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Investigations on microalgal oil production from *Arthrospira platensis*: towards a more sustainable biodiesel production

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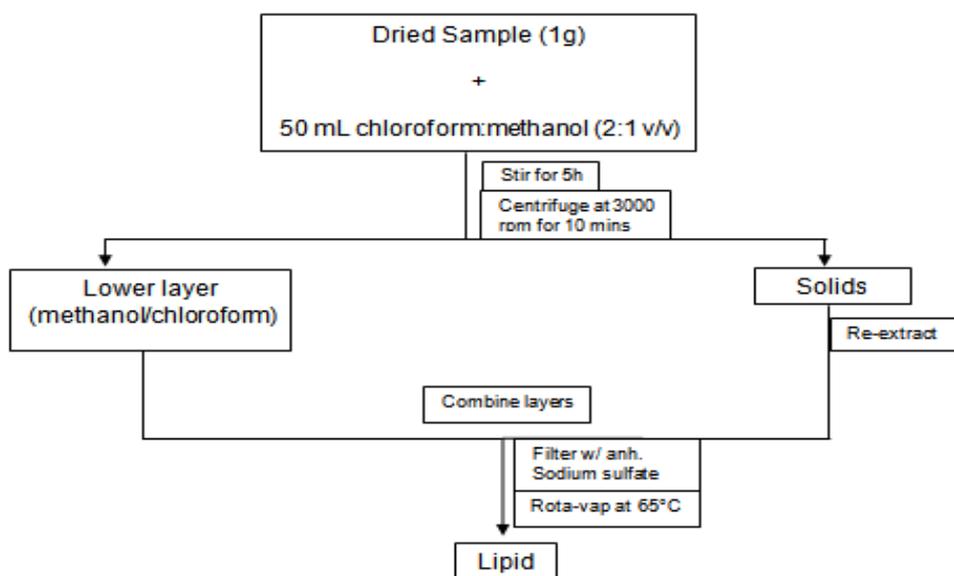


Figure 1S. Schematic diagram of extraction of lipid of dried *Spirulina*

Table 1S. Average optical density for different culture media.

	Absorbance, nm ($\lambda_{\text{max}}=680$ nm)					
Time (Day)	CONTROL	NP (-)	N(-)	P(-)	NPL	N(-)PL
1	0.242	0.242	0.246	0.252	0.236	0.241
2	0.254	0.237	0.254	0.242	0.265	0.243
3	0.285	0.247	0.274	0.249	0.259	0.264
6	0.367	0.322	0.374	0.331	0.365	0.354
7	0.387	0.343	0.385	0.372	0.390	0.370
8	0.429	0.418	0.377	0.388	0.387	0.414
10	0.521	0.437	0.476	0.444	0.455	0.460
11	0.531	0.449	0.446	0.467	0.463	0.471
13	0.549	0.488	0.538	0.490	0.506	0.491
14	0.613	0.583	0.681	0.555	0.569	0.543
16	0.619	0.570	0.674	0.585	0.555	0.582
17	0.680	0.671	0.701	0.614	0.635	0.608
21	0.804	0.682	0.806	0.662	0.710	0.682
23	0.832	0.729	0.767	0.778	0.702	0.704
29	1.097	0.920	0.972	0.783	0.811	0.816
36	1.457	1.348	1.133	1.206	1.000	1.115

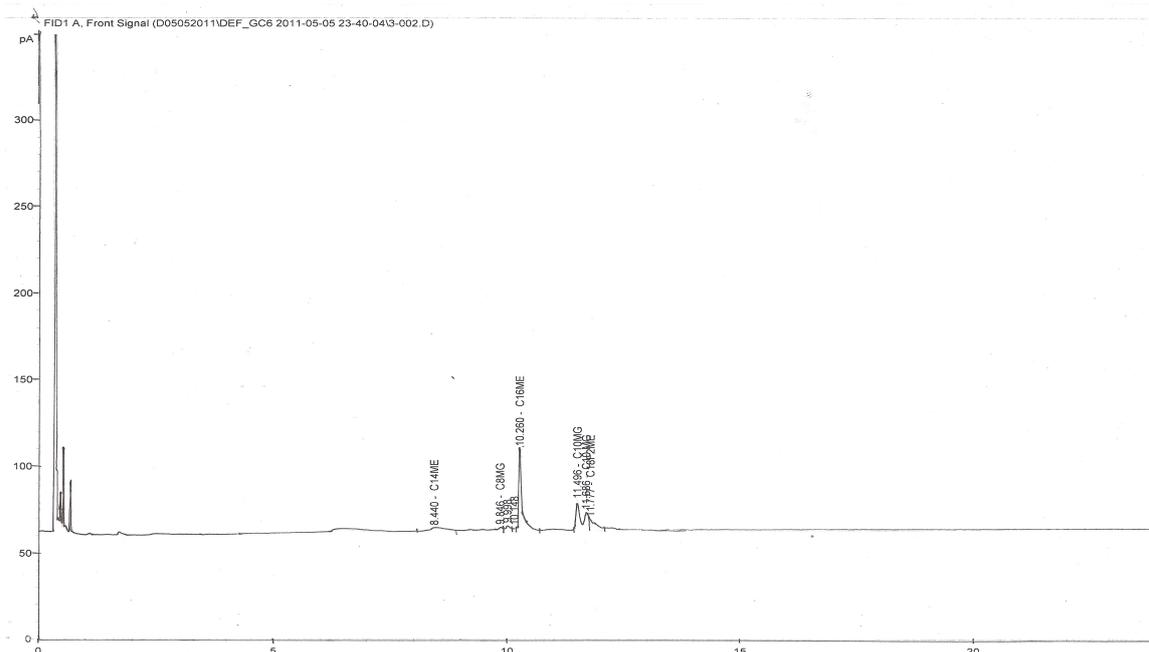
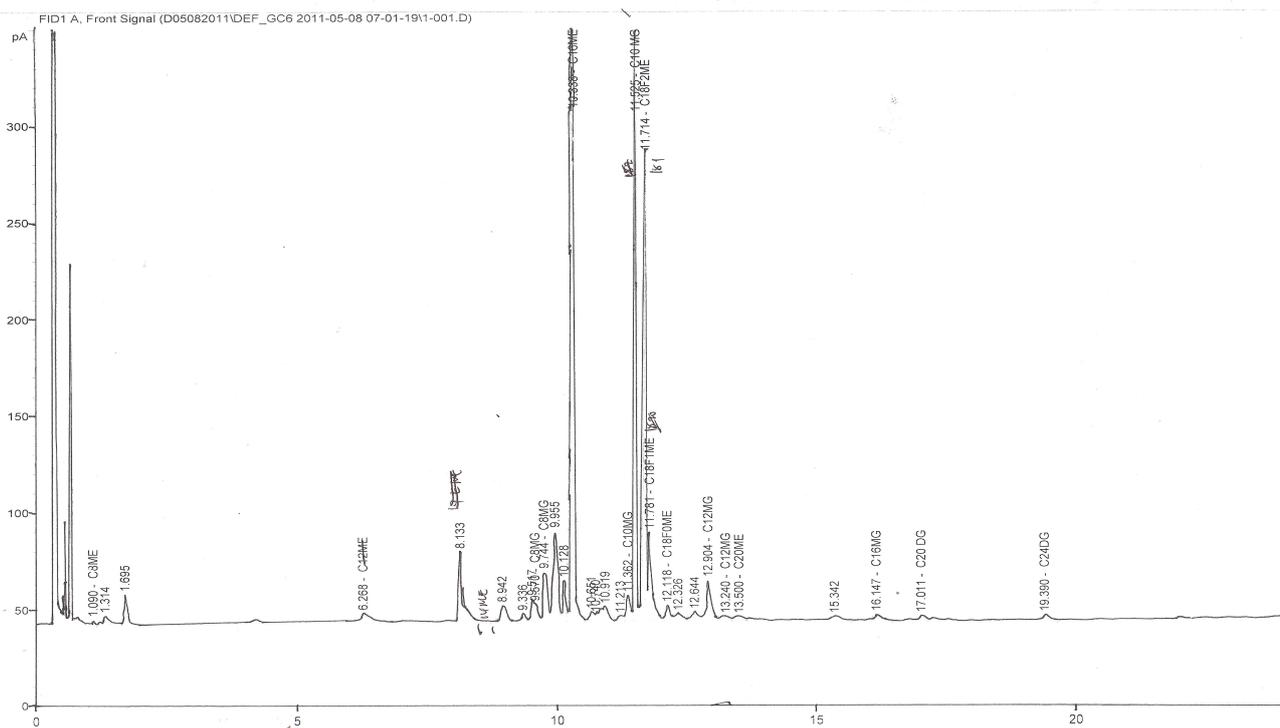


Figure 2S TOP) Chromatogram of CONTROL; BOTTOM) Chromatogram of NP(-).

Table 2S. A summary for the average algal biomass, lipid and lipid content for different culture media.

Culture medium	Biomass (g/L)	Lipid (g)	Lipid content (% w/w)
CONTROL	0.81	6.4×10^{-3}	3.8
NP(-)	0.68	8.7×10^{-3}	5.5
N(-)	0.54	1.99×10^{-2}	8.4
P (-)	0.51	3.93×10^{-2}	17.7
NPL	0.51	2.0×10^{-2}	9.1
N(-)PL	0.48	4.3×10^{-2}	20.3



Figure 3S. Spirulina grown at six-different culture media.



Figure 4S. Extraction of lipids with 2:1 v/v chloroform:methanol.

Table 3S. One-factor ANOVA: Optical density at different culture media

CONTROL		NP (-)		N(-)		P(-)		NPL		N(-)PL	
X ₁	X ₁ ²	X ₂	X ₂ ²	X ₃	X ₃ ²	X ₄	X ₄ ²	X ₅	X ₅ ²	X ₆	X ₆ ²
0.242	0.058564	0.242	0.058564	0.246	0.060516	0.252	0.063504	0.236	0.055696	0.241	0.058081
0.254	0.064516	0.237	0.056169	0.254	0.064516	0.242	0.058564	0.265	0.070225	0.243	0.059049
0.285	0.081225	0.247	0.061009	0.274	0.075076	0.249	0.062001	0.259	0.067081	0.264	0.069696
0.367	0.134689	0.322	0.103684	0.374	0.139876	0.331	0.109561	0.365	0.133225	0.354	0.125316
0.387	0.149769	0.343	0.117649	0.385	0.148225	0.372	0.138384	0.390	0.1521	0.370	0.1369
0.429	0.184041	0.418	0.174724	0.377	0.142129	0.388	0.150544	0.387	0.149769	0.414	0.171396
0.521	0.271441	0.437	0.190969	0.476	0.226576	0.444	0.197136	0.455	0.207025	0.460	0.2116
0.531	0.281961	0.449	0.201601	0.446	0.198916	0.467	0.218089	0.463	0.214369	0.471	0.221841
0.549	0.301401	0.488	0.238144	0.538	0.289444	0.490	0.2401	0.506	0.256036	0.491	0.241081
0.613	0.375769	0.583	0.339889	0.681	0.463761	0.555	0.308025	0.569	0.323761	0.543	0.294849
0.619	0.383161	0.570	0.3249	0.674	0.454276	0.585	0.342225	0.555	0.308025	0.582	0.338724
0.680	0.462400	0.671	0.450241	0.701	0.491401	0.614	0.376996	0.635	0.403225	0.608	0.369664
0.804	0.646416	0.682	0.465124	0.806	0.649636	0.662	0.438244	0.710	0.5041	0.682	0.465124
0.832	0.692224	0.729	0.531441	0.767	0.588289	0.778	0.605284	0.702	0.492804	0.704	0.495616
1.097	1.203409	0.920	0.8464	0.972	0.944784	0.783	0.613089	0.811	0.657721	0.816	0.665856
1.457	2.122849	1.348	1.817104	1.133	1.283689	1.206	1.454436	1.000	1	1.115	1.243225

9.667	7.413835	8.686	5.977612	9.104	6.221	8.418	5.376	8.308	4.995	8.358	5.168	SUMS
0.604	0.463365	0.543	0.373601	0.569	0.389	0.526	0.336	0.519	0.312	0.522	0.323	MEANS
16		16		16		16		16		16		N

ΣX _t	52.541	df (Between groups)	5
ΣX _t ²	35.151919	df (Within groups)	90
N _t	96	MS _b	0.017866
SS _t	6.39612	MS _w	0.070075
SS _b	0.08933	F	0.254952
SS _w	6.306791	Critical Value at 0.01 level	3.23
		Critical Value at 0.05 level	2.32

p > 0.05

Table 4S. Optical density monitoring for the six different culture media

	Optical Density $\lambda_{\text{max}} = 680 \text{ nm}$					
Time (Day)	CONTROL	NP (-)	N(-)	P(-)	NPL	N(-)PL
1	0.242	0.241	0.246	0.252	0.236	0.241
	0.242	0.241	0.246	0.253	0.236	0.241
	0.241	0.244	0.246	0.252	0.236	0.241
Average	0.242	0.242	0.246	0.252	0.236	0.241
2	0.255	0.237	0.254	0.240	0.266	0.243
	0.253	0.236	0.254	0.242	0.265	0.244
	0.253	0.236	0.253	0.243	0.263	0.244
Average	0.254	0.237	0.254	0.242	0.265	0.243
3	0.287	0.247	0.274	0.248	0.257	0.267
	0.284	0.247	0.275	0.249	0.258	0.261
	0.283	0.247	0.272	0.250	0.261	0.264
Average	0.285	0.247	0.274	0.249	0.259	0.264
6	0.369	0.322	0.369	0.332	0.367	0.358
	0.366	0.322	0.375	0.331	0.365	0.354
	0.366	0.324	0.380	0.330	0.362	0.351
Average	0.367	0.322	0.374	0.331	0.365	0.354
7	0.384	0.342	0.384	0.376	0.389	0.368
	0.387	0.343	0.386	0.375	0.391	0.370
	0.390	0.345	0.387	0.366	0.391	0.371
Average	0.387	0.343	0.385	0.372	0.390	0.370
8	0.427	0.412	0.376	0.388	0.393	0.417
	0.430	0.416	0.377	0.389	0.388	0.413
	0.431	0.428	0.377	0.387	0.381	0.410
Average	0.429	0.418	0.377	0.388	0.387	0.414
10	0.536	0.436	0.475	0.439	0.459	0.457
	0.520	0.438	0.474	0.446	0.444	0.459
	0.509	0.438	0.479	0.448	0.431	0.465
Average	0.521	0.437	0.476	0.444	0.445	0.460
11	0.534	0.448	0.445	0.462	0.465	0.470
	0.530	0.451	0.445	0.468	0.463	0.469
	0.530	0.449	0.447	0.470	0.462	0.473
Average	0.531	0.449	0.446	0.467	0.463	0.471
13	0.551	0.487	0.539	0.491	0.506	0.485
	0.545	0.489	0.538	0.488	0.506	0.491
	0.550	0.487	0.536	0.490	0.506	0.495
Average	0.549	0.488	0.538	0.490	0.506	0.491

	Optical Density λ_{\max} = 680 nm					
Time (Day)	CONTROL	NP (-)	N(-)	P(-)	NPL	N(-)PL
14	0.615	0.579	0.666	0.560	0.563	0.544
	0.613	0.584	0.682	0.555	0.570	0.541
	0.611	0.586	0.695	0.550	0.574	0.545
Average	0.613	0.583	0.681	0.555	0.569	0.543
16	0.620	0.569	0.673	0.588	0.553	0.575
	0.618	0.569	0.673	0.585	0.555	0.583
	0.618	0.571	0.676	0.582	0.556	0.589
Average	0.619	0.570	0.674	0.585	0.555	0.582
17	0.675	0.666	0.705	0.617	0.632	0.612
	0.681	0.672	0.700	0.616	0.634	0.609
	0.683	0.677	0.699	0.610	0.638	0.603
Average	0.680	0.671	0.701	0.614	0.635	0.608
21	0.806	0.680	0.806	0.661	0.717	0.678
	0.805	0.684	0.807	0.663	0.707	0.685
	0.801	0.683	0.805	0.663	0.705	0.684
Average	0.804	0.682	0.806	0.662	0.710	0.682
23	0.833	0.730	0.730	0.789	0.701	0.710
	0.828	0.730	0.728	0.777	0.701	0.705
	0.836	0.728	0.728	0.768	0.704	0.697
Average	0.832	0.729	0.729	0.778	0.702	0.704
29	1.094	0.914	0.980	0.785	0.806	0.815
	1.099	0.920	0.970	0.784	0.813	0.815
	1.099	0.924	0.966	0.782	0.816	0.819
Average	1.097	0.920	0.972	0.783	0.811	0.816
36	1.459	1.346	1.134	1.204	0.996	1.118
	1.456	1.351	1.134	1.207	1.001	1.115
	1.454	1.346	1.133	1.208	1.003	1.111
Average	1.457	1.348	1.133	1.206	1.000	1.115

Table 5S. *Spirulina* biomass at different culture media

Culture medium	Culture volume (L)	Wt. evaporating dish (g)	Trial	Wt. dried algae + evap.dish (g)	Wt. of <i>Spirulina</i> (g)	Biomass (g/L)
CONTROL	0.206	47.9081	1	48.0746	0.1665	0.8082
			2	48.0754	0.1673	0.8121
			3	48.0763	0.1682	0.8165
			Average	48.0754	0.1673	0.8121
NP(-)	0.235	42.7587	1	42.9166	0.1579	0.6719
			2	42.9169	0.1582	0.6732
			3	42.9176	0.1589	0.6762
			Average	42.9170	0.1583	0.6736
N(-)	0.443	44.0314	1	44.2676	0.2362	0.5332
			2	44.2691	0.2377	0.5366
			3	44.2691	0.2377	0.5366
			Average	44.2686	0.2372	0.5354
P(-)	0.434	52.5684	1	52.7897	0.2213	0.5099
			2	52.7910	0.2226	0.5129
			3	52.7908	0.2224	0.5124
			Average	52.7905	0.2221	0.5118
NPL	0.432	58.5563	1	58.7769	0.2206	0.5106
			2	58.7773	0.2210	0.5116
			3	58.7778	0.2215	0.4896
			Average	58.7773	0.2210	0.5116
N(-)PL	0.442	54.5939	1	54.8049	0.2110	0.4774
			2	54.8053	0.2114	0.4780
			3	54.8057	0.2118	0.4792
			Average	54.8053	0.2114	0.4780

Table 6S. Lipid content of *Spirulina* grown under different culture media

Culture medium	Trial	Wt. of beaker (g)	Wt. of beaker+lipid (g)	Wt. of lipid (g)	Ave. Lipid content (%)
CONTROL	1	26.9589	26.9645	5.60×10^{-3}	3.825
	2	26.9582	26.9647	6.50×10^{-3}	
	3	26.9579	26.9648	6.90×10^{-3}	
	Average	26.9583	26.9647	6.40×10^{-3}	
NP(-)	1	29.4059	29.4150	9.10×10^{-3}	5.496
	2	29.4061	29.4149	8.80×10^{-3}	
	3	29.4059	29.4143	8.40×10^{-3}	
	Average	29.4060	29.4147	8.70×10^{-3}	
N(-)	1	30.5796	30.5994	1.98×10^{-2}	8.390
	2	30.5795	30.5994	1.98×10^{-2}	
	3	30.5795	30.5995	2.00×10^{-2}	
	Average	30.5795	30.5994	1.99×10^{-2}	
P(-)	1	28.6758	28.7150	3.92×10^{-2}	17.70
	2	28.6756	28.7147	3.91×10^{-2}	
	3	28.6751	28.7148	3.97×10^{-2}	
	Average	28.6755	28.7148	3.93×10^{-2}	
NPL	1	27.0102	27.0304	2.02×10^{-2}	9.050
	2	27.0103	27.0301	1.98×10^{-2}	
	3	27.0104	27.0303	1.99×10^{-2}	
	Average	27.0103	27.0303	2.00×10^{-2}	
N(-)PL	1	29.4589	29.5019	4.30×10^{-2}	20.34
	2	29.4584	29.5019	4.35×10^{-2}	
	3	29.4588	29.5015	4.27×10^{-2}	
	Average	29.4587	29.5017	4.30×10^{-2}	