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## APPENDIX

### Appendix 1

Bold Basal Modified Medium (Spencer, 1989)  
contained the following components in gram per litre.

NaCl	12.5
CaCl <sub>2</sub> .2H <sub>2</sub> O	12.5
MgSO <sub>4</sub> .7H <sub>2</sub> O	38
K <sub>2</sub> HPO <sub>4</sub>	93
KH <sub>2</sub> PO <sub>4</sub>	44
EDTA	25
FeCl <sub>3</sub>	2.5
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.35
ZnSO <sub>4</sub>	4.4
MnCl <sub>2</sub>	0.73
CuSO <sub>4</sub>	0.77
CoCl <sub>3</sub>	0.23
(NH <sub>2</sub> ) <sub>2</sub> CO	0.12

In the mixotrophic condition 1.4 g/l of sodium acetate was added. The pH was adjusted to 7.3. The medium was autoclaved at 15 lb/in<sup>2</sup> for 20 minutes.

## Appendix 2

The Basal Medium (Kakizono, Kobayashi, and Nagai, 1992) contained the following components in gram per litre.

MgCl <sub>2</sub> .6H <sub>2</sub> O	0.2
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.01
CaCl <sub>2</sub> .2H <sub>2</sub> O	0.02
Yeast extract	2.0

In the mixotrophic condition 1.2 g/l of sodium acetate was added. The pH was adjusted to 6.8. The medium was autoclaved at 15 lb/in<sup>2</sup> for 20 minutes.



### Appendix 3

Medium for *H. lacustris* ATCC 30453 (Eduard et al, 1993)  
contained the following components in milligram per litre.

$K_2HPO_4$	75
$KH_2PO_4$	175
$CaCl_2 \cdot 2H_2O$	25
NaCl	25
$MgSO_4 \cdot 7H_2O$	75
$NaNO_3$	250
EDTA	25
$FeCl_3$	2.5
$Na_2MoO_4 \cdot 2H_2O$	0.35
$ZnSO_4$	4.4
$MnCl_2$	0.73
$CoCl_3$	0.23

In the mixotrophic condition 1g/l of sodium acetate was added. The pH was adjusted to 7.0. The medium was autoclaved at 15 lb/in<sup>2</sup> for 20 minutes.

## Appendix 4

### Determining of Specific Growth Rate (Black, 1996)

Specific growth rate can be described by the equation

$$\ln N - \ln N_0 = \mu (t - t_0)$$

To solve for  $\mu$ , take the logarithm of both side of the equation, and rearrange the terms

$$\log N - \log N_0 = \frac{\mu}{2.303} (t - t_0)$$

$$\mu = \frac{2.303 \log \frac{N}{N_0}}{t - t_0}$$

when  $N_0$  = number of algal at zero time  
 $N$  = number of algal at the end of a selected period of time  
 $t_0$  = time at zero  
 $t$  = time at the end of a selected period  
 $\mu$  = specific growth rate

## Appendix 5

The linear models procedure and Duncan's multiple range test for effect of sodium chloride.

<b>Dependent Variable: ASTA</b>					
		Sum of	Mean		
Source	DF	Squares	Square	F Value	Pr > F
Model	9	572.2230667	63.5803407	13.80	0.0001
Error	20	92.1434133	4.6071707		
Corrected Total	29	664.3664800			
Source	DF	Type I SS	Mean Square	F Value	Pr > F
LIGHT	4	63.9877467	15.9969367	3.47	0.0261
NaCl	5	508.2353200	101.6470640	22.06	0.0001

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 20    MSE = 4.607171

Number of Means    2       3       4       5

Critical Range    2.582   2.711   2.801   2.853

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	LIGHT
A	6.310	6	100
A			
A	6.098	6	50
B A	4.802	6	20
B			
B	3.028	6	200
B			
B	2.872	6	150

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 20    MSE = 4.607171

Number of Means    2        3        4        5        6

Critical Range    2.828   2.970   3.068   3.125   3.171

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	NaCl
A	10.890	5	0.2
A			
A	9.786	5	0
B	2.692	5	0.4
B			
B	2.534	5	0.8
B			
B	1.164	5	1.2
B			
B	0.666	5	1.6

## Appendix 6

The linear models procedure and Duncan's multiple range test for effect of carbon and nitrogen content.

### Dependent Variable: ASTA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	817.9117400	116.8445343	22.59	0.0001
Error	12	62.0605600	5.1717133		
Corrected Total	19	879.9723000			

  

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LIGHT	4	55.0982000	13.7745500	2.66	0.0844
CARBON	1	53.1288600	53.1288600	10.27	0.0076
NITROGEN	2	709.6846800	354.8423400	68.61	0.0001

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 12    MSE = 5.171713

Number of Means    2        3        4        5

Critical Range    3.497    3.663    3.774    3.833.

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	LIGHT
	A	14.393	4	50
	A			
B	A	13.143	4	150
B	A			
B	A	12.650	4	100
B	A			
B	A	12.568	4	20
B				
B		9.372	4	200

Duncan's Multiple Range Test for variable: ASTA

Harmonic Mean of cell sizes =7.5

Number of Means 2

Critical Range 2.554

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	CARBON
A	13.366	15	43.8
B	9.602	50	

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05 df = 12 MSE = 5.171713

Harmonic Mean of cell sizes = 6

Number of Means 2 3

Critical Range 2.855 2.991

Means with the same letter are not significantly different

Duncan Grouping	Mean	N	NITROGEN
A	15.887	10	0
B	12.542	5	21.9
C	5.384	5	43.8



## Appendix 7

The linear models procedure and Duncan's multiple range test for effect of sodium acetate.

### Dependent Variable: ASTA

Source	DF	Squares	Square	F Value	Pr > F
Model	7	1531.669725	218.809961	22.61	0.0001
Error	12	116.140370	9.678364		
Corrected Total	19	1647.810095			

  

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LIGHT	4	97.554870	24.388717	2.52	0.0964
SO	3	1434.114855	478.038285	49.39	0.0001

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05 df = 12 MSE = 9.678364

Number of Means      2      3      4      5

Critical Range      4.784    5.011    5.163    5.243

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	LIGHT
A	19.177	4	50
A			
B A	17.227	4	100
B A			
B A	16.765	4	150
B A			
B A	15.205	4	20
B			
B	12.573	4	200

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05 df = 12 MSE = 9.678364

Number of Means 2 3 4

Critical Range 4.279 4.482 4.618

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	SO
A	26.248	5	21.9
B	21.712	5	43.8
C	12.508	5	0
D	4.290	5	87.6

## Appendix 8

The linear models procedure and Duncan's multiple range test for effect of temperature and light intensity supplemented with 0.2 % (w/v) sodium chloride.

### Dependent Variable: ASTA

Sum of	Mean				
Source	DF	Squares	Square	F Value	Pr > F
Model	5	279.8361167	55.9672233	31.38	0.0003
Error	6	10.6997833	1.7832972		
Corrected	11	290.5359000			
Total					
Source	DF	Type I SS	Mean Square	F Value	Pr > F
LIGHT	2	32.5261500	16.2630750	9.12	0.0152
TENA	3	247.3099667	82.4366556	46.23	0.0002

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05 df = 6 MSE = 1.783297

Number of Means    2       3

Critical Range     2.311   2.395

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	LIGHT
A	10.042	4	50
A			
B A	8.310	4	100
B			
B	6.022	4	140

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 6    MSE = 1.783297

Number of Means    2        3        4

Critical Range    2.668    2.765    2.810

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TENA
A	14.353	3	22
B	10.040	3	25
C	5.900	3	30
D	2.207	3	35



## Appendix 9

The linear models procedure and Duncan's multiple range test for effect of temperature and light intensity, supplemented with 21.9 mM sodium acetate.

### Dependent Variable: ASTA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1324.620183	264.924037	26.68	0.0005
Error	6	59.588283	9.931381		
Corrected Total	11	1384.208467			

  

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LIGHT	2	7.659317	3.829658	0.39	0.6957
TESO	3	1316.960867	438.986956	44.20	0.0002

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 6    MSE = 9.931381

Number of Means    2            3

Critical Range      5.453    5.652

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	LIGHT
A	17.377	4	50
A			
A	16.720	4	100
A			
A	15.453	4	140



Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 6    MSE = 9.931381

Number of Means    2        3        4

Critical Range    6.296    6.526    6.632

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TESO
A	29.767	3	22
B	22.187	3	25
C	12.170	3	30
D	1.943	3	35

## Appendix 10

The linear models procedure and Duncan's multiple range test for effect of ferrous sulphate.

### Dependent Variable: ASTA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	62.26760833	12.45352167	7.81	0.0133
Error	6	9.57068333	1.59511389		
Corrected Total	11	71.83829167			

  

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SO219	2	0.30311667	0.15155833	0.10	0.9107
IRON	3	61.96449167	20.65483056	12.95	0.0050

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 6    MSE = 1.595114

Number of Means    2        3

Critical Range      2.185    2.265

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	SO219
A	24.875	4	40
A			
A	24.632	4	20
A			
A	24.490	4	60

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 6    MSE = 1.595114

Number of Means    2        3        4

Critical Range    2.523    2.615    2.658

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	IRON
A	28.377	3	225
B	24.363	3	450
B			
B	23.663	3	0
B			
B	22.260	3	900

**Dependent Variable: ASTA**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	186.9928250	37.3985650	5.73	0.0277
Error	6	39.1504667	6.5250778		
Corrected Total	11	226.1432917			

  

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SO438	2	100.9520667	50.4760333	7.74	0.0218
IRON	3	86.0407583	28.6802528	4.40	0.0585

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 6    MSE = 6.525078

Number of Means    2            3

Critical Range      4.420      4.581

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	SO438
A	33.893	4	40
A			
A	33.598	4	20
B	27.597	4	60

Duncan's Multiple Range Test for variable: ASTA

Alpha = 0.05    df = 6    MSE = 6.525078

Number of Means    2        3        4

Critical Range    5.104    5.290    5.376

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	IRON
	A	35.287	3	0
	A			
B	A	32.980	3	225
B	A			
B	A	30.320	3	450
B				
B		28.197	3	900



## **BIOGRAPHY**

Miss Worapa Heepchantree was born on July 15 (1969) in Bangkok, Thailand. She graduated with a Bachelor of Science Degree in Biology from Faculty of Science, Srinakarintarawirote University, Bangkhen Campus in 1991 and studied for a Master Degree in Biotechnology Programme in 1993.