Scenedesmus rubescens heterotrophic production strategies for added value biomass

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Supplementary information

Table S1 - Screening method by Plackett-Burman design in actual level of variables through Minitab® software for *Scenedesmus rubescens*

		Concentration (mM)											
Trial	**Nitrogen source**	**N**	**Mg**	**Ca**	**P**	**Fe**	**Cu**	**Zn**	**Mn**	**Mo**	**Co**	**Ni**	**B**
1	Ammonium	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
2	Ammonium	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
3	Nitrate	60	3	0.3	1	0.1	0.06	0.003	0.08	0.020	0.005	0	0.1
4	Nitrate	20	3	0.3	10	0.1	0.06	0.060	0.03	0.001	0.020	0.02	0.1
5	Ammonium	20	0.5	0.3	10	0.02	0.06	0.003	0.08	0.020	0.020	0.02	0.1
6	Nitrate	60	0.5	0.3	10	0.1	0.01	0.060	0.08	0.001	0.005	0	0.1
7	Nitrate	60	0.5	1.7	10	0.02	0.01	0.003	0.03	0.020	0.005	0.02	0.1
8	Ammonium	60	0.5	1.7	10	0.1	0.06	0.003	0.03	0.020	0.020	0	0.5
9	Ammonium	60	3	0.3	10	0.1	0.01	0.003	0.03	0.001	0.020	0	0.5
10	Ammonium	20	3	0.3	10	0.02	0.06	0.060	0.08	0.020	0.005	0	0.5
11	Ammonium	20	0.5	0.3	1	0.02	0.01	0.003	0.03	0.001	0.005	0	0.1
12	Nitrate	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
13	Ammonium	60	3	1.7	10	0.02	0.01	0.060	0.08	0.001	0.020	0.02	0.1
14	Nitrate	20	3	1.7	10	0.1	0.01	0.003	0.08	0.020	0.005	0.02	0.5
15	Nitrate	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
16	Nitrate	20	0.5	1.7	10	0.02	0.06	0.060	0.03	0.001	0.005	0	0.5
17	Nitrate	60	3	1.7	1	0.02	0.06	0.060	0.03	0.020	0.020	0	0.1
18	Nitrate	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
19	Ammonium	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
20	Nitrate	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
21	Ammonium	60	0.5	1.7	1	0.1	0.06	0.060	0.08	0.001	0.005	0.02	0.5
22	Ammonium	20	3	1.7	1	0.1	0.06	0.003	0.03	0.001	0.005	0.02	0.1
23	Ammonium	20	0.5	1.7	1	0.1	0.01	0.060	0.08	0.020	0.020	0	0.1
24	Ammonium	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
25	Ammonium	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
26	Ammonium	40	1.75	1	5.5	0.06	0.03	0.032	0.06	0.011	0.013	0.01	0.3
27	Nitrate	60	0.5	0.3	1	0.02	0.06	0.003	0.08	0.001	0.020	0.02	0.5
28	Ammonium	60	3	0.3	1	0.02	0.01	0.060	0.03	0.020	0.005	0.02	0.5
29	Nitrate	20	3	1.7	1	0.02	0.01	0.003	0.08	0.001	0.020	0	0.5
30	Nitrate	20	0.5	0.3	1	0.1	0.01	0.060	0.03	0.020	0.020	0.02	0.5

Table S2 - Responses functions for screening method composition for heterotrophic cultivation of *Scenedesmus rubescens*. Minitab® software was used

Trials	Biomass concentration (g/L)	Global productivity (g/L/day)	Maximum productivity (g/L/day)
1	9.62	0.089	0.277
2	6.50	0.060	0.142
3	4.41	0.041	0.079
4	12.09	0.112	1.243
5	13.82	0.128	0.344
6	6.90	0.064	0.176
7	10.77	0.100	1.114
8	9.79	0.091	0.166
9	7.31	0.061	0.131
10	7.55	0.070	0.150
11	6.43	0.060	0.142
12	9.62	0.089	0.277
13	11.51	0.091	0.560
14	14.73	0.136	1.235
15	9.21	0.085	0.322
16	12.34	0.114	1.206
17	6.02	0.056	0.109
18	9.21	0.085	0.322
19	6.50	0.060	0.133
20	9.21	0.085	0.322
21	4.33	0.040	0.081
22	5.04	0.047	0.093
23	5.35	0.050	0.120
24	6.91	0.064	0.152
25	6.50	0.060	0.133
26	6.91	0.064	0.152
27	4.91	0.045	0.096
28	3.81	0.035	0.059
29	6.08	0.056	0.212
30	7.53	0.0697	0.150

Table S3 – Optimized culture medium developed in this work for *Scenedesmus rubescens* (macro and micronutrients)

0037 SA medium	
Component	Concentration (mM)
$(NH_4)_2SO_4$	60
NaH_2PO_4	26
K_2HPO_4	24
$MgSO_4.7H_2O$	1.75
Citric acid	6.25
$FeSO_4.7H_2O$	0.06
$CaCl_2.2h_2O$	0.3
H_3BO_3	0.1
$ZnSO_4$	0.003
$MnCl_2.4H_2O$	0.03
$Na_2Mo_4.2H_2O$	0.03
$CuSO_4.2H_2O$	0.00325
$NiCl_2.6H_2O$	0.02
$Co(NO_3)_2.6H_2O$	0.01

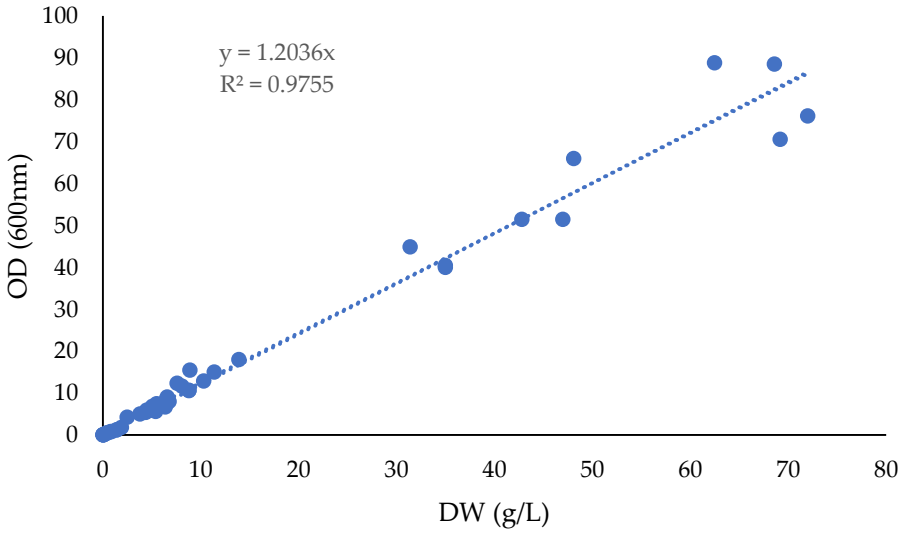

Figure S1 – Calibration Curve. Optical density of *S. rubescens* suspensions (in water) measured at λ= 600 nm versus dry biomass concentration (g/L), for heterotrophic growth.