

**THE CULTURE OF COCCOLITHOPHORID ALGAE FOR CARBON
DIOXIDE BIOREMEDIATION**

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**This thesis is presented for the degree of Doctor of Philosophy of Murdoch
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I declare that this thesis is my own account of my research and contains work which has not been previously submitted for a degree at any tertiary institution.

Navid R Moheimani

"Biology occupies a position among the sciences at once marginal and central. Marginal because-the living world constituting but a tiny and very "special" part of the universe-it does not seem likely that the study of living beings will ever uncover general laws applicable outside the biosphere. But if the ultimate aim of the whole of science is indeed, as I believe, to clarify man's relationship to the universe, then biology must be accorded a central position . . ."

Jacques Monod (1910-1976)

French biologist, 1965 Nobel Prize, resistance leader in WW2
from *Jacques Monod Chance and Necessity Alfred A. Knopf, New York, 1971, p xi.*

I dedicate this work to all scientists who suffered long nights in the name of science for increasing humans' knowledge of the world around them.

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ABSTRACT

The culture of coccolithophorid algae is an attractive option for sequestration or recycling of CO₂ as they can fix carbon by photosynthesis as well as in calcium carbonate scales known as coccoliths. They also produce high amounts of lipids which have a potential application as a renewable fuel.

Five species of coccolithophorids (*Pleurochrysis carterae*, CCMP647, *Pleurochrysis sp.* CCMP1211, *Gephyrocapsa oceanica* CS-335/2, *Emiliana huxleyi* CCMP371, and *Emiliana huxleyi* CS-369) were screened for their ability to grow at high temperature. All species grew up to 28°C except *E. huxleyi* CS-369. However, *Pleurochrysis sp.* CCMP 1211 which was found to clump and can therefore not be recommended for large-scale cultivation. The salinity tolerance of these species was also examined.

Growth of *P. carterae*, *G. oceanica*, and *E. huxleyi* in laboratory scale closed photobioreactors (plate, carboy, airlift, and tubular photobioreactors) showed the plate photobioreactor to be the best closed cultivation system. The highest productivities were achieved by *P. carterae* in the plate photobioreactor and were 0.54 g.L⁻¹.d⁻¹, 0.12 g.L⁻¹.d⁻¹, 0.06 g.L⁻¹.d⁻¹ for total dry weight, lipid and CaCO₃ respectively.

The growth of *P. carterae* and *E. huxleyi* was also examined in an outdoor raceway pond. The *E. huxleyi* culture was easily contaminated resulting in the loss of the culture in less than three weeks, but *P. carterae* grew well over a period of 13 months. The overall total dry weight productivity of *P. carterae* was 0.19 g.L⁻¹.d⁻¹ with lipid and CaCO₃ contents of up to 33% and 10% of dry weight respectively. There was little protozoan and bacterial contamination. Medium pH increased to pH 11 during the day and was found to be a reliable variable for maintaining the health of the culture. A maximum pH achieved during the day of less than pH 8.5 indicated the imminent

collapse of the culture. Heavy rain and low temperature were the main reasons for culture loss in mid winter, whereas high temperature during summer favoured *P. carterae* growth. A comparison of the growth of *P. carterae* and *Dunaliella salina* MUR8 in the raceway ponds showed no significant differences between these two species with regard to areal total dry weight productivity and lipid content.

The effects of several limiting factors were also examined. A reduction in medium pH resulting from CO₂ addition inhibited the growth of *E. huxleyi* in the plate photobioreactor, whereas *P. carterae* growth and productivities increased in the pH range of pH 7.7 to 8.0 in the plate photobioreactor and pH 9.1 to 9.6 in the outdoor raceway pond. The best operational pond depth for outdoor raceway culture of *P. carterae* was between 16 cm and 21 cm. Early morning temperatures, especially during the winter, highly affected the growth of *P. carterae* in the raceway pond, whereas artificially increasing the medium temperature improved the health of the culture but resulted in little increase in productivity. Photosynthesis of *P. carterae* was found to be highly inhibited by high oxygen concentration in the medium irrespective of temperature or irradiance.

An economic model of *P. carterae* in a 63 ha raceway plant resulted in a cost for the biomass of between 7.35 Aus\$.Kg⁻¹ and 14.17 Aus\$.Kg⁻¹ depending on the harvesting method used.

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ABBREVIATIONS

Following are the abbreviations for all Chapters except Chapter 7:

CDR	Carbon dioxide removal
CV	Coccolith vesicle
RB	Reticular body
G	Golgi apparatus
cs	Coccolithosome
DMS	Dimethyl sulphide
DMSP	Dimethyl sulfoniopropionate
C _i	Inorganic carbon
CCM	Carbon concentrating mechanism
CA _{ext}	External carbonic anhydrase
AE	Anion exchange protein
CER	Cortical endoplasmic reticulum
t _d	Doubling time
EPA	Eicosapentaenoic acid
PUFA	Polyunsaturated fatty acid
DHA	Docosahexaenoic acid
GLA	Gamma linoleic acid
AA	Arachidonic acid
μ	Specific growth rate (generation time)
GF	Glass fibre
CDT _{il}	Concentric draught –tube internal loop
PAR	Photosynthetic active radiation
Re _i	Reynolds number
PSII	Photosystem II
RUBP	Ribulose biphosphate
3-PGA	3-phosphoglyceraldehyde
PSU	Photosynthetic unit
Rubisco	Ribulose-1,5-biphosphate carboxylase/oxygenase

Abbreviations used in the Economic Model (Chapter 7):

E_{rec}	Energy received
Pr_{afdw}	Ash free dry weight
$C_{biomass, fixed}$	Carbon fixed in biomass
$C_{coccolith, fixed}$	Carbon fixed in coccolith
$C_{air, in}$	Total carbon air inlet
$C_{air, out}$	Total carbon air outlet
C_{in}	CO ₂ input
$V_{air, in}$	Total volume of air inlet
CO_{2air}	CO ₂ content of air
C_{CO_2}	Carbon content of CO ₂
t_{air}	Time period of aeration
$V_{co_2, in}$	CO ₂ volume input
C_{uptake}	Total carbon uptake
C_{BM}	Carbon content of species
$C_{coccolith}$	Carbon content of coccolith
$Pr_{total dry weight}$	Total dry weight production
Pr_{cc}	Total coccolith production
Pr_{ash}	Total ash production
$C_{utility}$	Carbon Utility
<i>PNUM</i>	Number of pond
<i>LENGTH</i>	Pond Length
<i>WIDTH</i>	Pond width
<i>DEPTH</i>	Pond depth
<i>ODEPTH</i>	Pond Operating Depth
<i>SPA</i>	Single Pond Area
<i>TPA</i>	Total Pond Area
<i>TPHA</i>	Total Pond Area (per hectare)
<i>TEA</i>	Total Extra Area
<i>TCA</i>	Total Company Area
<i>SPV</i>	Single Pond Volume
<i>TPV</i>	Total Pond Volume
<i>SPREP</i>	Site Preparation
<i>CSYS</i>	Culture System
<i>ENGF</i>	Engineering Fees
<i>CONTING</i>	Contingency

<i>Land</i>	Land
<i>TCAP</i>	Total Capital
<i>HE</i>	Harvesting efficiency
<i>PPH</i>	Proportion of pond harvested
<i>PMR</i>	Proportion of medium recycled
<i>NTPSY</i>	Number of times ponds set up per year
<i>PDW</i>	Proportion of down time
<i>EV</i>	Rate of evaporation
<i>TGD</i>	Total Growth Days
<i>DT</i>	Doubling time
<i>VP_{UpH}</i>	Volumetric productivity at unregulated pH
<i>VP_{9.6}</i>	Volumetric productivity at pH 9.6
<i>VP₉</i>	Volumetric productivity at pH 9
<i>AP_{UpH}</i>	Areal productivity at unregulated pH
<i>Ap_{9.6}</i>	Areal productivity at pH 9.6
<i>Ap₉</i>	Areal productivity at pH 9
<i>AVP_{UpH}</i>	Annual productivity at unregulated pH
<i>AVP_{9.6}</i>	Annual productivity at pH 9.6
<i>AVP₉</i>	Annual productivity at pH 9
<i>AEV</i>	Annual extra volume culture media need
<i>ACH</i>	Average cell at harvest
<i>NCM</i>	NaNO ₃ concentration in medium
<i>NN</i>	Nitrogen content of NaNO ₃
<i>NPC</i>	Nitrogen content of culture media
<i>NC</i>	Cell Nitrogen
<i>NUC</i>	Used N from culture media
<i>NRR</i>	Remaining N in residual
<i>NARC</i>	N need to add to remaining culture media in the reactor
<i>NAHC</i>	N need to add to harvested culture media
<i>NPE</i>	N need per extra volume
<i>NGN</i>	N need to get to the N concentration annually
<i>Ncost</i>	N Costs
<i>Ancost</i>	Annual N cost
<i>PCM</i>	NaH ₂ PO ₄ . H ₂ O concentration in media
<i>PN</i>	P content of Na ₂ PO ₄ .H ₂ O
<i>PPC</i>	P percentage in culture media

<i>PC</i>	Cell P
<i>PUC</i>	Used P from culture media
<i>PRR</i>	Remaining P in residual
<i>PARC</i>	P need to add to remaining culture media in the reactor
<i>PAHC</i>	P need to add to harvested culture media
<i>PPE</i>	P need per extra volume
<i>PGP</i>	P need to get to the P concentration annually
<i>Pcost</i>	P costs
<i>Apcost</i>	Annual P cost
<i>FWC</i>	Fresh water cost
<i>ADE</i>	Average days with evaporation
<i>ARE</i>	Average rainy days
<i>Sa</i>	Salinity
<i>TFWC</i>	Total Fresh water cost
<i>Sco</i>	NaCl costs
<i>AR</i>	Average rain
<i>TS</i>	Total NaCl in whole system
<i>AD</i>	Addition water to whole system
<i>TVaR</i>	Total volume after rain
<i>NaA</i>	NaCl concentration after TVaR
<i>NaD</i>	NaCl addition
<i>Asco</i>	Annual NaCl cost
<i>LCS</i>	Labour cost supervisor
<i>LCST</i>	Labour cost senior technician
<i>LCTD</i>	Labour cost technician (day team)
<i>LCTS</i>	Labour cost technician (shift teams)
<i>TLC</i>	Total labour cost
<i>PoC</i>	Power cost
<i>PoU</i>	Power usage
<i>TPoC</i>	Total power cost
<i>PU_{upH}</i>	Price per Kg algae unharvested at unregulated pH
<i>PU_{pH9.6}</i>	Price per Kg algae unharvested at pH 9.6
<i>PU_{pH9}</i>	Price per Kg algae unharvested at pH 9