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APPENDICES

APPENDIX A

STATISTICAL RESULTS

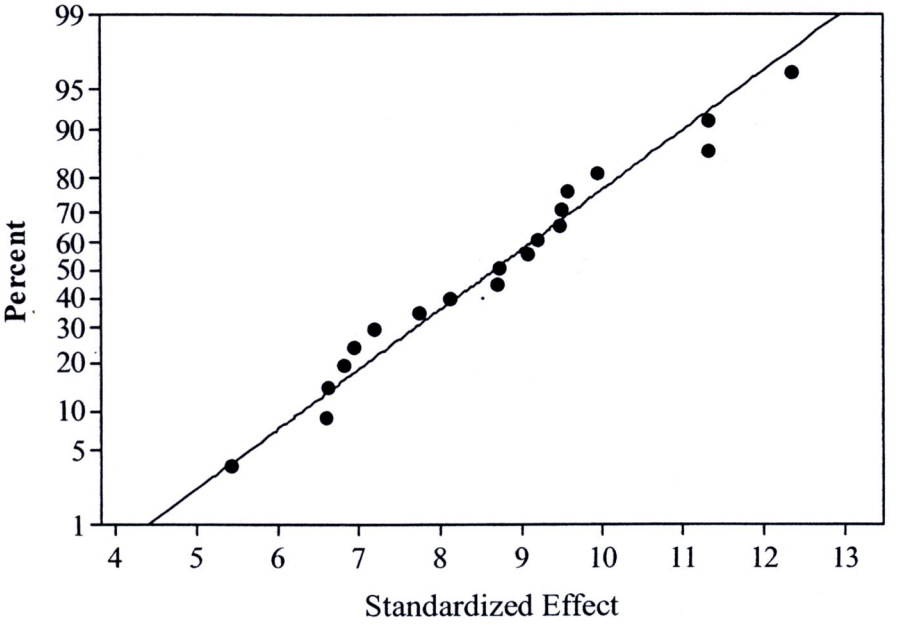

Figure A1 Normal probability plot of biomass from the data screening of factors influencing growth using fractional factorial design (p-value 0.675, AD = 0.259)

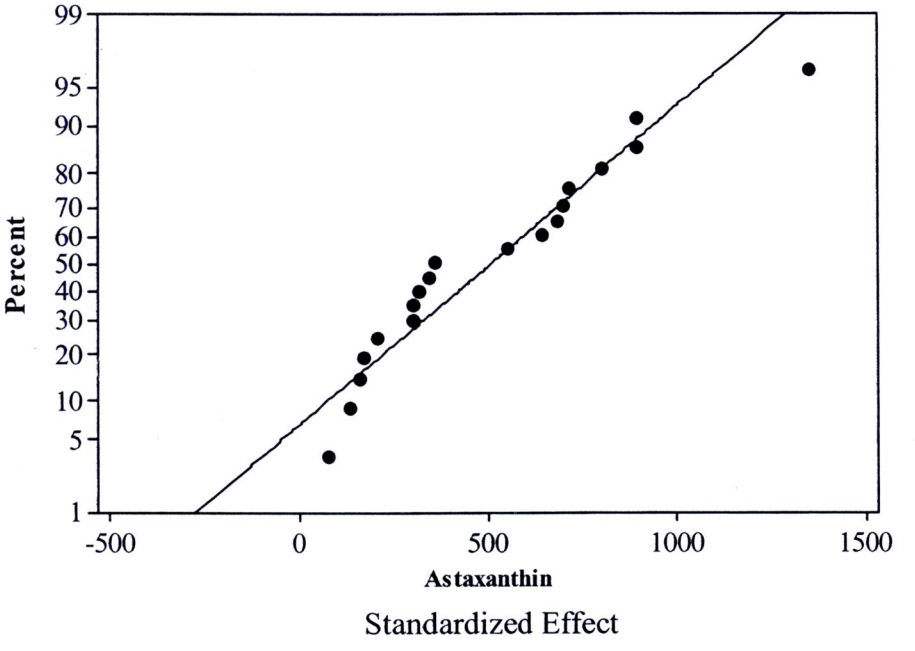

Figure A2 Normal probability plot of astaxanthin of the data from screening of factors influencing growth using fractional factorial design (p-value = 0.138, AD = 0.547)

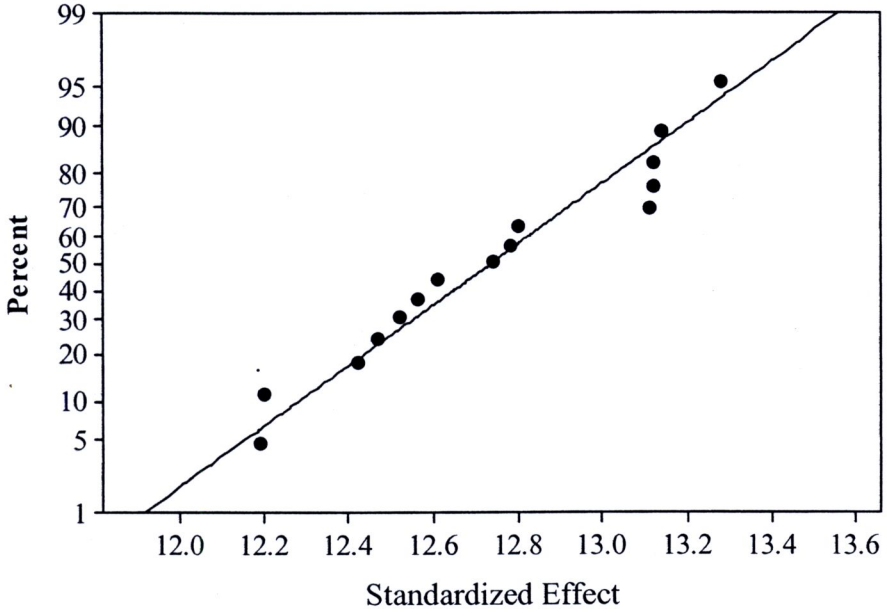

Figure A3 Normal probability plot of astaxanthin of the data from optimization of growth using Doehlert design (p-value = 0.317, AD = 0.401)

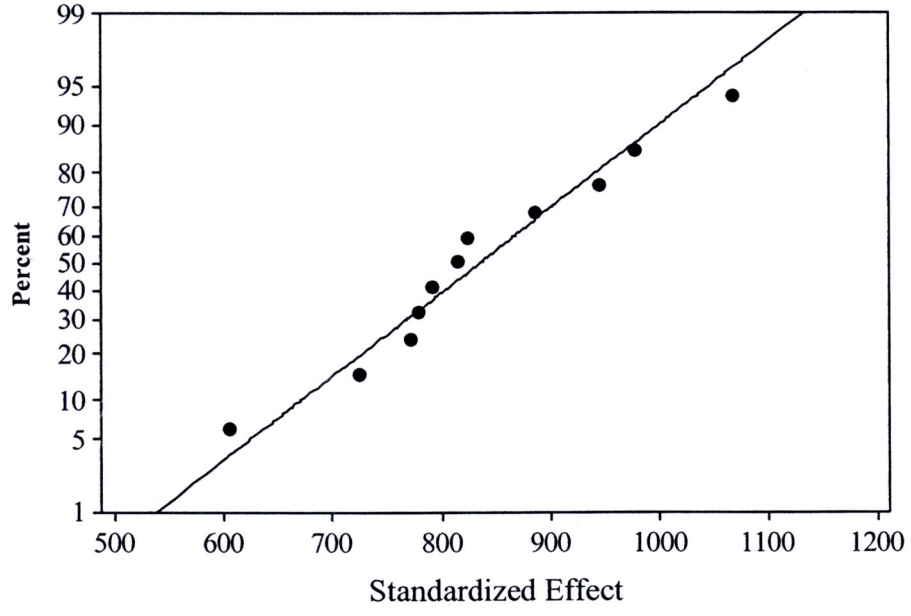

Figure A4 Normal probability plot of astaxanthin of the data from screening factors influencing astaxanthin production (p-value = 0.719, AD = 0.237)

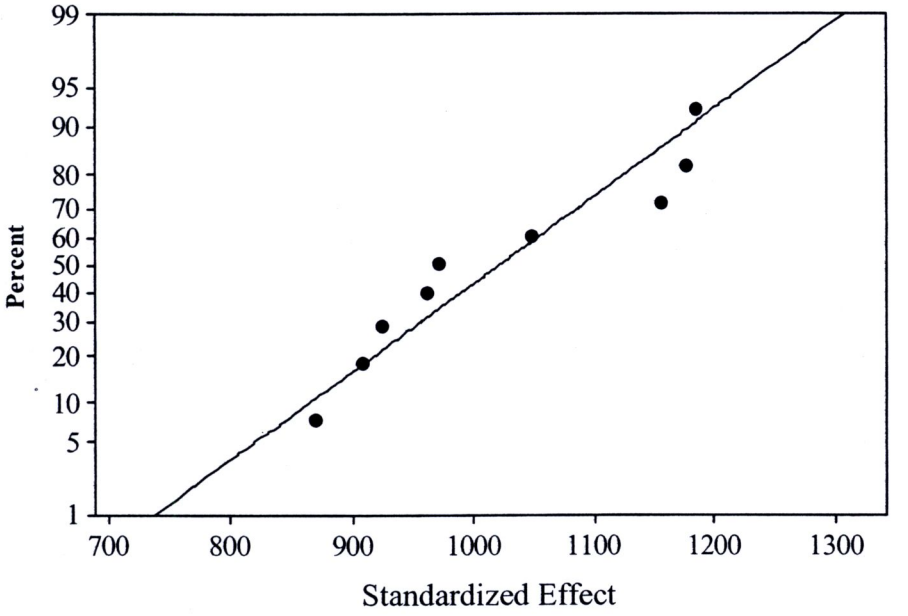

Figure A5 Normal probability plot of astaxanthin of the data from optimization of astaxanthin production using Doehlert design (p-value = 0.191, AD = 0.464)

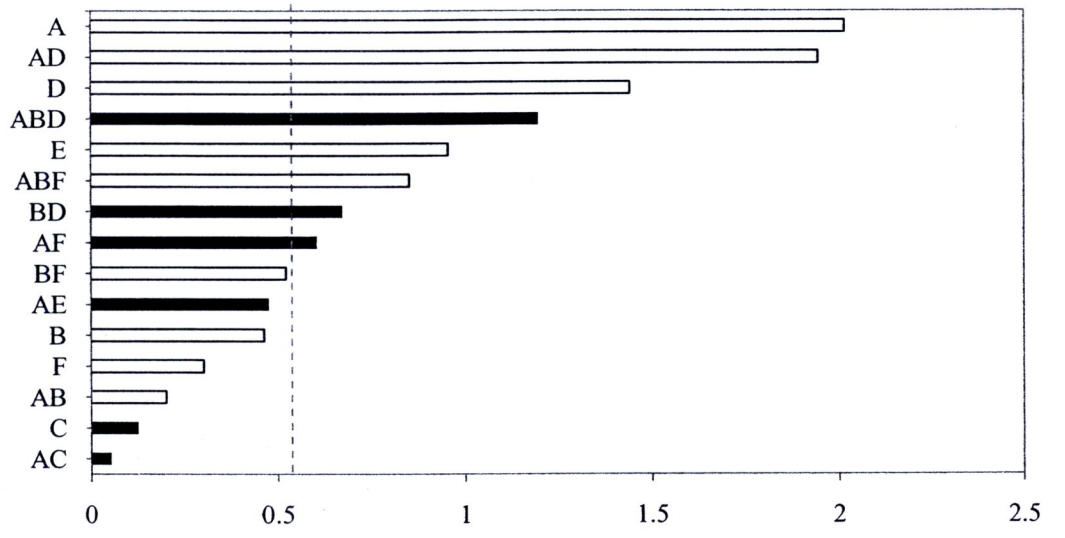

Figure A6 Pareto charts depicting the influence of sucrose (A), glucose (B), $(NH_4)_2SO_4$ (C), KNO_3 (D), *n*-hexadecane (E), and pH (F) on growth by *X. dendrorhous*. Open bars represent positive effect, while close bars represent negative effect. The dot line show significant line of each chart. (Alias pattern AD/EF, BD/CF, AF/DE, BF/CD, AE/BC, AC/BE, AB/CE)

Table A1 Regressive analysis for astaxanthin production using fractional factorial design (FFD)

Term	Effect	Coefficient	*t*	p
Constant		540.9	102.30	0.000*
A	-175.0	-87.5	-16.55	0.004*
B	-590.8	-295.4	-55.87	0.000*
C	11.2	5.6	1.06	0.401
D	-98.6	-49.3	-9.32	0.011*
E	81.9	40.9	7.74	0.016*
F	-0.9	-0.4	-0.08	0.942
AB	20.9	10.4	1.97	0.187
AC	-0.4	-0.2	-0.04	0.974
AD	52.8	26.4	4.99	0.038*
AE	-192.3	-96.2	-18.19	0.003*
AF	-29.2	-14.6	-2.76	0.110
BD	158.0	79.0	14.94	0.004*
BF	-116.4	-58.2	-11.00	0.008*
ABD	-70.0	-35.0	-6.62	0.022*
ABF	24.5	12.2	2.32	0.147

$S = 21.1515$ $R^2 = 99.96\%$ R^2-(adj) = 99.61%

APPENDIX B

STANDARD CURVE

1. Biomass Standard Curve

Table B1 Dry cell weight and corresponding optical density at 660 nm

No.	Dry Cell Wight (g/L)	OD 660
1	0	0
2	0.733	0.093
3	1.400	0.199
4	2.133	0.340
5	2.633	0.455
6	3.333	0.572
7	3.867	0.665
8	4.233	0.735
9	4.967	0.824

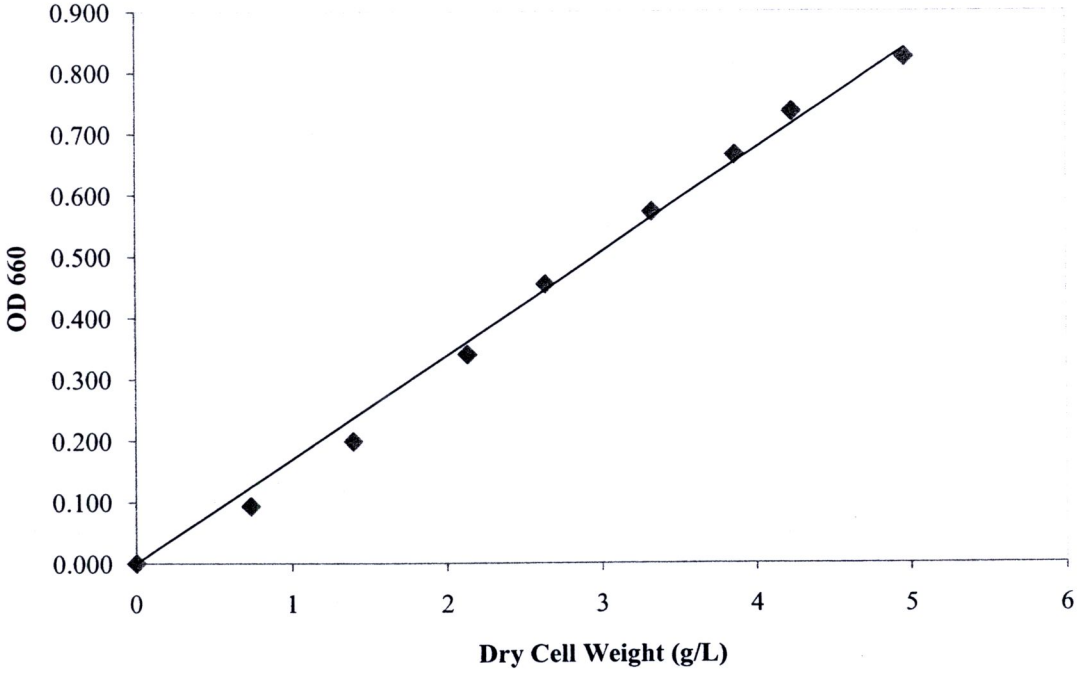

Figure B1 Correlation between dry cell weight and optical density at 660 nm with corresponding equation of y = 0.1689x (R^2 = 0.9946)

2. Sucrose Standard Curve

Table B2 Sucrose concentration and corresponding area detected by HPLC at retention time of 8.3 minutes

No.	Sucrose Concentration (g/L)	Area
1	0	0
2	10	2,332,627.00
3	20	4,820,304.50
4	40	9,542,929.00
5	60	14,104,550.50
6	80	17,560,651.50
7	100	19,646,648.50

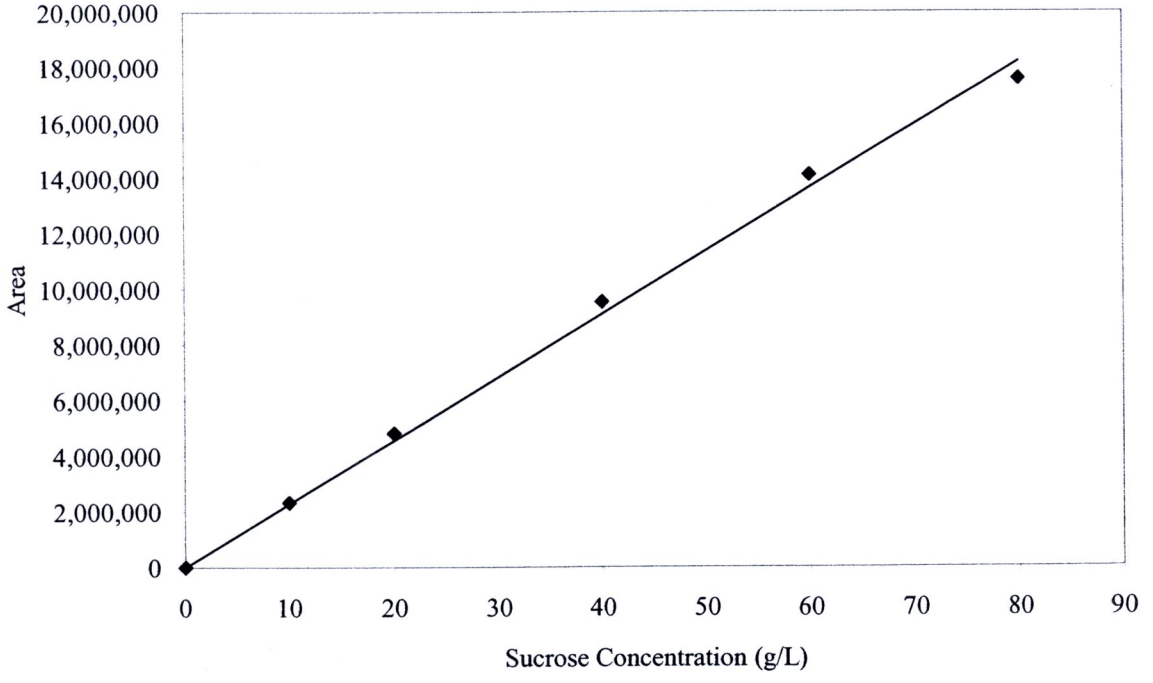

Figure B2 Correlation between sucrose concentration and total area under peaks detected with corresponding equation of y = 227,486x (R^2 = 0.9944)

3. Glucose Standard Curve

Table B3 Glucose concentration and corresponding area detected by HPLC at retention time of 10.2 minutes

No.	Glucose Concentration (g/L)	Area
1	0	0
2	10	3,145,033.00
3	20	6,255,206.00
4	40	12,927,836.50
5	60	19,166,467.50
6	80	23,795,851.50
7	100	26,355,580.00

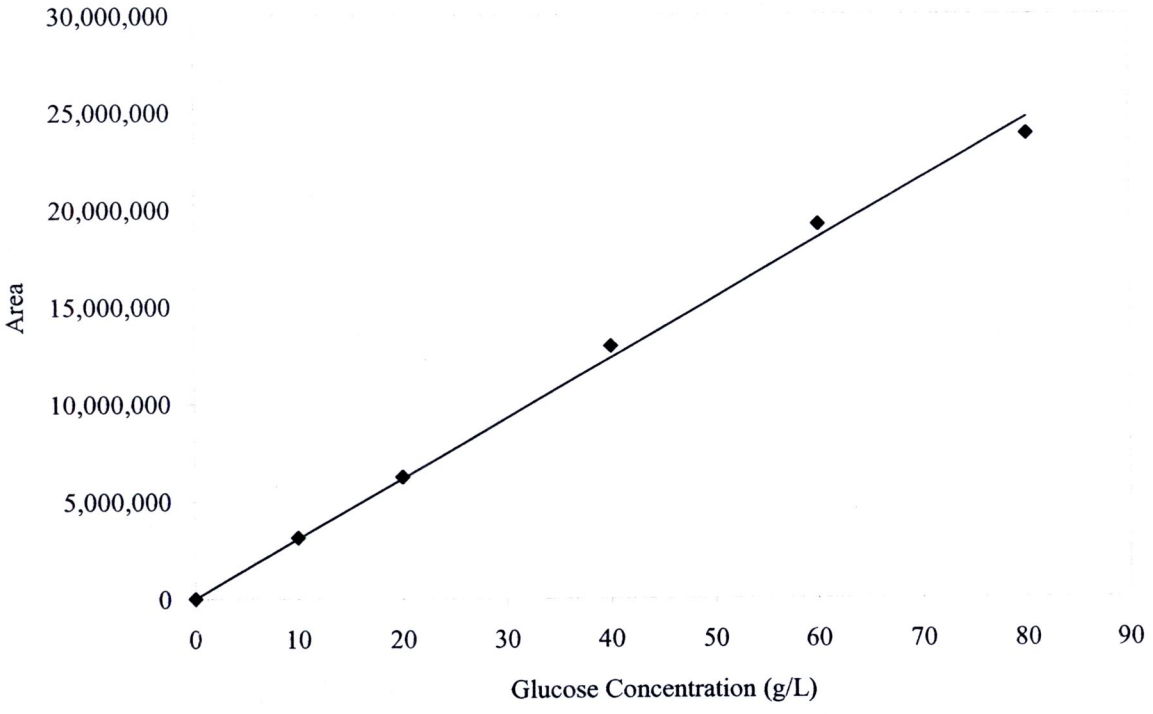

Figure B3 Correlation between glucose concentration and total area under peaks detected with corresponding equation of y = 308,043x (R^2 = 0.9947)

4. Fructose Standard Curve

Table B4 Fructose concentration and corresponding area detected by HPLC at retention time of 11.9 minutes

No.	Fructose Concentration (g/L)	Area
1	0	0
2	10	3,073,857.00
3	20	6,204,216.00
4	40	12,129,338.00
5	60	18,005,256.00
6	80	24,053,747.00
7	100	27,514,082.50

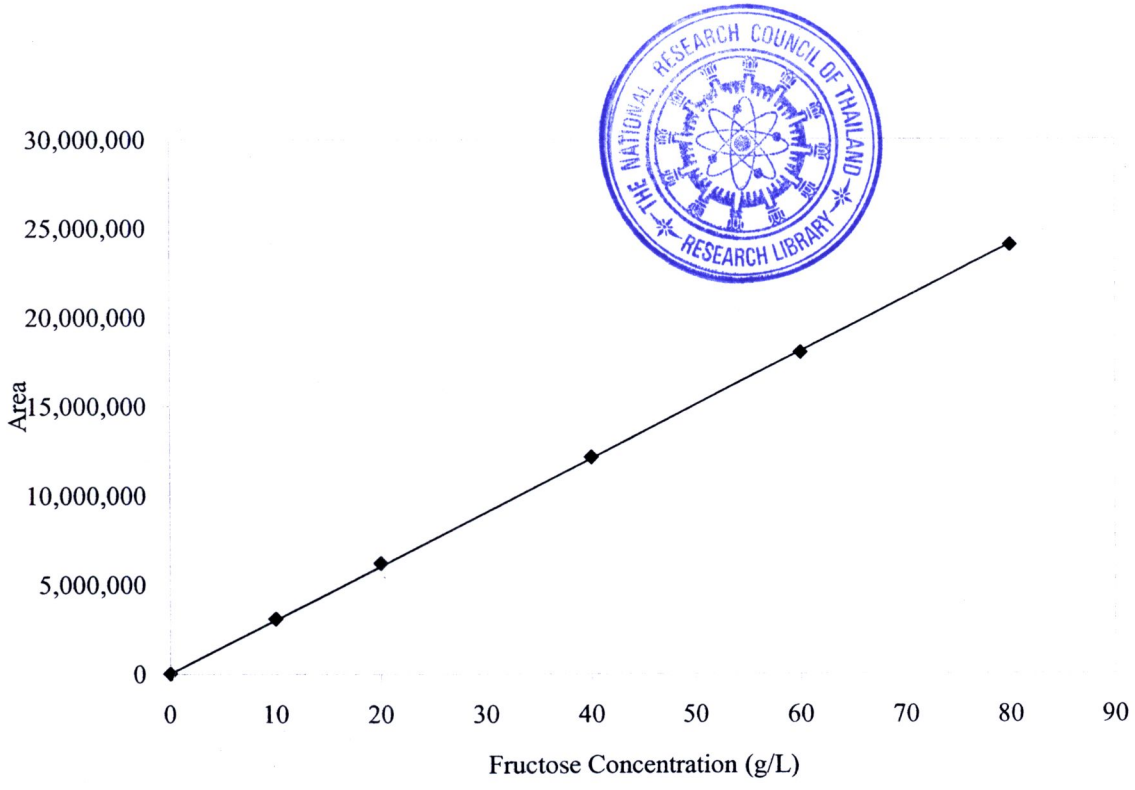

Figure B4 Correlation between fructose concentration and total area under peaks detected with corresponding equation of y = 301,208x (R^2 = 0.9998)

5. 1-kestose Standard Curve

Table B5 1-kestose concentration and corresponding area detected by HPLC at retention time of 7.3 minutes

No.	1-kestose Concentration (g/L)	Area
1	0	0
2	10	3,116,884
3	30	9,628,196
4	40	12,715,731
5	50	15,130,266

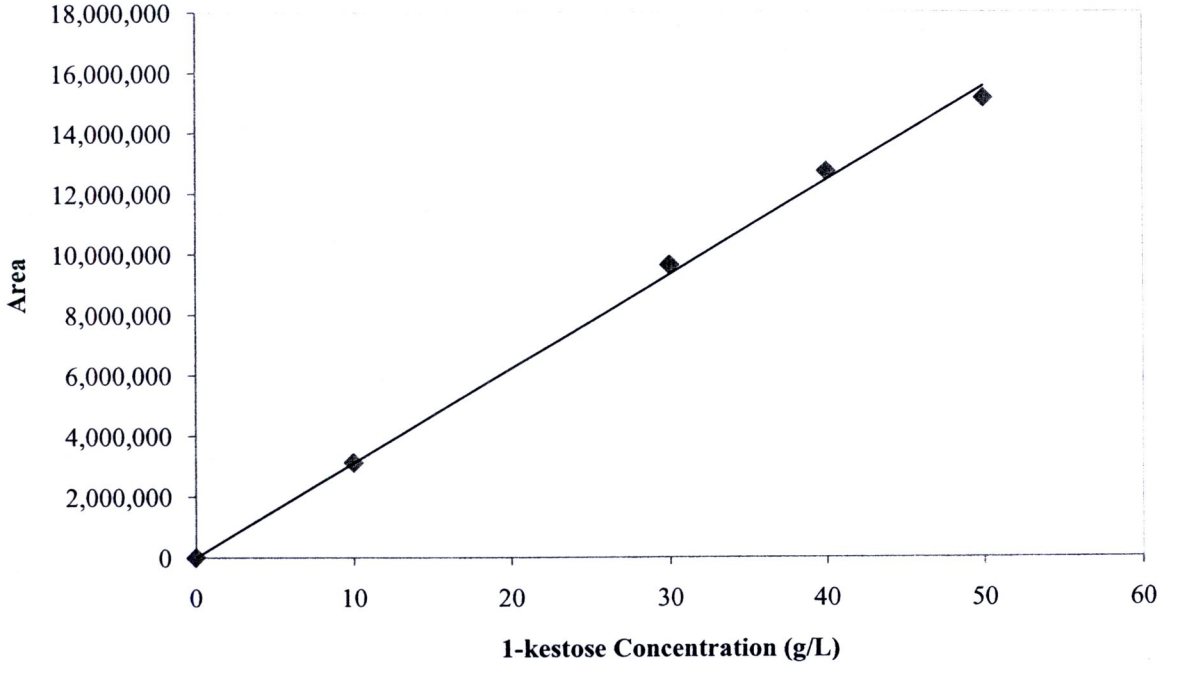

Figure B5 Correlation between 1-kestose concentration and total area under peaks detected with corresponding equation of y = 310,815x (R^2 = 0.9979)

6. Total Sugar Determination

Sugars were analyzed by the phenol-sulfuric assay as described by Dubois et al. (1956). Briefly, the standard glucose at the concentration of 0-100 µg/mL as well as various samples of 1000 µL were mixed with 1000 µL of 5% phenol concentration. Subsequently, 5 mL sulfuric acid concentrate was added, carefully mixed and incubated for 10 minutes at room temperature followed by soaked 5 min in ice water. The optical density was, then, measured at 485 nm.

Table B6 Glucose concentration and corresponding optical density at 485 nm

No.	Glucose Concentration (µg/mL)	OD 485
1	0	0.000
2	10	0.086
3	20	0.186
4	30	0.269
5	40	0.364
6	50	0.480
7	60	0.561
8	70	0.672
9	80	0.762
10	90	0.822
11	100	0.947

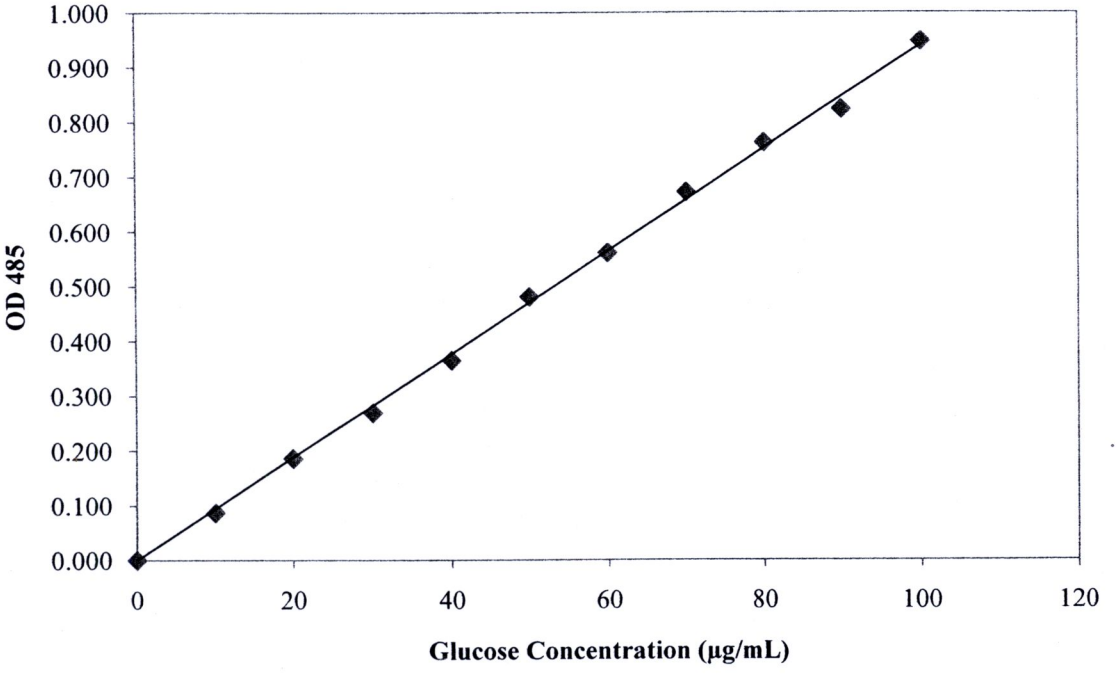

Figure B6 Correlation between glucose concentration and optical density at 485 nm with corresponding equation of y = 0.0094x (R^2 = 0.9996)

APPENDIX C

EXPERIMENTAL RESULTS

Table C1 Biomass, astaxanthin (content and concentration), total sugar, and TKN present in *X. dendrorhous* cultivated in YM medium

Time	Total sugar (g/l)	Dry weight (g/l)	Astaxanthin ($\mu g/g_{yeast}$)	Astaxanthin (g/L)	TKN (%)
0	10.47	0.00	0	0	0.146
12	8.96	1.10	50.02	0.06	n.d.
24	1.97	2.85	70.18	0.20	0.109
48	0.91	4.20	183.67	0.77	0.104
72	0.98	4.15	241.44	1.00	n.d.
96	0.82	4.15	231.78	0.96	n.d.
120	0.89	4.10	239.26	0.98	0.099
144	0.94	3.77	278.13	1.05	n.d.

Note: n.d. meaning not determine

Table C2 Biomass, astaxanthin (content and concentration), total sugar, and TKN present in *X. dendrorhous* cultivated in Pineapple juice base medium

Time	Total sugar (g/l)	Dry weight (g/l)	Astaxanthin ($\mu g/g_{yeast}$)	Astaxanthin (g/L)	TKN (%)
0	9.50	0.00	0.00	0.00	0.041
12	6.61	0.60	119.93	0.07	n.d.
24	5.13	2.00	168.25	0.34	0.031
48	0.94	4.03	219.52	0.89	0.029
72	1.35	4.25	222.92	0.95	n.d.
96	1.20	3.83	244.72	0.94	n.d.
120	1.49	3.85	222.63	0.86	0.026
144	1.38	3.77	241.47	0.91	n.d.

Note: n.d. meaning not determine

Table C3 Experimental designs required for 2_{IV}^{6-2} fractional factorial design together with responses in terms of biomass, astaxanthin content, and astaxanthin concentrations obtained at day 8 post inoculation

Run No.	A	B	C	D	E	F	Dry Weight (g/L)	Astaxanthin Content (μg/g $_{yeast}$)	Astaxanthin Concentration (mg/L)
1	-1	-1	-1	-1	-1	-1	6.62	896.53	5.94
2	1	-1	-1	-1	1	-1	7.76	713.75	5.54
3	-1	1	-1	-1	1	1	8.70	359.30	3.13
4	1	1	-1	-1	-1	1	9.51	136.12	1.29
5	-1	-1	1	-1	1	1	9.22	1,351.49	12.46
6	1	-1	1	-1	-1	1	5.43	896.82	4.87
7	-1	1	1	-1	-1	-1	6.61	209.22	1.38
8	1	1	1	-1	1	-1	8.74	158.39	1.38
9	-1	-1	-1	1	-1	1	6.83	686.29	4.69
10	1	-1	-1	1	1	1	11.34	641.78	7.28
11	-1	1	-1	1	1	-1	8.14	548.52	4.46
12	1	1	-1	1	-1	-1	9.96	300.37	2.99
13	-1	-1	1	1	1	-1	6.95	802.94	5.58
14	1	-1	1	1	-1	-1	12.35	701.17	8.66
15	-1	1	1	1	-1	1	7.21	173.29	1.25
16	1	1	1	1	1	1	11.33	78.80	0.89
17	0	0	0	0	0	0	9.59	302.55	2.90
18	0	0	0	0	0	0	9.49	315.27	2.99
19	0	0	0	0	0	0	9.09	343.85	3.13

Table C4 Experimental designs and corresponding responses in terms of dry cell weight obtained at day 8 post inoculation

Run No.	KNO_3	Sucrose	Hexadecane	Dry weight (g/L)	Astaxanthin ($\mu g/g_{yeast}$)	Astaxanthin (mg/L)
1	0	-1	0	12.42	643.30	7.99
2	1	0	0	13.28	718.93	9.55
3	0	1	0	13.14	654.47	8.60
4	-1	0	0	12.52	649.87	8.14
5	-0.5	-0.5	0.707	12.19	675.09	8.23
6	0.5	-0.5	0.707	12.47	721.81	9.00
7	0.5	0.5	0.707	12.74	778.29	9.92
8	-0.5	0.5	0.707	12.78	753.32	9.63
9	-0.5	-0.5	-0.707	12.56	744.58	9.36
10	0.5	-0.5	-0.707	12.20	800.32	9.76
11	0.5	0.5	-0.707	12.61	724.10	9.13
12	-0.5	0.5	-0.707	12.80	733.02	9.39
13	0	0	0	13.12	769.59	10.10
14	0	0	0	13.12	777.82	10.21
15	0	0	0	13.11	733.97	9.63

Table C5 Experimental designs required for 2_{IV}^{4-1} fractional factorial design together with responses in terms of cell and astaxanthin concentrations obtained at day 10 post inoculation

Run No.	A	B	C	D	Dry weight (g/L)	Astaxanthin (μg/g $_{yeast}$)	Astaxanthin (mg/L)
1	-1	-1	-1	-1	12.53	886.41	11.11
2	1	-1	-1	1	11.66	1068.94	12.46
3	-1	1	-1	1	11.74	946.71	11.11
4	1	1	-1	-1	10.54	978.99	10.32
5	-1	-1	i	1	11.63	778.17	9.05
6	1	-1	1	-1	9.76	772.47	7.54
7	-1	1	1	-1	10.99	606.72	6.67
8	1	1	1	1	10.50	725.69	7.62
9	0	0	0	0	11.86	823.35	9.76
10	0	0	0	0	11.88	815.29	9.68
11	0	0	0	0	11.32	792.43	8.97

Table C6 Experimental designs required for runs required for steepest ascent together with responses in terms of astaxanthin concentrations obtained at day 9 post inoculation

Steps	Path	Pyruvate	Tween20	Dry weight (g/L)	Astaxanthin (μg/g$_{yeast}$)	Astaxanthin (mg/L)
	X_0	25	0.55	-	-	-
	ΔX	5	-0.076	-	-	-
1	X_0	25	0.55	11.45	651.72	7.46
2	$X_0+\Delta X$	30	0.47	12.03	730.00	10.00
3	$X_0+2\Delta X$	35	0.40	11.53	805.57	9.29
4	$X_0+3\Delta X$	40	0.32	12.46	904.84	11.27
5	$X_0+4\Delta X$	45	0.25	11.71	1023.71	11.98
6	$X_0+5\Delta X$	50	0.17	12.57	1095.00	13.02
7	$X_0+6\Delta X$	55	0.09	11.87	1116.48	13.25
8	$X_0+7\Delta X$	60	0.02	11.47	1107.38	12.70

Table C7 Experimental designs and corresponding responses in terms of astaxanthin concentration obtained at day 9 post inoculation

Run No.	Pyruvate (X)	Tween20 (Y)	Dry weight (g/L)	Astaxanthin ($\mu g/g_{yeast}$)	Astaxanthin (mg/L)
1	0.5	0.866	11.01	908.43	10.00
2	0.5	-0.866	11.55	962.00	11.11
3	-0.5	0.866	11.59	869.90	10.08
4	-0.5	-0.866	11.05	1048.82	11.59
5	1	0	11.60	971.81	11.27
6	-1	0	11.67	925.17	10.79
7	0	0	11.54	1176.35	13.57
8	0	0	11.47	1155.83	13.25
9	0	0	11.66	1184.69	13.81

Table C8 Biomass concentration and astaxanthin production by *X. dendrorhous* in 2-liter fermentor

Time	Total Sugar (g/L)	Sucrose (g/L)	Glucose (g/L)	Fructose (g/L)	1-kestose (g/L)	TKN (%)	Dry Weight (g/L)	Astaxanthin Content (μg/g $_{yeast}$)	Astaxanthin Concentration (mg/L)
0	103.62	41.08	13.16	9.69	0	0.0483	0	0	0
3	78.51	39.30	13.97	10.15	4.56	N.d.	0.12	4.69	0.00
6	73.72	33.31	15.07	10.27	7.77	N.d.	0.17	5.60	0.00
9	57.16	19.08	17.78	10.73	14.75	N.d.	0.35	5.76	0.00
12	51.24	9.87	19.36	11.51	18.11	0.0378	0.76	22.90	0.02
16	41.77	7.01	19.01	12.02	17.54	0.0290	1.74	36.85	0.06
20	29.63	6.16	18.07	12.36	15.71	0.0354	2.71	102.62	0.28
24	19.52	5.46	16.81	12.44	13.34	0.0290	4.04	107.75	0.44
30	15.37	4.77	15.64	12.91	10.22	N.d.	5.92	177.75	1.05
36	12.57	4.11	14.86	13.08	7.35	0.0258	7.51	250.93	1.89
48	12.22	3.22	13.40	12.53	2.89	0.0258	9.46	419.43	3.97
72	12.82	3.02	8.31	7.23	0.08	N.d.	11.47	449.64	5.16
96	12.84	3.45	1.71	3.54	0.02	0.0403	12.61	522.19	6.59
120	12.73	2.16	0.44	0.88	0.01	0.0419	14.01	617.39	8.65
144	103.62	1.04	0.13	0.05	0.01	N.d.	16.65	679.35	10.79
168	78.51	0.97	0.14	0.05	0.01	0.0451	16.79	700.03	11.90
192	73.72	0.97	0.29	0.08	0	N.d.	17.43	692.30	12.06
216	57.16	0.97	0.33	0.08	0	N.d.	16.15	776.56	12.54
240	51.24	0.97	0.13	0.08	0	0.0419	14.97	906.57	13.57

Note: N.d. meaning not determine

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