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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<sup>-1</sup> d<sup>-1</sup> 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<sup>-1</sup> 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  $91 \mu\text{mol}\cdot\text{m}^{-2}\text{s}^{-1}$ , the productivities of biomass and lipid in the raceway were  $>61 \text{ mg L}^{-1} \text{ d}^{-1}$  and  $>8 \text{ mg L}^{-1} \text{ d}^{-1}$ , 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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## Abbreviations

<i>A</i>	Surface area for light absorption
Acetyl-CoA	Acetyl coenzyme A
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
$A_{xxx}$	Spectrophotometric absorbance at <i>xxx</i> nm
BG11	A medium formulation for growing cyanobacteria/microalgae
CCALA	Culture Collection of Autotrophic Organisms, Czech Republic
CCAP	Culture Collection of Algae and Protozoa, Argyll, United Kingdom
<i>D</i>	Dilution rate
<i>DCW</i>	Biomass dry cell weight
DF	Dilution factor
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
EPA	Eicosapentaenoic acid
<i>F</i>	Feed flow rate
FAME	Fatty acid methyl esters
F/2-Si	A medium formulation for growing diatoms
GC	Gas chromatography
G3P	Glyceraldehyde-3-phosphate
HRAP	High Rate Algal Ponds
<i>I</i>	Irradiance
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
$I_L$	Local irradiance at any depth <i>L</i> from the surface



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
$N_1$	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
$P_1$	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
$X_1$	Biomass concentration at time $t_1$
$X_2$	Biomass concentration at time $t_2$
y	y-Variable

$Y_N, Y_{XN}$	Biomass yield coefficient on nitrate
$Y_P, Y_{XP}$	Biomass yield coefficient on phosphate
$Y_{XL}$	Biomass yield coefficient on light

#### Greek symbols

$\mu$	Specific growth rate
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