

# EFFECT OF NITROGEN AND PHOSPHORUS ENRICHMENT ON THE GROWTH OF THREE TROPICAL CHLOROPHYTES

MALA SILVA RAMADHONA

INSTITUTE OF POSTGRADUATE STUDIES

UNIVERSITY OF MALAYA

KUALA LUMPUR

2003



# EFFECT OF NITROGEN AND PHOSPHORUS ENRICHMENT ON THE GROWTH OF THREE TROPICAL CHLOROPHYTES

#### MALA SILVA RAMADHONA

A DISSERTATION SUBMITTED TO THE
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF PHILOSOPHY

INSTITUTE OF POSTGRADUATE STUDIES

UNIVERSITY OF MALAYA

KUALA LUMPUR

2003

#### ACKNOWLEDGEMENTS

This project could not have been fulfilled without the assistance and support of various people and organisations.

Firstly, I want to praise and give thanks to my Almighty God, ALLAH SWT, for his mercy and blessings which he has bestowed upon me.

I am sincerely grateful to Professor Phang Siew Moi for her supervision, patience, enthusiasm, encouragement, and support throughout my project.

I especially wish to thank to my co-supervisor Dr. Chu Wan Loy for his valuable guidance, patience, encouragement, and suggestions that he has given to me throughout this project.

I also wish to express my gratitude to all my friends: Melor, Phaik Eem, Cheng Lee, Emie, Gan, Sing Lai, Hui Yin and all the members of the Algae Laboratory (past and present) for their help, support and delightful company and also for all my friends in the University of Malaya, thanks for everything.

Most of all I would like to thank to my beloved family: Papa (Marzuki) and Mama (Ulfah), for their love, support, care, patience, and understanding, and also for my brother (Ali) and sisters (Dewi and Maya). For my beloved Reza, thanks for the support and love that you have given to me. For my housemates, thanks for the happiness when we were together. Last but not least, to my lovely niece and nephews, Dita, Ariq and Hafis for the lovely and sweet days, I love you all.

#### ABSTRACT

The main objective of the present study was to assess the potential use of three tropical chlorophytes, namely Chlorella vulgaris UMACC 001. Scenedesmus quadricauda UMACC 041, and Ankistrodesmus convolutus UMACC 101 as test organisms for bioassays of nitrogen and phosphorus. Based on a growth study using Chlorella vulgaris UMACC 001, 1% BBM containing 0.03 mM NaN03 was chosen as the minimal culture medium. The growth of the cultures at 0.03, 0.15, 0.75, 3.75, and 18.75 mM NaNO3 and NH4Cl was monitored based on cell numbers and OD620 attained in 96 h tests, and compared with that in Dilution Water, which was without nitrogen and phosphorus. For the phosphate experiments, the cultures were grown at 0, 0.02. 0.10, 0.50, 2.50, and 12.5 mM phosphate (KH<sub>2</sub>PO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub>) in 1% BBM containing 0.03 or 18.75 mM NaNO3. There was no marked difference in the overall growth trends of the three chlorophytes with respect to the variations in nitrogen and phosphorus levels tested. However, the cell numbers and OD620 attained at 96 h by the three chlorophytes increased with increasing levels of nitrogen and phosphorus. The Percentage Growth Enhancement at 96 h (PGE-96) based on the percentage increase of cell numbers or OD620 at 96 h, relative to that attained in Dilution Water (arbitrarily given a PGE-value of 100%) could be used as a parameter in bioassay tests for nitrogen and phosphorus. The three chlorophytes have the potential to be used as test organisms in bioassays of nitrogen and phosphorus in tropical freshwater environments.

#### ABSTRAK

Objektif utama dari kajian ini adalah untuk menilai keupayaan penggunaan tiga ienis alga hiiau tropika dinamakan Chlorella vulgaris UMACC 001, Scenedesmus quadricauda UMACC 041 dan Ankistrodesmus convolutus UMACC 101 sebagai organisma uiian untuk bioassav nitrogen dan fosfat. Berdasarkan kajian pertumbuhan menggunakan Chlorella vulgaris UMACC 001, Bold's Basal Medium 1% yang mengandungi 0.03 mM NaNO<sub>3</sub> telah dipilih sebagai media minimal untuk kultur. Pertumbuhan kultur pada 0.03, 0.15, 0.75, 3.75 dan 18.75 mM NaNO₃ atau NH₄Cl telah dipantau berdasarkan kepada bilangan sel dan OD620 yang diperolehi dalam ujian 96 jam, dibandingkan dengan di dalam air pencairan, yang mana ia tidak mengandungi nitrogen dan fosfat. Untuk eksperimen fosfat, kultur ditumbuhkan pada 0, 0.02, 0.10, 0.50, 2.50 and 12.5 mM fosfat (KH<sub>2</sub>PO<sub>4</sub> dan K<sub>2</sub>HPO<sub>4</sub>) di dalam BBM 1% yang mengandungi 0.03 atau 18.75 mM NaNO<sub>3</sub> Tidak terdapat perbezaan yang ketara pada keseluruhan tren pertumbuhan ketiga-tiga alga hijau merujuk kepada variasi tahap nitrogen dan fosfat yang diuji. Walau bagaimanapun, bilangan sel dan OD620 yang diperolehi pada 96 jam (PGE-96), oleh ketiga-tiga alga hijau ini bertambah dengan peningkatan tahap nitrogen dan fosforus. Peratus pertambahan pertumbuhan pada 96 jam (PGE-96) berdasarkan kepada peratus pertambahan bilangan sel atau OD620 pada 96 iam adalah berkait dengan yang diperolehi dalam air pencairan (nilai PGE yang diberikan adalah 100%) boleh digunakan sebagai parameter di dalam ujian bioassay untuk nitrogen and fosfat. Ketiga-tiga alga hijau mempunyai potensi untuk digunakan sebagai organisma ujian dalam bioassay nitrogen dan fosfat di dalam persekitaran air tawar tropika.

			Page
Ackn	owledge	ements	i
Abstı	ract		ii
Abstı	rak		iii
Table	e of con	tents	iv
List o	of abbre	viations	xi
List o	of tables		xii
List o	of figure	s	xix
List o	of apper	ndices	xxvi
1.0	Introd	uction	1
	1.1.	Environmental pollution	3
	1.1.1.	Water pollution	3
	1.1.2	The state of Malaysian freshwater environment	5
	1.2.	Nitrogen	5
	1.2.1	Phosphorus	6
	1.3.	Eutrophication	6
	1.4.	Tolerance of tropical microalgae to nitrogen enrichment	7
	1.5.	Objectives	8

# Table of Contents

2.0.	Litera	ture review	10
	2.1.	Freshwater ecosystems	10
	2.2.	Nitrogen in the environment	10
	2.3.	Nitrogen enrichment	12
	2.4.	Phosphorus in the environment	13
	2.5.	Phosphorus enrichment	15
	2.6.	Biological indicators	19
	2.7.	Algae as biological indicators	23
	2.8.	Monitoring	27
	2.9.	Biological monitoring	28
	2.10.	Algae as biomonitors	29
	2.11.	Nitrogen content in freshwater algae	32
	2.12.	Nitrogen metabolism in algae	33
	2.13.	Nitrate and nitrite assimilation	34
	2.14.	Ammonium assimilation	35
	2.15.	Effect of nitrogen sources on the growth and physiology	
		of microalgae	36
	2.16.	Phosphorus content in freshwater algae	41
	2.17.	Phosphate metabolism in algae	41
	2.18.	Phosphorus uptake and assimilation	44
	2.19.	Effect of phosphorus on the growth and physiology	
		of microalgae	46

# **Table of Contents**

3.0.	Mate	rials and methods	50
	3.1.	Preparation of glassware	50
	3.2.	Microalgal cultures	50
	3.2.1.	Test organisms	5
	3.2.2.	Maintenance of stock cultures	5
	3.2.3.	Preparation of inoculum	52
	3.3.	Preparation of stock solutions	54
	3.4.	Test procedures *	5
	3.4.1.	Experiment 1: Selection of minimal culture medium	56
	3.4.2.	Experiments 2&3: Effect of NaNO <sub>3</sub> and NH <sub>4</sub> Cl levels on	
		short term growth (96h) of three chlorophytes	56
	3.4.3.	Experiments 4&5: Effect of phosphate (K <sub>2</sub> HPO <sub>4</sub> and	
		KH <sub>2</sub> PO <sub>4</sub> ) levels on short term growth (96h)	
		of three chlorophytes in low-NaNO $_3$ and low-NH $_4$ Cl medium	57
	3.4.4.	Experiments 6&7: Effect of phosphate ( $K_2HPO_4$ and	
		KH <sub>2</sub> PO <sub>4</sub> ) levels on short term growth (96h)	
		of three chlorophytes in high-NaNO $_3$ and high-NH $_4$ Cl medium	58
	3.5.	Cell count	59
	3.6.	Specific growth rate	59
	3.7.	Percentage Growth Enhancement	60
	3.8.	Linear regression analysis	60

4.0

Resul	ts	61
4.1.	Experiment 1: Selection of minimal culture medium	61
4.2.	Experiment 2: Effect of NaNO <sub>3</sub> levels on the short term	
	growth (96h) of three chlorophytes	63
4.2.1.	l.Specific growth rate (μ) based on cell number	66
4.2.1.2	P.Percentage Growth Enhancement (PGE) based on	
	cell numbers	68
4.2.2.	Absorbance value (OD <sub>620</sub> )	70
4.2.2.1	. Specific growth rate ( $\mu$ ) based on OD <sub>620</sub>	72
4.2.2.2	2. Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	74
4.2.3.	Linear regression analysis	74
4.3.	Experiment 3: Effect of NH <sub>4</sub> Cl levels on the short term	
	growth (96h) of three chlorophytes	79
4.3.1.2	Specific growth rate (μ) based on cell number	81
4.3.1.3	.Percentage Growth Enhancement (PGE) based on	
	cell numbers	83
4.3.2.	Absorbance value (OD <sub>620</sub> )	83
4.3.2.1	. Specific growth rate $(\overset{\iota}{\mu})$ based on $OD_{620}$	87
4.3.2.2	. Percentage Growth Enhancement (PGE) based on $OD_{620}$	89
4.3.3.	Linear regression analysis	89
4.4.	Experiment 4: Effect of phosphate levels on the short term	
	growth (96h) of three chlorophytes in low-NaNO <sub>3</sub>	
	medium	94
	4.1. 4.2. 4.2.1. 4.2.2. 4.2.2. 4.2.3. 4.3.1.3 4.3.1.3 4.3.2. 4.3.2.1 4.3.2.2 4.3.3.	<ul> <li>4.2. Experiment 2: Effect of NaNO<sub>3</sub> levels on the short term growth (96h) of three chlorophytes</li> <li>4.2.1.1.Specific growth rate (μ) based on cell number</li> <li>4.2.1.2.Percentage Growth Enhancement (PGE) based on cell numbers</li> <li>4.2.2. Absorbance value (OD<sub>620</sub>)</li> <li>4.2.2.1. Specific growth rate (μ) based on OD<sub>620</sub></li> <li>4.2.2.2. Percentage Growth Enhancement (PGE) based on OD<sub>620</sub></li> <li>4.2.3. Linear regression analysis</li> <li>4.3. Experiment 3: Effect of NH<sub>4</sub>CI levels on the short term growth (96h) of three chlorophytes</li> <li>4.3.1.2.Specific growth rate (μ) based on cell number</li> <li>4.3.1.3.Percentage Growth Enhancement (PGE) based on cell numbers</li> <li>4.3.2. Absorbance value (OD<sub>620</sub>)</li> <li>4.3.2.1. Specific growth rate (μ) based on OD<sub>620</sub></li> <li>4.3.2.2. Percentage Growth Enhancement (PGE) based on OD<sub>620</sub></li> <li>4.3.3. Linear regression analysis</li> <li>4.4. Experiment 4: Effect of phosphate levels on the short term growth (96h) of three chlorophytes in low-NaNO<sub>3</sub></li> </ul>

4.4.1.2	Specific growth rate $(\mu)$ based on cell number	97
4.4.1.3	Percentage Growth Enhancement (PGE) based on	
	cell numbers	99
4.4.2.	Absorbance value (OD <sub>620</sub> )	99
4.4.2.1	. Specific growth rate ( $\mu$ ) based on OD <sub>620</sub>	103
4.4.2.2	. Percentage Growth Enhancement (PGE) based on $OD_{620}$	105
4.4.3.	Linear regression analysis	105
4.5.	Experiment 5: Effect of phosphate levels on the short term	
	growth (96h) of three chlorophytes in low-NH $_4$ Cl	
	medium	110
4.5.1.2	Specific growth rate (μ) based on cell number	113
4.5.1.3	.Percentage Growth Enhancement (PGE) based on	
	cell numbers	115
4.5.2.	Absorbance value (OD <sub>620</sub> )	115
4.5.2.1	. Specific growth rate (µ) based on $OD_{620}$	119
4.5.2.2	2. Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	121
4.5.3.	Linear regression analysis	121
4.6.	Experiment 6: Effect of phosphate levels on the short term	
	growth (96h) of three chlorophytes in high-NaNO $_3$	
	medium	126
4.6.1.2	2.Specific growth rate (µ) based on cell number	128
4.6.1.3	B.Percentage Growth Enhancement (PGE) based on	
	cell numbers	130

5.0.

4.6.2	2. Absorbance value (OD <sub>620</sub> )	130
4.6.2	2.1. Specific growth rate (μ) based on OD <sub>620</sub>	134
4.6.2	2.2. Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	136
4.6.3	3. Linear regression analysis	137
4.7.	Experiment 7: Effect of phosphate levels on the short term	
	growth (96h) of three chlorophytes in high-NH <sub>4</sub> Cl	
	medium	140
4.7.1	.2.Specific growth rate (μ) based on cell number	142
4.7.1	.3.Percentage Growth Enhancement (PGE) based on	
	cell numbers	144
4.7.2	Absorbance value (OD <sub>620</sub> )	144
4.7.2	.1. Specific growth rate ( $\mu$ ) based on OD <sub>620</sub>	148
4.7.2	.2. Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	150
4.7.3	. Linear regression analysis	151
Discu	ussion	154
5.1.	Selection of minimal culture medium for testing	154
5.2.	Growth response of the three chlorophytes to variations	
	in nitrogen and phosphorus levels	155
5.3.	Potential use the chlorophytes for bioassay	160
5.4.	Areas for further studies	161

# Table of Contents

6.0. Conclusion	164
References	166
Appendices	190

## List of Abbreviations

Full name	Abbreviation
Weight/ volume	w/v
Bold Basal's Medium	BBM
Degree Celcius	°C
Hour	Н
Optical Density at 620 nm -	OD <sub>620</sub>
Rotation per minute	rpm
Dilution Water	DW
4-(2-Hydroxyethyl)-piperazine-1-	
ethane-sulfonic acid	HEPES

	,	Page
Table 1:	Nitrogen in the environment	11
Table 2 :	Phosphorus in the environment	14
Table 3 :	The Swedish eutrophication classification system	18
	according to algal biomass in the lakes	
Table 4 :	Nitrogen concentrations in freshwater algae (% dry mass)	32
Table 5 :	Effect of nitrogen sources on the growth and physiology of	38
	microalgae	
Table 6 :	Phosphorus concentrations in freshwater algae (% dry	41
	mass)	
Table 7 :	Kinetic parameter values for orthophosphate uptake by	46
	algae	
Table 8 :	Effect of phosphorus on the growth and physiology of	48
	microalgae	
Table 9 :	Composition of Bold Basal Medium (BBM)	53
Table 10:	Composition of Dilution Water	54
Table 11:	Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	62
	of Chlorella vulgaris UMACC 001 cultured in different	
	concentrations of BBM compared with that in Dilution	
	Water	
Table 12:	Specific growth rates (µ) calculated within different growth	66
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different NaNO <sub>3</sub> concentrations	
Table 13:	Specific growth rates (µ) calculated within different growth	67
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different NaNO <sub>3</sub> concentrations	
Table 14:	Specific growth rates (µ) calculated within different growth	67
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different NaNO <sub>3</sub> concentrations	

Table 15:	Percentage Growth Enhancement (PGE) based on cell	69
	numbers attained at 12 h and 96 h of the three	
	chlorophytes grown at different levels of NaNO <sub>3</sub>	
Table 16:	Specific growth rates (µ) calculated within different growth	72
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different NaNO <sub>3</sub> concentrations	
Table 17:	Specific growth rates (µ) calculated within different growth	73
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different NaNO <sub>3</sub> concentrations	
Table 18:	Specific growth rates (µ) calculated within different growth	73
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different NaNO <sub>3</sub> concentrations	
Table 19:	Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	75
	attained at 12 h and 96 h of the three chlorophytes grown	
	at different levels of NaNO <sub>3</sub>	
Table 20:	Linear regression coefficients between OD <sub>620</sub> and cell	78
	numbers of three chlorophytes grown at different levels of	
	NaNO <sub>3</sub>	
Table 21:	Specific growth rates (µ) calculated within different growth	81
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different NH <sub>4</sub> Cl concentrations	
Table 22:	Specific growth rates (µ) calculated within different growth	82
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different NH₄Cl concentrations	
Table 23:	Specific growth rates (µ) calculated within different growth	82
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different NH <sub>4</sub> Cl concentrations	
Table 24:	Percentage Growth Enhancement (PGE) based on cell	84
	number attained at 12 h and 96 h of the three chlorophytes	
	grown at different levels of NH <sub>4</sub> Cl	

Table 25:	Specific growth rates (µ) calculated within different growth	87
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different NH <sub>4</sub> CI concentrations	
Table 26:	Specific growth rates (µ) calculated within different growth	88
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different NH <sub>4</sub> Cl concentrations	
Table 27:	Specific growth rates (µ) calculated within different growth	88
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different NH <sub>4</sub> Cl concentrations	
Table 28:	Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	90
	attained at 12 h and 96 h of the three chlorophytes grown	
	at different levels of NH₄Cl	
Table 29:	Linear regression coefficients between OD <sub>620</sub> and cell	93
	numbers of three chlorophytes grown at different levels of	
	NH <sub>4</sub> CI	
Table 30:	Specific growth rates (µ) calculated within different growth	97
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different PO <sub>4</sub> concentrations	
Table 31:	Specific growth rates (µ) calculated within different growth	98
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different PO <sub>4</sub> concentrations	
Table 32:	Specific growth rates (µ) calculated within different growth	98
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different PO <sub>4</sub> concentrations	
Table 33:	Percentage Growth Enhancement (PGE) based on cell	100
	numbers attained at 12 h and 96 h of the three	
	chlorophytes grown at different levels of phosphate in low-	
	NaNO <sub>3</sub> (0.03 mM) medium	
Table 34:	Specific growth rates (µ) calculated within different growth	103
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different PO <sub>4</sub> concentrations	

Table 35:

	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different PO <sub>4</sub> concentrations	
Table 36:	Specific growth rates (µ) calculated within different growth	104
41	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different PO <sub>4</sub> concentrations	
Table 37:	Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	106
	attained at 12 h and 96 h of the three chlorophytes grown	
	at different levels of phosphate in low-NaNO <sub>3</sub> (0.03 mM)	
	medium	
Table 38:	Linear regression coefficients between OD <sub>620</sub> and cell	109
	numbers of three chlorophytes grown at different levels of	
	phosphate in medium containing low NaNO <sub>3</sub>	
Table 39:	Specific growth rates (µ) calculated within different growth	113
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different PO₄ concentrations	
Table 40:	Specific growth rates (µ) calculated within different growth	114
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different PO <sub>4</sub> concentrations	
Table 41:	Specific growth rates (µ) calculated within different growth	114
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different PO <sub>4</sub> concentrations	
Table 42:	Percentage Growth Enhancement (PGE) based on cell	116
	numbers attained at 12 h and 96 h of the three	
	chlorophytes grown at different levels of phosphate in low-	
	NH <sub>4</sub> CI (0.03 mM) medium	
Table 43:	Specific growth rates (µ) calculated within different growth	119
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different PO <sub>4</sub> concentrations	
		-

Specific growth rates (µ) calculated within different growth

104

Table 11.	opositio growth rates (p) salediated within directiff growth	120
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different PO <sub>4</sub> concentrations	
Table 45:	Specific growth rates (µ) calculated within different growth	120
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different PO <sub>4</sub> concentrations	
Table 46:	Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	122
	attained at 12 h and 96 h of the three chlorophytes grown	
	at different levels of phosphate in low-NH <sub>4</sub> Cl (0.03 mM)	
	medium	
Table 47:	Linear regression coefficients between OD <sub>620</sub> and cell	125
	numbers of three chlorophytes grown at different levels of	
	phosphate in medium containing low NH <sub>4</sub> Cl	
Face		
Table 48:	Specific growth rates (µ) calculated within different growth	128
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different PO <sub>4</sub> concentrations	
Table 49:	Specific growth rates (µ) calculated within different growth	129
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different PO <sub>4</sub> concentrations	
Table 50:	Specific growth rates (µ) calculated within different growth	129
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different PO <sub>4</sub> concentrations	
Table 51:	Percentage Growth Enhancement (PGE) based on cell	131
	numbers attained at 12 h and 96 h of the three	
	chlorophytes grown at different levels of phosphate in	
	high-NaNO <sub>3</sub> (18.75 mM) medium	
Table 52:	Specific growth rates (µ) calculated within different growth	134
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different PO <sub>4</sub> concentrations	

Table 44: Specific growth rates (µ) calculated within different growth

120

Table 53:	Specific growth rates (µ) calculated within different growth	135
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different PO <sub>4</sub> concentrations	
Table 54:	Specific growth rates (µ) calculated within different growth	135
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different PO <sub>4</sub> concentrations	
Table 55:	Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	136
	attained at 12 h and 96 h of the three chlorophytes grown	
	at different levels of phosphate in high-NaNO <sub>3</sub> (18.75 mM)	
	medium	
Table 56:	Linear regression coefficients between OD <sub>620</sub> and cell	139
	numbers of three chlorophytes grown at different levels of	
	phosphate in medium containing high NaNO <sub>3</sub>	
Table 57:	Specific growth rates (µ) calculated within different growth	142
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different PO <sub>4</sub> concentrations	
Table 58:	Specific growth rates (µ) calculated within different growth	143
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different PO <sub>4</sub> concentrations	
Table 59:	Specific growth rates (µ) calculated within different growth	143
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different PO <sub>4</sub> concentrations	
Table 60:	Percentage Growth Enhancement (PGE) based on cell	145
	numbers attained at 12 h and 96 h of the three	
	chlorophytes grown at different levels of phosphate in	
	high-NH₄Cl (18.75 mM) medium	
Table 61:	Specific growth rates (µ) calculated within different growth	148
	periods of Chlorella vulgaris UMACC 001 cultured at	
	different PO <sub>4</sub> concentrations	

Table 62:	Specific growth rates (µ) calculated within different growth	149
	periods of Scenedesmus quadricauda UMACC 041	
	cultured at different PO <sub>4</sub> concentrations	
Table 63:	Specific growth rates (µ) calculated within different growth	149
	periods of Ankistrodesmus convolutus UMACC 101	
	cultured at different PO <sub>4</sub> concentrations	
Table 64:	Percentage Growth Enhancement (PGE) based on OD <sub>620</sub>	150
	attained at 12 h and 96 h of the three chlorophytes grown	
	at different levels of phosphate in high-NH <sub>4</sub> Cl (18.75 mM)	
	medium	
Table 65:	Linear regression coefficients between OD <sub>620</sub> and cell	153
	numbers of three chlorophytes grown at different levels of	
	phosphate in medium containing high NH₄Cl	
Table 66:	Nitrogen levels in various culture media and test media	155
Table 67:	A summary of growth responses of the three chlorophytes	157
	to nitrogen and phosphorus levels in the medium	

		1 age
Figure 1:	Outlined of study	9
Figure 2:	Microalgae used in thepresent study	51
Figure 3:	Semi-logarithmic growth curves based on OD <sub>620</sub> of Chlorella vulgaris UMACC 001 cultured at different concentrations of Bols Basal Medium (BBM) compared to Dilution Water	61
Figure 4:	Semi-logarithmic growth curves based on cell numbers of <i>Chlorella vulgaris</i> UMACC 001 cultured at different concentrations of NaNO <sub>3</sub>	64
Figure 5:	Semi-logarithmic growth curves based on cell numbers of <i>Scenedesmus quadricauda</i> UMACC 041 cultured at different concentrations of NaNO <sub>3</sub>	64
Figure 6:	Semi-logarithmic growth curves based on cell numbers of <i>Ankistrodesmus convolutus</i> UMACC 101 cultured at different concentrations of NaNO <sub>3</sub>	65
Figure 7:	Semi-logarithmic growth curves based on OD <sub>620</sub> of <i>Chlorella vulgaris</i> UMACC 001 cultured at different concentrations of NaNO <sub>3</sub>	70

Page

Figure 8:	Semi-logarithmic growth curves based on OD <sub>620</sub> of	71
	Scenedesmus quadricauda UMACC 041 cultured	
	at different concentrations of NaNO <sub>3</sub>	
Figure 9:	Semi-logarithmic growth curves based on $OD_{620}$ of	71
	Ankistrodesmus convolutus UMACC 101 cultured	
	at different concentrations of NaNO <sub>3</sub>	
Figure 10:	Linear regression analysis between OD <sub>620</sub> and cell	77
	numbers of the three chlorophytes grown at	
	different levels of NaNO <sub>3</sub>	
Figure 11:	Semi-logarithmic growth curves based on cell	79
	numbers of Chlorella vulgaris UMACC 001	
	cultured at different concentrations of NH <sub>4</sub> Cl	
Figure 12:	Semi-logarithmic growth curves based on cell	80
	numbers of Scenedesmus quadricauda UMACC	
	041 cultured at different concentrations of NH <sub>4</sub> Cl	
Figure 13:	Semi-logarithmic growth curves based on cell	80
,	numbers of Ankistrodesmus convolutus UMACC	
	101 cultured at different concentrations of NH <sub>4</sub> Cl	*
Figure 14:	Semi-logarithmic growth curves based on OD <sub>620</sub>	85
	of Chlorella vulgaris UMACC 001 cultured at	
	different concentrations of NH <sub>4</sub> Cl	

# List of Figures Figure 15:

Figure 15.	Serni-logarithmic growth curves based on OD620 of	65
	Scenedesmus quadricauda UMACC 041 cultured	
	at different concentrations of NH <sub>4</sub> Cl	
Figure 16:	Semi-logarithmic growth curves based on OD <sub>620</sub> of	86
	Ankistrodesmus convolutus UMACC 101 cultured	
	at different concentrations of NH₄Cl	
Figure 17:	Linear regression analysis between OD <sub>620</sub> and cell	92
	numbers of the three chlorophytes grown at	
	different levels of NH <sub>4</sub> Cl	
Figure18:	Semi-logarithmic growth curves based on cell	95
	numbers of Chlorella vulgaris UMACC 001	
	cultured at different levels of PO <sub>4</sub> in medium	
	containing 0.03 mM NaNO₃	
Figure 19:	Semi-logarithmic growth curves based on cell	95
	numbers of Scenedesmus quadricauda UMACC	
	041 cultured at different levels of PO <sub>4</sub> in medium	
	containing 0.03 mM NaNO <sub>3</sub>	
Figure 20:	Semi-logarithmic growth curves based on cell	96
	numbers of Ankistrodesmus convolutus UMACC	
	101 cultured at different levels of PO <sub>4</sub> in medium	
	containing 0.03 mM NaNO <sub>3</sub>	
	a 2 - 2 - 2 - 2 - 2 - 2	

Semi-logarithmic growth curves based on OD<sub>620</sub> of 85

Figure 21:	Semi-logarithmic growth curves based on OD <sub>620</sub>	101
	of Chlorella vulgaris UMACC 001 cultured at	
,	different levels of PO <sub>4</sub> in medium containing 0.03	
	mM NaNO <sub>3</sub>	,
Figure 22:	Semi-logarithmic growth curves based on OD <sub>620</sub> of	101
	Scenedesmus quadricauda UMACC 041 cultured	
	at different levels of PO <sub>4</sub> in medium containing	
	0.03 mM NaNO <sub>3</sub>	
Figure 23:	Semi-logarithmic growth curves based on OD <sub>620</sub> of	102
	Ankistrodesmus convolutus UMACC 101 cultured	
	at different levels of PO <sub>4</sub> in medium containing	
	0.03 mM NaNO₃	
Figure 24:	Linear regression analysis between OD <sub>620</sub> and cell	108
	numbers of the three chlorophytes grown at	
	different levels of phosphate	
Figure 25:	Semi-logarithmic growth curves based on cell	111
	numbers of Chlorella vulgaris UMACC 001	
400 - 4	cultured at different levels of PO <sub>4</sub> in medium	
	containing 0.03 mM NH₄Cl	
Figure 26:	Semi-logarithmic growth curves based on cell	111
	numbers of Scenedesmus quadricauda UMACC	
	041 cultured at different levels of PO <sub>4</sub> in medium	
	containing 0.03 mM NH <sub>4</sub> Cl	

	0 11 10 1 -1	112
Figure 27:	Semi-logarithmic growth curves based on cell	112
	numbers of Ankistrodesmus convolutus UMACC	
	101 cultured at different levels of PO <sub>4</sub> in medium	
4	containing 0.03 mM NH <sub>4</sub> Cl	
Figure 28:	Semi-logarithmic growth curves based on OD <sub>620</sub>	117
	of Chlorella vulgaris UMACC 001 cultured at	
	different levels of PO <sub>4</sub> in medium containing 0.03	
	mM NH₄CI	
Figure 29:	Semi-logarithmic growth curves based on OD <sub>620</sub> of	117
	Scenedesmus quadricauda UMACC 041 cultured	
	at different levels of PO <sub>4</sub> in medium containing	
	0.03 mM NH <sub>4</sub> Cl	
Figure 30:	Semi-logarithmic growth curves based on OD <sub>620</sub> of	118
	Ankistrodesmus convolutus UMACC 101 cultured	
	at different levels of PO <sub>4</sub> in medium containing	
-	0.03 mM NH <sub>4</sub> Cl	
Figure 31:	Linear regression analysis between OD <sub>620</sub> and cell	124
	numbers of the three chlorophytes grown at	-
	different levels of phosphate	
Figure 32:	Semi-logarithmic growth curves based on cell	126
	numbers of Chlorella vulgaris UMACC 001	
	cultured at different levels of PO <sub>4</sub> in medium	
	containing 18.75 mM NaNO <sub>3</sub>	4

Figure 33:	Semi-logarithmic growth curves based on cell	127
	numbers of Scenedesmus quadricauda UMACC	
	041 cultured at different levels of PO <sub>4</sub> in medium	
	containing 18.75 mM NaNO₃	
Figure 34:	Semi-logarithmic growth curves based on cell	127
	numbers of Ankistrodesmus convolutus UMACC	
	101 cultured at different levels of PO <sub>4</sub> in medium	
	containing 18.75 mM NaNO <sub>3</sub>	
Figure 35:	Semi-logarithmic growth curves based on OD <sub>620</sub>	132
	of Chlorella vulgaris UMACC 001 cultured at	
	different levels of PO <sub>4</sub> in medium containing 18.75	
	mM NaNO₃	
Figure 36:	Semi-logarithmic growth curves based on OD <sub>620</sub> of	132
	Scenedesmus quadricauda UMACC 041 cultured	
	at different levels of PO <sub>4</sub> in medium containing	
	18.75 mM NaNO₃	
Figure 37:	Semi-logarithmic growth curves based on OD <sub>620</sub> of	133
	Ankistrodesmus convolutus UMACC 101 cultured	
	at different levels of PO <sub>4</sub> in medium	
	containing18.75 mM NaNO₃	
Figure 38:	Linear regression analysis between OD <sub>620</sub> and cell	138
	numbers of the three chlorophytes grown at	
	different levels of phosphate	

Figure 39:	Semi-logarithmic growth curves based on cell numbers of <i>Chlorella vulgaris</i> UMACC 001 cultured at different levels of PO <sub>4</sub> in medium containing 18.75 mM NH <sub>4</sub> Cl	140
Figure 40:	Semi-logarithmic growth curves based on cell numbers of Scenedesmus quadricauda UMACC 041 cultured at different levels of PO <sub>4</sub> in medium containing 18.75 mM NH <sub>4</sub> Cl	141
Figure 41:	Semi-logarithmic growth curves based on cell numbers of <i>Ankistrodesmus convolutus</i> UMACC 101 cultured at different levels of PO <sub>4</sub> in medium containing 18.75 mM NH <sub>4</sub> Cl	141
Figure 42:	Semi-logarithmic growth curves based on OD <sub>620</sub> of <i>Chlorella vulgaris</i> UMACC 001 cultured at different levels of PO <sub>4</sub> in medium containing 18.75 mM NH <sub>4</sub> Cl	146
Figure 43:	Semi-logarithmic growth curves based on OD <sub>620</sub> of Scenedesmus quadricauda UMACC 041 cultured at different levels of PO <sub>4</sub> in medium containing 18.75 mM NH <sub>4</sub> Cl	146

Figure 44:	Semi-logarithmic growth curves based on OD <sub>620</sub> of Ankistrodesmus convolutus UMACC 101 cultured at different levels of PO <sub>4</sub> in medium containing18.75 mM NH <sub>4</sub> Cl	147
Figure 45:	Linear regression analysis between OD <sub>620</sub> and cell numbers of the three chlorophytes grown at different levels of phosphate	152

		9-
Appendix 1:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of <i>Chlorella</i>	189
	vulgaris UMACC 001 grown at different NaNO <sub>3</sub>	
	levels	
Appendix 2:	OD <sub>620</sub> of <i>Chlorella vulgaris</i> UMACC 001 grown	189
	at different NaNO₃ levels	
Appendix 3:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of	190
	Scenedesmus quadricauda UMACC 041 grown	
	at different NaNO₃ levels	
Appendix 4:	OD <sub>620</sub> of Scenedesmus quadricauda UMACC	190
	041 grown at different NaNO <sub>3</sub> levels	
Appendix 5:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of	191
	Ankistrodesmus convolutus UMACC 101 grown	
	at different NaNO <sub>3</sub> levels	
Appendix 6:	OD <sub>620</sub> of Ankistrodesmus convolutus UMACC	191
	101 grown at different NaNO₃ levels	41
Appendix 7:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Chlorella	192
	vulgaris UMACC 001 grown at different NH <sub>4</sub> Cl	
	levels	
Appendix 8:	OD <sub>620</sub> of Chlorella vulgaris UMACC 001 grown	192
	at different NH₄Cl levels	

Page

Con count (meanixo.b x 10 mm) of	193
Scenedesmus quadricauda UMACC041 grown	
at different NH₄Cl levels	
OD <sub>620</sub> of Scenedesmus quadricauda UMACC	193
041 grown at different NH <sub>4</sub> Cl levels	
Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of	194
Ankistrodesmus convolutus UMACC 101 grown	
at different NH <sub>4</sub> Cl levels	
OD <sub>620</sub> of Ankistrodesmus convolutus UMACC	194
101 grown at different NH₄Cl levels	
Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of <i>Chlorella</i>	195
vulgaris UMACC 001 grown at different levels of	
PO4 <sup>3-</sup> in low- NaNO <sub>3</sub> (0.03 mM) medium	
OD <sub>620</sub> of of Chlorella vulgaris UMACC 001	195
grown at different levels of PO4 <sup>3-</sup> in low- NaNO <sub>3</sub>	
(0.03 mM) medium	
Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of	196
Scenedesmus quadricauda UMACC 041 grown	
at different levels of PO4 <sup>3-</sup> in low- NaNO <sub>3</sub> (0.03	
mM) medium	
OD <sub>620</sub> of of Scenedesmus quadricauda UMACC	196
041 grown at different levels of PO43- in low-	
	at different NH <sub>4</sub> Cl levels  OD <sub>620</sub> of Scenedesmus quadricauda UMACC 041 grown at different NH <sub>4</sub> Cl levels  Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Ankistrodesmus convolutus UMACC 101 grown at different NH <sub>4</sub> Cl levels  OD <sub>620</sub> of Ankistrodesmus convolutus UMACC 101 grown at different NH <sub>4</sub> Cl levels  Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Chlorella vulgaris UMACC 001 grown at different levels of PO4 <sup>3-</sup> in low- NaNO <sub>3</sub> (0.03 mM) medium  OD <sub>620</sub> of of Chlorella vulgaris UMACC 001 grown at different levels of PO4 <sup>3-</sup> in low- NaNO <sub>3</sub> (0.03 mM) medium  Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Scenedesmus quadricauda UMACC 041 grown at different levels of PO4 <sup>3-</sup> in low- NaNO <sub>3</sub> (0.03 mM) medium

Appendix 9: Cell count (mean±S.D x 10<sup>5</sup> ml<sup>-1</sup>) of

193

Appendix 17:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Ankistrodesmus convolutus UMACC 101 grown at different levels of PO4 <sup>3-</sup> in low- NaNO <sub>3</sub> (0.03 mM) medium	197
Appendix 18:	OD <sub>620</sub> of <i>Ankistrodesmus convolutus</i> UMACC 101 grown at different levels of PO4 <sup>3-</sup> in low- NaNO <sub>3</sub> (0.03 mM) medium	197
Appendix 19:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of <i>Chlorella</i> vulgaris UMACC 001 grown at different levels of PO4 <sup>3</sup> · in low- NH <sub>4</sub> Cl (0.03 mM) medium	198
Appendix 20:	OD <sub>620</sub> of <i>Chlorella vulgaris</i> UMACC 001 grown at different levels of PO4 <sup>3-</sup> in low- NH <sub>4</sub> Cl (0.03 mM) medium	198
Appendix 21:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Scenedesmus quadricauda UMACC 041 grown at different levels of PO4 <sup>3-</sup> in low- NH <sub>4</sub> Cl (0.03 mM) medium	199
Appendix 22:	OD <sub>620</sub> of Scenedesmus quadricauda UMACC 041 grown at different levels of PO4 <sup>3-</sup> in low-NH <sub>4</sub> Cl (0.03 mM) medium	199

Appendix 23:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Anikstrodesmus convolutus UMACC101 grown at different levels of PO4 <sup>3-</sup> in low- NH <sub>4</sub> Cl (0.03 mM) medium	200
Appendix 24:	OD <sub>620</sub> of <i>Anikstrodesmus convolutus</i> UMACC101 grown at different levels of PO4 <sup>3-</sup> in low- NH <sub>4</sub> Cl (0.03 mM) medium	200
Appendix 25:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of <i>Chlorella</i> vulgaris UMACC 001 grown at different levels of PO4 <sup>3-</sup> in high- NaNO <sub>3</sub> (18.75 mM) medium	201
Appendix 26:	OD <sub>620</sub> of <i>Chlorella vulgaris</i> UMACC 001 grown at different levels of PO4 <sup>3-</sup> in high- NaNO <sub>3</sub> (18.75 mM) medium	201
Appendix 27:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Scenedesmus quadricauda UMACC 041 grown at different levels of PO4 <sup>3-</sup> in high- NaNO <sub>3</sub> (18.75 mM) medium	202
Appendix 28:	OD <sub>620</sub> of <i>Scenedesmus quadricauda</i> UMACC 041 grown at different levels of PO4 <sup>3-</sup> in high- NaNO <sub>3</sub> (18.75 mM) medium	202

Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Ankistrodemus convolutus UMACC 101 grown at different levels of PO4 <sup>3-</sup> in high- NaNO <sub>3</sub> (18.75 mM) medium	203
OD <sub>620</sub> of <i>Ankistrodemus convolutus</i> UMACC 101 grown at different levels of PO4 <sup>3-</sup> in high-NaNO <sub>3</sub> (18.75 mM) medium	203
Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of <i>Chlorella</i> vulgaris UMACC 001 grown at different levels of PO4 <sup>3-</sup> in high- NH <sub>4</sub> Cl (18.75 mM) medium	204
OD <sub>620</sub> of of <i>Chlorella vulgaris</i> UMACC 001 grown at different levels of PO4 <sup>3-</sup> in high- NH <sub>4</sub> Cl (18.75 mM) medium	204
Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Scenedesmus quadricauda UMACC 041 grown at different levels of PO4 <sup>3-</sup> in high- NH <sub>4</sub> Cl (18.75 mM) medium	205
OD <sub>620</sub> of Scenedesmus quadricauda UMACC 041 grown at different levels of PO4 <sup>3-</sup> in high-NH <sub>4</sub> Cl (18.75 mM) medium	205
	Ankistrodemus convolutus UMACC 101 grown at different levels of PO4 <sup>3-</sup> in high- NaNO <sub>3</sub> (18.75 mM) medium  OD <sub>620</sub> of Ankistrodemus convolutus UMACC 101 grown at different levels of PO4 <sup>3-</sup> in high-NaNO <sub>3</sub> (18.75 mM) medium  Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Chlorella vulgaris UMACC 001 grown at different levels of PO4 <sup>3-</sup> in high-NH <sub>4</sub> Cl (18.75 mM) medium  OD <sub>620</sub> of of Chlorella vulgaris UMACC 001 grown at different levels of PO4 <sup>3-</sup> in high-NH <sub>4</sub> Cl (18.75 mM) medium  Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of Scenedesmus quadricauda UMACC 041 grown at different levels of PO4 <sup>3-</sup> in high-NH <sub>4</sub> Cl (18.75 mM) medium  OD <sub>620</sub> of Scenedesmus quadricauda UMACC 041 grown at different levels of PO4 <sup>3-</sup> in high-NH <sub>4</sub> Cl (18.75 mM) medium

Appendix 35:	Cell count (mean±S.D x 10 <sup>5</sup> ml <sup>-1</sup> ) of	206
	Ankistrodesmus convolutus UMACC 101 grown	
	at different levels of PO4 <sup>3-</sup> in high- NH₄Cl (18.75	
* *	mM) medium	
Appendix 36:	OD <sub>620</sub> of Ankistrodesmus convolutus UMACC	206
	101 grown at different levels of PO43- in high-	
-	NH₄CI (18.75 mM) medium	
Appendix 37:	Regression analysis between cell number and	207
×	OD <sub>620</sub> of <i>Chlorella vulgaris</i> UMACC 001 grown	
	in Bold's Basal Medium.	
		7