal operational conditions)</td>
<td>130</td>
</tr>
<tr>
<td>4.3.1.1a Analysis of C. vulgaris crude oil from raceway batch-1</td>
<td>138</td>
</tr>
<tr>
<td>4.3.1.1b Fractionation of C. vulgaris lipids into different lipid classes</td>
<td>138</td>
</tr>
<tr>
<td>4.3.1.1c Fatty acid profile of C. vulgaris oil</td>
<td>139</td>
</tr>
<tr>
<td>4.3.1.1c Concentrations of certain elements in C. vulgaris oil</td>
<td>141</td>
</tr>
<tr>
<td>4.3.1.2 Effect of low irradiance</td>
<td>143</td>
</tr>
<tr>
<td>4.3.1.3 Effect of nitrate stress</td>
<td>147</td>
</tr>
<tr>
<td>4.3.1.3a Effect of ≤21% of normal initial nitrate level on C. vulgaris</td>
<td>148</td>
</tr>
<tr>
<td>4.3.1.3b Effect of 10% of normal initial nitrate on C. vulgaris</td>
<td>156</td>
</tr>
<tr>
<td>4.3.1.3c Effect of 22% of normal initial nitrate level on N. salina</td>
<td>160</td>
</tr>
<tr>
<td>4.3.1.3d Lipid accumulation</td>
<td>166</td>
</tr>
<tr>
<td>4.3.2 Raceway continuous culture</td>
<td>169</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>179</td>
</tr>
</tbody>
</table>
SUMMARY AND CONCLUSION................................................................. 179

5.1 Summary .................................................................................. 179

5.2 Conclusion ................................................................................ 181

REFERENCES .................................................................................. 185

APPENDIX ........................................................................................ 225

Experimental data .......................................................................... 225
LIST OF FIGURES

Figure 2.1 Some examples of microalgae belonging to different classes: a) *Haematococcus pluvialis*, Chlorophyta (CCALA – Culture Collection of Autotrophic Organisms, Czech Republic); b) *Porphyridium cruentum*, Rhodophyta (CCALA – Culture Collection of Autotrophic Organisms, Czech Republic); c) *Dunaliella salina*, Chlorophyta (UTEX – The Culture Collection of Algae of University of Texas, Austin); d) *Chlorella vulgaris*, Chlorophyta (UTEX – The Culture Collection of Algae of University of Texas, Austin); e) *Phaeodactylum tricornutum*, Bacillariophyta (NCMA – Provasoli-Guillard National Center of Marine algae and Microbiota) and f) *Nannochloropsis salina*, Eustigmatophyte (www.sb-roscoff.fr/Phyto/gallery/main.php).

Figure 2.2 Microalgae applications in various fields. Modified from Dufossé et al. (2005).

Figure 2.3 Conversion of solar energy into chemical energy by oxygenic photosynthesis in the chloroplast. Modified from Campbell and Reece (2005).

Figure 2.4 Light intensity levels and its effect on growth. Adapted from Ogbonna and Tanaka (2000).

Figure 2.5 Microalgal metabolic pathways contributing to production of lipids. Based on Radakovits et al. (2010).

Figure 2.6 Some designs of open and closed microalgal production systems: a) Centre-pivot ponds used for the production of *Chlorella*, Taiwan (www.pureplanet.de); b) Algae wastewater treatment raceway pond at the NIWA's research site, Christchurch, New Zealand (National Institute of Water and Atmospheric Research Ltd); c) *Dunaliella bardawil* cultivation in raceway ponds (Nature Beta Technologies Ltd, Eilat, Israel, subsidiary of Nikken Sohonsha Co. Gifu, Japan); d) Bubble column photobioreactors growing algal strains for different pigments at Arizona State
Figure 2.7 Commercial algal biomass production in raceways: a) and b) Cultivation of *Spirulina* (a blue-green cyanobacterium) and *Haematococcus pluvialis* (an orange pigment producing green alga) by Cyanotech Corporation, Kona, Hawaii (www.cyanotech.com); c) *Dunaliella bardawil* cultivation in raceway ponds (Nature Beta Technologies Ltd, Eilat, Israel, subsidiary of Nikken Sohonsha Co. Gifu, Japan). 36

Figure 2.8 Light profile in a 0.3 m deep raceway at a dry biomass concentration of 0.5 g L$^{-1}$. The profile was calculated for a suspension of the marine diatom *Phaeodactylum tricornutum* at an incident irradiance level of 2,000 μmol·m$^{-2}$·s$^{-1}$ at the surface of the raceway. The zones of different metabolic activity are: the photoinhibited zone ($I_L ≥ 800$ μmol·m$^{-2}$·s$^{-1}$); the light-saturated zone ($170 ≤ I_L ≤ 800$ μmol·m$^{-2}$·s$^{-1}$); the light-limited zone ($4 ≤ I_L ≤ 170$ μmol·m$^{-2}$·s$^{-1}$); and the dark zone ($I_L ≤ 4$ μmol·m$^{-2}$·s$^{-1}$). $I_L$ is the local irradiance at any depth $L$ from the surface. Source: Chisti (2012). 39

Figure 3.1 Sparged Duran bottle cultures. 55

Figure 3.2 Raceway and paddlewheel. Dimensions in mm. 57

Figure 3.3 Raceway pond (empty). 58

Figure 3.4 Raceway paddlewheel with transparent protective cover. 59

Figure 3.5 The raceway main control panel, the heat exchanger and the paddlewheel motor. 59

Figure 3.6 Raceway with LEDs in day mode of operation. 61

Figure 3.7 Diurnal light output profile of raceway LED array: a) at peak value of 100% light level, the day-night averaged irradiance was 280 μmol·m$^{-2}$·s$^{-1}$; b) at the peak
output set to 50% of full light level, the day-night averaged irradiance was 165 μmol·m⁻²·s⁻¹. ..........................62

Figure 3.8 Irradiance on the surface of culture broth in the raceway at various output settings of the LED array. ..........................63

Figure 3.9 Variation of irradiance at different depths of freshwater in the raceway: a) at different LED light output levels; b) at different fluorescent light levels. The total depth was 0.23 m. ..........................64

Figure 3.10 Irradiance profile of fluorescent light at different depths in the raceway culture broth of C. vulgaris with 0.5 g L⁻¹ of biomass concentration. The total culture depth was 0.23 m. The fluorescent light output level was 100%..........................65

Figure 3.11 Impeller speed versus motor potentiometer settings. ..........................66

Figure 3.12 Relationship between impeller rpm and the liquid flow velocity............67

Figure 3.13 Continuous flow centrifugation – Algal broth from the black tank is pumped to the centrifuge where the biomass is retained in the bowl shown on the right. The biomass-free effluent leaves the centrifuge and is collected in a waste bottle. .......70

Figure 3.14 Spectrophotometric calibration curve for freshwater C. vulgaris obtained under continuous light in Duran bottle using the standard BG11 medium.................73

Figure 3.15 Spectrophotometric calibration curve (averaged) for C. vulgaris (b) obtained under continuous light in Duran bottles using BG11 seawater medium with different initial nitrate concentrations (0.1 to 1.1 g L⁻¹). ..............................................74

Figure 3.16 Spectrophotometric calibration curve (averaged) for C. vulgaris (c) obtained under 12 h:12 h light-dark cycle in Duran bottles using BG11 seawater medium with different nitrate concentrations (0.1 to 1.1 g L⁻¹). ..............................................74

Figure 3.17 Spectrophotometric calibration curve for C. vulgaris (d) obtained under continuous light in raceway in BG11 seawater medium..........................75
Figure 3.18 Spectrophotometric calibration curve for *C. vulgaris* (e) obtained under 12 h: 12 h diurnal light-dark cycle in raceway in BG11 seawater medium............................. 75

Figure 3.19 Spectrophotometric calibration curve (averaged) for *N. salina* (a) obtained under continuous light in Duran bottle using BG11 seawater medium with different initial nitrate concentrations (0.1 to 1.1 g L\(^{-1}\))................................................................. 76

Figure 3.20 Spectrophotometric calibration curve (averaged) for *N. salina* (b) obtained under 12 h:12 h light-dark cycle in Duran bottles using BG11 seawater medium with different initial nitrate concentrations (0.1 to 1.1 g L\(^{-1}\))................................................................. 76

Figure 3.21 Spectrophotometric calibration curve for *C. fusiformis* obtained under continuous light in Duran bottle using BG11 seawater medium................................. 77

Figure 3.22 Spectrophotometric calibration curve for *T. subcordiformis* obtained under continuous light in Duran bottle using BG11 seawater medium......................... 77

Figure 3.23 Nitrate standard curve prepared with dilutions of BG11 medium in seawater.................................................................................................................. 79

Figure 3.24 Phosphate standard curve. .................................................................................. 81

Figure 3.25 Total lipids (crude oil) after evaporation of chloroform................................. 83

Figure 3.26 Fluorescence confocal microscope images of algae stained with Nile Red: (a) *C. vulgaris*; (b) *N. salina*; (c) *C. fusiformis*; (d) *T. subcordiformis*......................... 84

Figure 3.27 Light microscopic images of: (a) *C. vulgaris*; (b) *N. salina*; (c) *C. fusiformis*; (d) *T. subcordiformis*. .................................................................................. 86

Figure 4.1 Growth profile of: a) *C. vulgaris*, irradiance of 105 \(\mu\)mol-m\(^{-2}\)-s\(^{-1}\); b) *N. salina*, irradiance of 124 \(\mu\)E-m\(^{-2}\)-s\(^{-1}\); c) *T. subcordiformis*, irradiance of 124 \(\mu\)mol-m\(^{-2}\)-s\(^{-1}\); d) *C. fusiformis*, irradiance of 105 \(\mu\)mol-m\(^{-2}\)-s\(^{-1}\). All algae were grown in BG11 seawater medium, 24 ± 2 °C, bubbled with 5% (v/v) CO\(_2\) in air. Silicate was added to the medium for *C. fusiformis*. ................................................................................. 92
Figure 4.2 Growth profiles of *C. vulgaris* in freshwater and different seasalt concentrations .................................................................95

Figure 4.3 *C. vulgaris* cultures on day 23: (a, b) Freshwater culture in duplicate bottles; (c) culture with a salt concentration of 40 g L$^{-1}$ (control, salinity 37 ppt); (d, e) cultures with salt concentrations of 50 g L$^{-1}$ (salinity 46 ppt) in duplicates; (f, g) cultures with salt concentrations of 60 g L$^{-1}$ (salinity 57 ppt) in duplicates. Low biomass concentration can be seen in cultures with a salt concentration of 60 g L$^{-1}$. The exact biomass concentrations are given in Figure 4.2. .................................................................95

Figure 4.4 Growth profiles of *N. salina* under different seasalt concentrations ..........98

Figure 4.5. *N. salina* cultures on day 23: (a, b) cultures with a salt concentration of 60 g L$^{-1}$ (salinity 57 ppt) in duplicates; (c, d) cultures with salt concentrations of 50 g L$^{-1}$ (salinity 47 ppt) in duplicates; (e) culture with a salt concentration of 40 g L$^{-1}$ (control, salinity 37 ppt). ............................................................................................................98

Figure 4.6 Growth profiles of *C. fusiformis* under different seasalt concentrations. ....100

Figure 4.7 *C. fusiformis* cultures on day 4: (a) culture with a salt concentration of 40 g L$^{-1}$ (control, salinity 37 ppt); (b, c) cultures with salt concentrations of 50 g L$^{-1}$ in duplicates (salinity 47 ppt); (d, e) cultures with salt concentrations of 60 g L$^{-1}$ (salinity 57 ppt). ............................................................................................................101

Figure 4.8 Growth and nutrient consumption profiles of *C. vulgaris* in Duran bottles: a) control, i.e. the standard phosphate concentration in BG11 seawater medium; b) twice the normal initial phosphate concentration in the BG11 seawater medium..............105

Figure 4.9 Growth and nutrient consumption profiles of *N. salina* in Duran bottles: a) control, i.e. standard phosphate concentration in BG11 seawater medium; b) twice the normal initial phosphate level in BG11 seawater medium. .................................................108
Figure 4.10 Growth and nutrient consumption profiles of *C. vulgaris* in Duran bottles with different initial nitrate concentrations of 100% (control), 50%, 20% and 10% in BG11 seawater medium: a-1) and b-1) biomass concentrations; a-2) and b-2) nitrate concentration; a-3) and b-3) phosphate concentration. All ‘a’ culture profiles were obtained under continuous light. All ‘b’ culture profiles were obtained under a 12 h:12 h light/dark cycle. In b-2 and b-3 the N and P consumption profiles were obtained only from the control bottles.

Figure 4.11 Growth and nutrient consumption profiles of *N. salina* in Duran bottles with different initial nitrate concentrations of 100% (control), 50%, 20% and 10% in BG11 seawater medium: a-1) and b-1) biomass concentrations; a-2) and b-2) nitrate concentration; a-3) and b-3) phosphate concentration. All ‘a’ culture profiles were obtained under continuous light. All ‘b’ culture profiles were obtained under a 12 h:12 h light/dark cycle. (In b-2 and b-3 the N and P consumption profiles were obtained only from the control bottles.)

Figure 4.12 Different colors of algal broth as a consequence of different initial nitrate levels in the media: i) *C. vulgaris* (on day 52); ii) *N. salina* (on day 49). (a, b) 100% initial nitrate; (c, d) 50% initial nitrate; (e, f) 20% initial nitrate; (g, h) 10% initial nitrate. All samples labeled “L” had been continuously illuminated. All samples labeled “LD” had been illuminated by a 12 h:12 h light/dark cycle. For biomass concentrations in individual samples, see Table 4.7 and Table 4.9.

Figure 4.13 Duran bottles cultures of two microalgae on day 46: (i) *C. vulgaris* (a, b, c, d) 20% initial nitrate, (e, f, g, h) 10% initial nitrate; (ii) *N. salina* (a, b) 20% initial nitrate, (c, d, e, f) 10% initial nitrate. All bottles labeled “L” had been continuously illuminated. All bottles labeled “LD” had been illuminated by a 12 h:12 h light/dark cycle.
Figure 4.14 Light microscopic images (day 46) of *C. vulgaris* cells grown in continuous light with an initial nitrate concentration of: (a) 100% (control) and (b) 10%. .................. 128

Figure 4.15 Light microscopic images (day 49) of *N. salina* cells grown in continuous light with an initial nitrate concentration of: (a) 100% (control) and (b) 10%. .............. 129

Figure 4.16 Growth and nutrient consumption profiles of *C. vulgaris* in raceway: a) Batch-1 with continuous irradiance of 91 $\mu$mol-m$^{-2}$s$^{-1}$ from fluorescent light; b) Batch-2, same as batch-1; c) Batch-3 cultured under LED light (100% light output at midday (day-night averaged irradiance of 280 $\mu$mol-m$^{-2}$s$^{-1}$), 12 h:12 h light/dark cycle). All batches started with 5-6% (v/v) inoculum except batch-1 for which the inoculum size was 10.5% (v/v). ........................................................................................................... 132

Figure 4.17 Fluorescent confocal microscopic image of *C. vulgaris* culture (stained with Nile Red) from Raceway batch-3 (image taken at day 34 of growth cycle, some debris can also be seen in the image). ........................................................................................................... 136

Figure 4.18. Growth and nutrient consumption profiles of *C. vulgaris* in raceway batch-4 with LED light (50% of full light output at midday (day-night averaged irradiance of 165 $\mu$mol-m$^{-2}$s$^{-1}$), 12 h:12 h light/dark cycle). ................................................................. 144

Figure 4.19 *C. vulgaris* growth and nutrient consumption profiles under nitrate stress: a) raceway batch-5 grown with ~20% of normal initial nitrate in BG11 (seawater); b) raceway batch-6 grown with ~18% of normal initial nitrate in BG11 (seawater). ....... 151

Figure 4.20 Growth and nutrient consumption profiles of *C. vulgaris* in raceway batch-7 with ~10% of normal initial nitrate in BG11 (seawater)................................. 156

Figure 4.21 Growth and nutrient consumption profiles of *N. salina* in raceway batch-8 with ~22% of normal initial nitrate in BG11 (seawater). Vertical dotted lines demarcate periods of different settings of paddlewheel speed on the potentiometer: 5, 6, 7 and 9 (the corresponding velocities were 0.21± 0.02 m s$^{-1}$, 0.23 ± 0.02 m s$^{-1}$, 0.27 ± 0.04 m s$^{-1}$ and 0.32 ± 0.03 m s$^{-1}$, respectively). ................................................................................................. 162
Figure 4.22 Fluorescent microscopy: (a) *C. vulgaris* at day 81; (b) *N. salina* at day 103.

Figure 4.23 Transmission electron microscopy of algal cells from raceway: (a) *C. vulgaris* on day 111 (batch-6); (b) *N. salina* on day 108 (batch-8). L, represents lipid droplets in cells; S, represents starch; N, represents nucleus. Images taken at Manawatu Microscopy and Imaging Centre, Massey University (sample prepared by glutaraldehyde-osmium tetroxide standard fixation protocol).

Figure 4.24 *C. vulgaris* culture in continuous raceway operation (arrows indicate steady states): a) biomass concentration at steady states 1-8, where SS-1 = continuous light at 91 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), SS-2 = continuous light at 46 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), SS-3 = same conditions as SS-1, SS-4 = light/dark cycle (14 h:10 h) at illumination level of 91 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), SS-5 = same conditions as SS-1, SS-6 = continuous light at 91 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), SS-7 = continuous light at 46 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), SS-8 = same conditions as SS-6; a1) oscillation in biomass concentration at SS-4 (due to 14 h: 10 h day/night cycle); b) growth and nutrient profile of *C. vulgaris* culture in continuous raceway.
LIST OF TABLES

Table 2.1 Summary of \textit{C. vulgaris} studies on lipid production ........................................ 30
Table 2.2 Summary of raceway algal cultivation ........................................................................ 41
Table 3.1 Microalgal strains ........................................................................................................ 49
Table 3.2 Components of BG11 medium .................................................................................. 51
Table 4.1 Summary of findings from preliminary screening of the microalgae ................. 93
Table 4.2 Biomass characteristics of \textit{C. vulgaris} under different salinities ....................... 96
Table 4.3 Biomass characteristics of \textit{N. salina} grown under different salinities .......... 99
Table 4.4 Biomass characteristics of \textit{C. fusiformis} grown under different salinities \textsuperscript{a} .. 102
Table 4.5 \textit{C. vulgaris} Duran bottle batch culture kinetics (different phosphate concentrations) .............................................................................................................. 106
Table 4.6 \textit{N. salina} Duran bottle batch culture kinetics (different phosphate concentrations) ............................................................................................................................... 109
Table 4.7 \textit{C. vulgaris} Duran bottles culture kinetic parameters (different initial nitrate concentrations in BG11 seawater medium) \textsuperscript{1} ......................................................... 116
Table 4.8 Lipid content, calorific value and elemental content of \textit{C. vulgaris} biomass (different initial nitrate concentrations) .............................................................................................................. 117
Table 4.9 \textit{N. salina} Duran bottles culture kinetic parameters (different initial nitrate concentrations in BG11 seawater medium) \textsuperscript{1} ................................................................. 121
Table 4.10 Lipid content, calorific value and elemental content of \textit{N. salina} biomass (different initial nitrate concentrations) .............................................................................................................. 122
Table 4.11 \textit{C. vulgaris} raceway batch culture kinetics (standard BG11 medium) ......... 134
Table 4.12 Lipid contents and calorific values of biomass samples ...................................... 136
Table 4.13 Elements (\%, w/w) in \textit{C. vulgaris} biomass from various raceway batches 138
Table 4.14 Fractionation of lipids from raceway batch-1 ....................................................... 139
Table 4.15 Fatty acid profile of crude oil of *C. vulgaris* (raceway batch-1) .............. 140

Table 4.16 Elemental content of crude *C. vulgaris* oil from the raceway batch-1 ...... 142

Table 4.17 *C. vulgaris* raceway batch culture kinetics (low irradiance condition) ...... 145

Table 4.18 Biomass characteristics of *C. vulgaris* at various harvesting times in the
raceway batch-4 ........................................................................................................ 146

Table 4.19 *C. vulgaris* raceway batch culture kinetics (low initial nitrate concentration)
.............................................................................................................................. 152

Table 4.20 Biomass characteristics at various harvesting times in the raceway batch-5
(~20% of normal initial nitrate) ................................................................................ 153

Table 4.21 Biomass characteristics at various harvesting times in the raceway batch-6
(~18% of normal initial nitrate) ................................................................................ 155

Table 4.22 *C. vulgaris* raceway batch culture kinetics (~10% of normal initial nitrate in
BG11) ........................................................................................................................ 157

Table 4.23 Biomass characteristics of *C. vulgaris* at various harvesting times in the
raceway batch-7 (10% of normal initial nitrate) ......................................................... 159

Table 4.24 *N. salina* raceway batch-8<sup>a</sup> culture kinetics (at different rotational speeds<sup>1</sup>)
................................................................................................................................. 163

Table 4.25 Biomass characteristics of *N. salina* at various harvesting times in the
raceway batch-8 (20% of normal initial nitrate) .......................................................... 1635

Table 4.26 *C. vulgaris* raceway continuous culture kinetics (standard BG11 seawater
medium) ....................................................................................................................... 172

Table 4.27 Biomass characteristics at various steady states (Figure 4.24) ................. 173
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Surface area for light absorption</td>
</tr>
<tr>
<td>Acetyl-CoA</td>
<td>Acetyl coenzyme A</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>$A_{xxx}$</td>
<td>Spectrophotometric absorbance at $xxx$ nm</td>
</tr>
<tr>
<td>BG11</td>
<td>A medium formulation for growing cyanobacteria/microalgae</td>
</tr>
<tr>
<td>CCALA</td>
<td>Culture Collection of Autotrophic Organisms, Czech Republic</td>
</tr>
<tr>
<td>CCAP</td>
<td>Culture Collection of Algae and Protozoa, Argyll, United Kingdom</td>
</tr>
<tr>
<td>D</td>
<td>Dilution rate</td>
</tr>
<tr>
<td>DCW</td>
<td>Biomass dry cell weight</td>
</tr>
<tr>
<td>DF</td>
<td>Dilution factor</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
</tr>
<tr>
<td>F</td>
<td>Feed flow rate</td>
</tr>
<tr>
<td>FAME</td>
<td>Fatty acid methyl esters</td>
</tr>
<tr>
<td>F/2-Si</td>
<td>A medium formulation for growing diatoms</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>G3P</td>
<td>Glyceraldehyde-3-phosphate</td>
</tr>
<tr>
<td>HRAP</td>
<td>High Rate Algal Ponds</td>
</tr>
<tr>
<td>I</td>
<td>Irradiance</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively coupled plasma mass spectrometry</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>Inductively coupled plasma optical emission spectrometry</td>
</tr>
<tr>
<td>$I_L$</td>
<td>Local irradiance at any depth $L$ from the surface</td>
</tr>
</tbody>
</table>
ISO  International Organization for Standardization
L  Depth from the surface
LED  Light emitting diode
MBL  A medium formulation for growing cyanobacteria/microalgae
NA  Not available
NADPH  Reduced form of nicotinamide adenine dinucleotide phosphate
ND  Not determined
$N_f$  Final nitrate concentration
NCMA  Provasoli-Guillard National Center of Marine algae and Microbiota, Maine, USA
$N_i$  Initial nitrate concentration
NIWA  National Institute of Water and Atmospheric Research Ltd, New Zealand
$N_{i1}$  Nitrate concentration at time $t_1$
N11  A medium formulation for growing cyanobacteria/microalgae
$N_2$  Nitrate concentration at time $t_2$
PAR  Photosynthetically active radiation (light in the wavelength range of 400-700 nm)
$P_b$  Biomass productivity
PBR  Photobioreactor
$P_f$  Final phosphate concentration
PFD  Photon-flux density
$P_i$  Initial phosphate concentration
$P_l$  Lipid productivity
ppt  Part per thousand
PUFA  Polyunsaturated fatty acid
$P_f$  Phosphate concentration at time $t_1$
\( P_2 \)  
Phosphate concentration at time \( t_2 \)

\( q_N \)  
Average biomass specific nitrate consumption rate

\( q_P \)  
Average biomass specific phosphate consumption rate

RNA  
Ribonucleic acid

rpm  
Revolutions per minute

RTD  
Resistance temperature device

RuBP  
Ribulose-1,5-bisphosphate

S  
Surface area

\( t \)  
Duration time of a batch (i.e. the time required to attain the biomass concentration \( X_f \))

TAG  
Triacylglycerols

UTEX  
The Culture Collection of Algae of University of Texas, Austin, USA

V  
Volume

\( V \)  
Volume in the culture vessel

\( v/v \)  
Volume by volume

\( w \)  
Weight fraction of the lipids in the biomass

\( w/w \)  
Weight by weight

\( w_s \)  
Steady state weight fraction of the lipids in the biomass

x  
x-Variable

\( X \)  
Biomass concentration at any time \( t \)

\( X_i \)  
Initial biomass concentration

\( X_f \)  
Final biomass concentration

\( X_s \)  
Steady state biomass concentration

\( X_{f_1} \)  
Biomass concentration at time \( t_1 \)

\( X_2 \)  
Biomass concentration at time \( t_2 \)

y  
y-Variable
$Y_N, Y_{X/N}$  Biomass yield coefficient on nitrate

$Y_P, Y_{X/P}$  Biomass yield coefficient on phosphate

$Y_{X/L}$  Biomass yield coefficient on light

Greek symbols

$\mu$  Specific growth rate