

SUPPLEMENATRY DOCUMENTS

Comprehensive Analysis of Biomass from *Chlorella sorokiniana* Cultivated with Industrial Flue Gas as the Carbon Source

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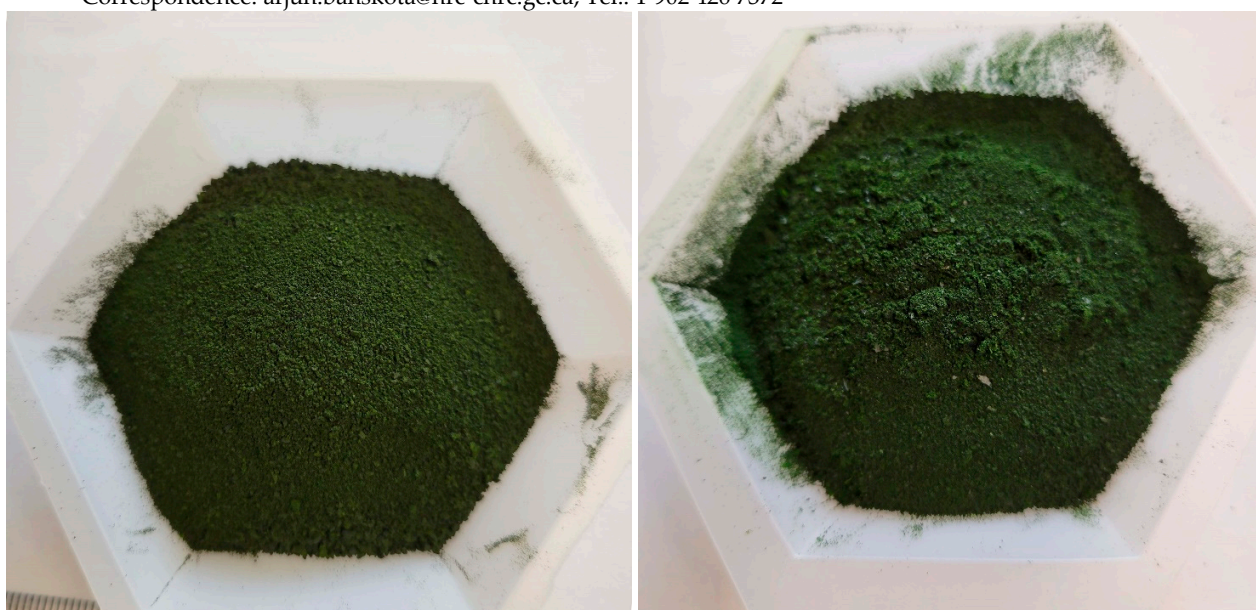
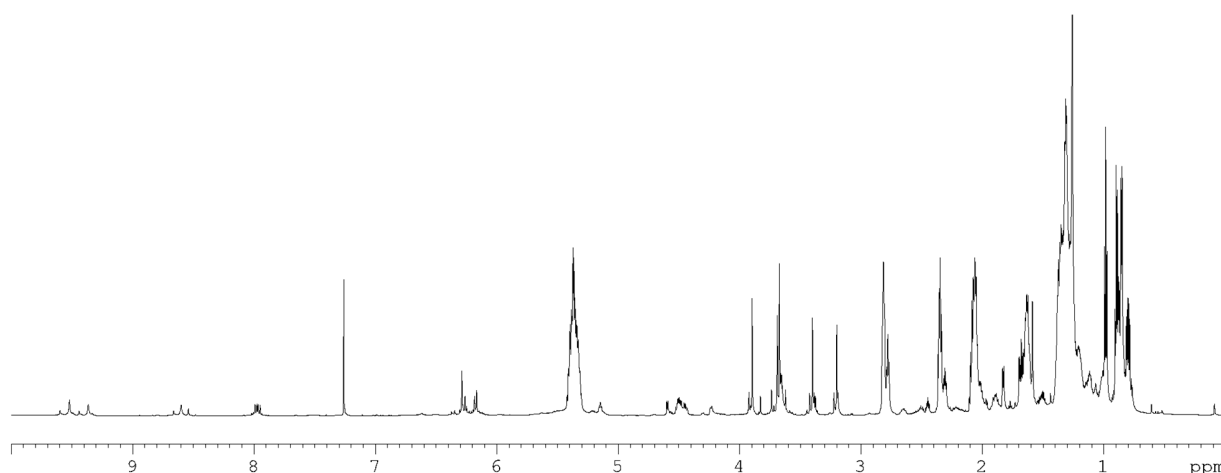


Figure S1. Freeze-dried algal biomass of *C. sorokiniana* cultured in 20 L (left) and 25000 L Photobioreactors (right).



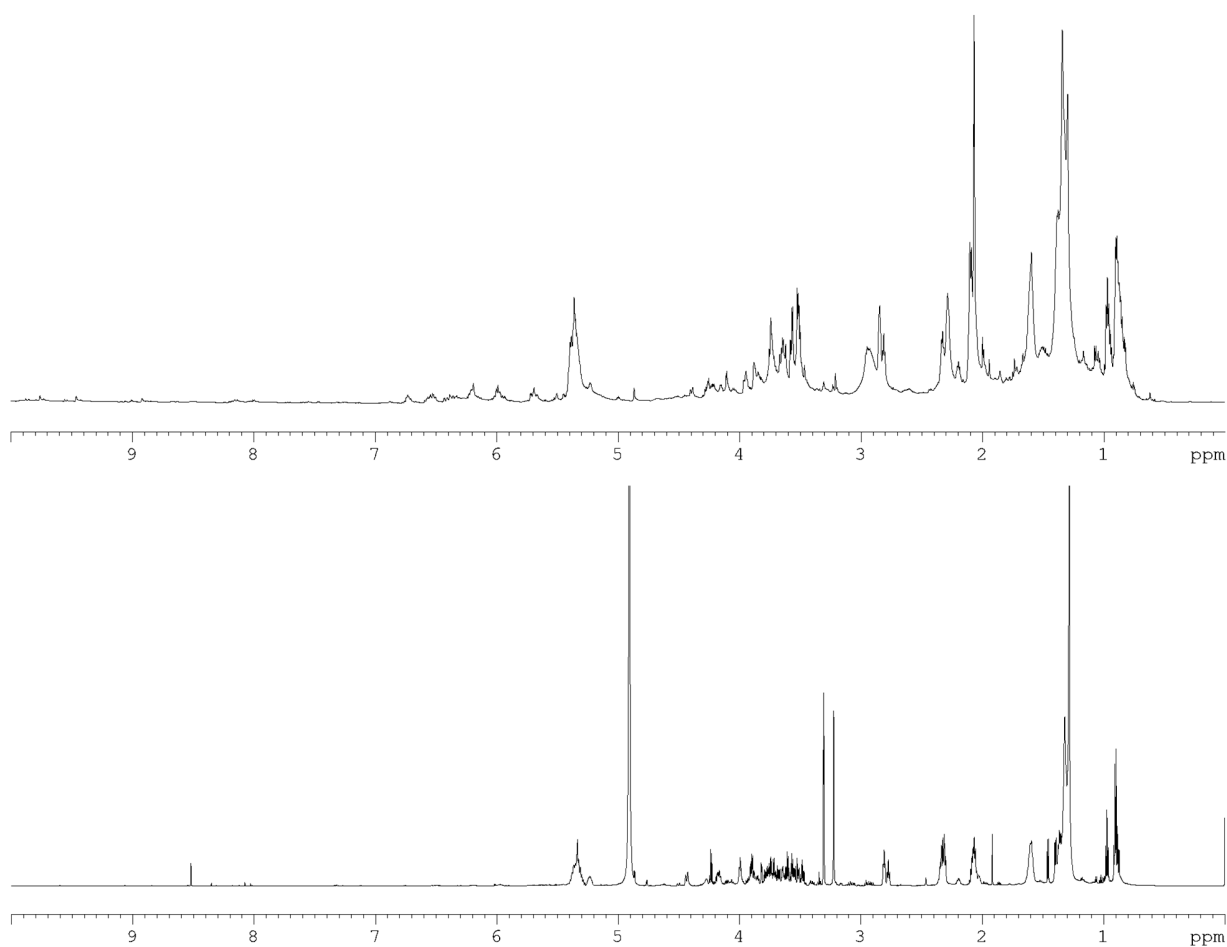


Figure S2. Proton NMR spectrum of [A] neutral lipid (top), glycolipid (middle) and phospholipid (bottom) fractions from lipid extract from *C. sorokiniana* cultured in a 25000 L Photobioreactor.

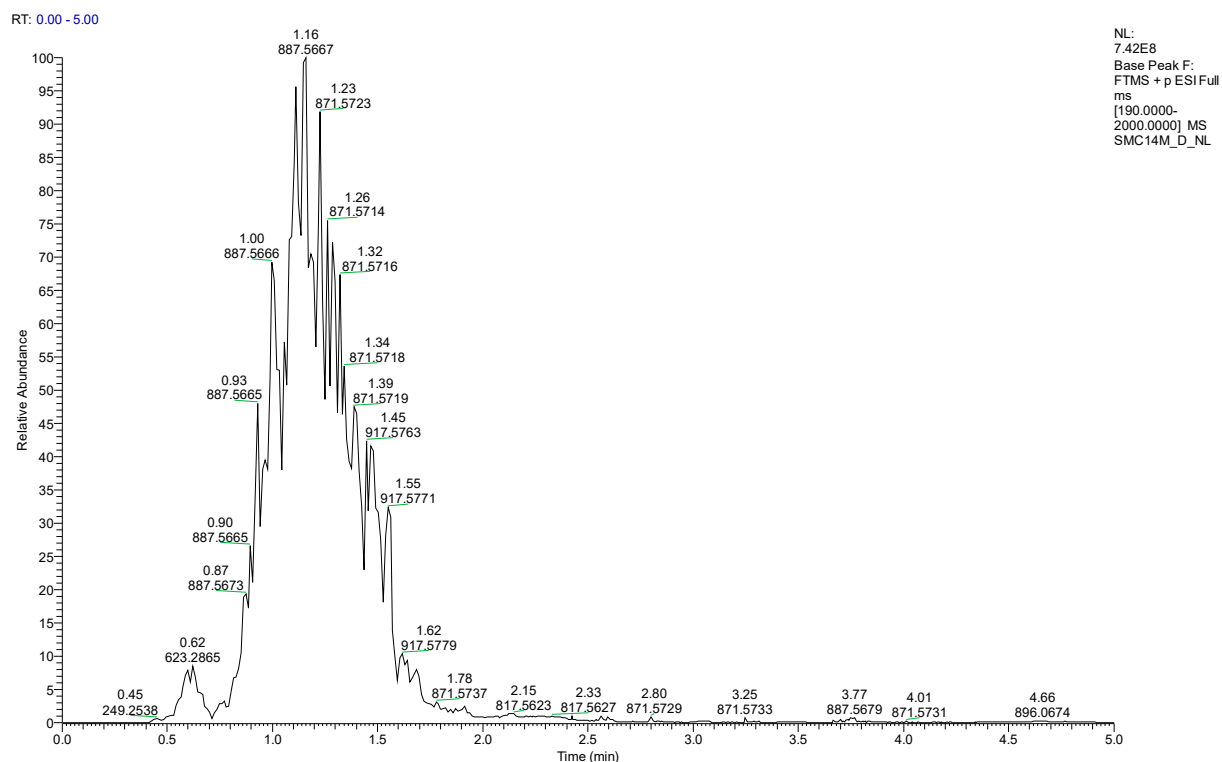
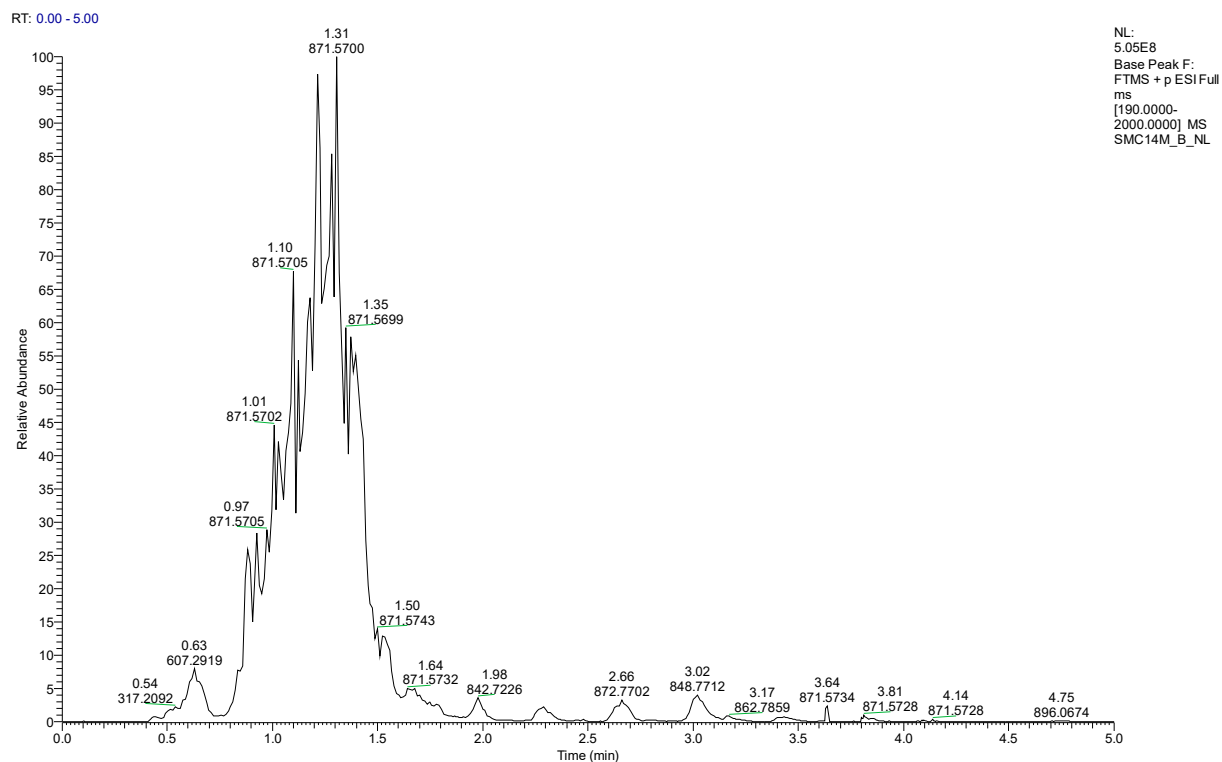
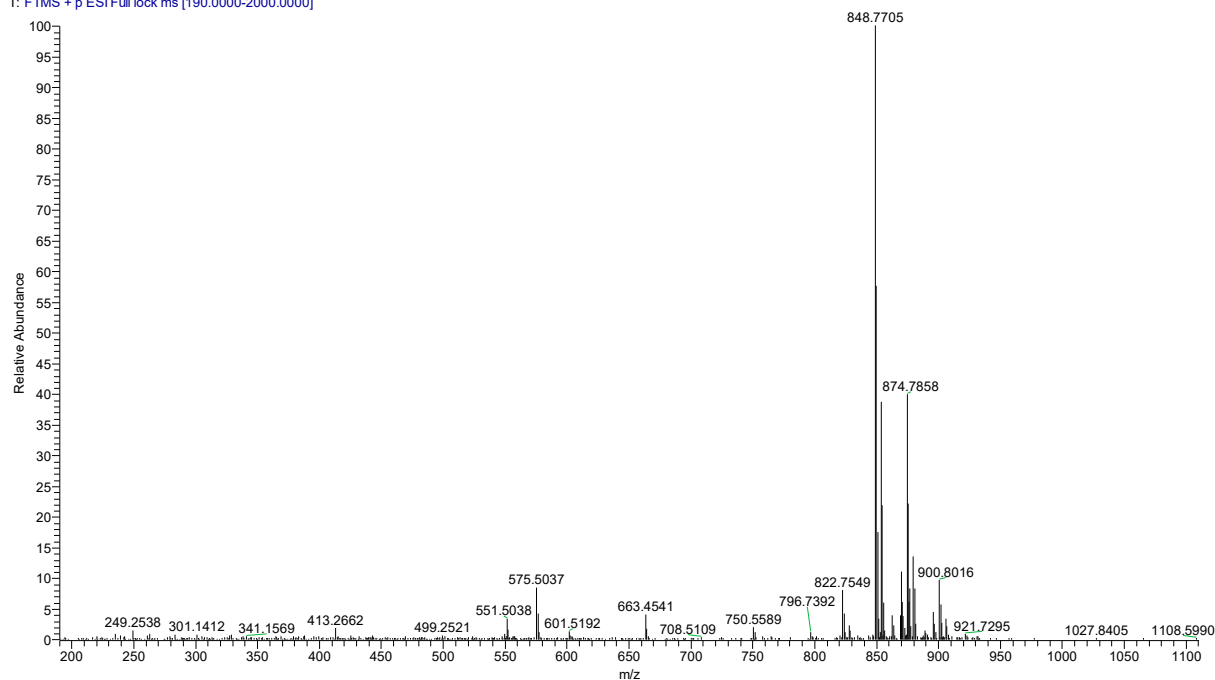


Figure S3. Total ion chromatograms (TICs) of the neutral lipid fraction of *C. sorokiniana* biomass cultured in 20 L (top) and 25000 L (bottom) Photobioreactors.

SMC14M_B_NL#941 RT: 3.03 AV: 1 NL: 1.58E7
T: FTMS + p ESI Full lock ms [190.0000-2000.0000]



SMC14M_B_NL#911-1107 RT: 2.94-3.65 AV: 8 NL: 2.64E5
T: Average spectrum MS2 848.77 (911-1107)

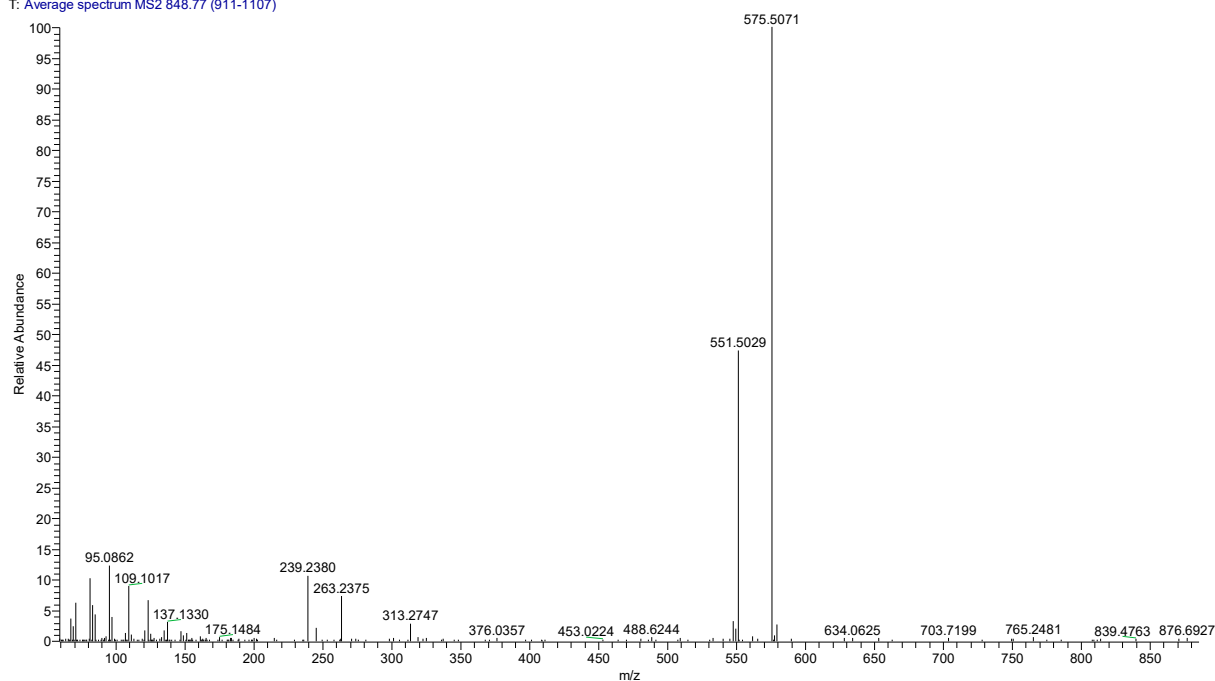
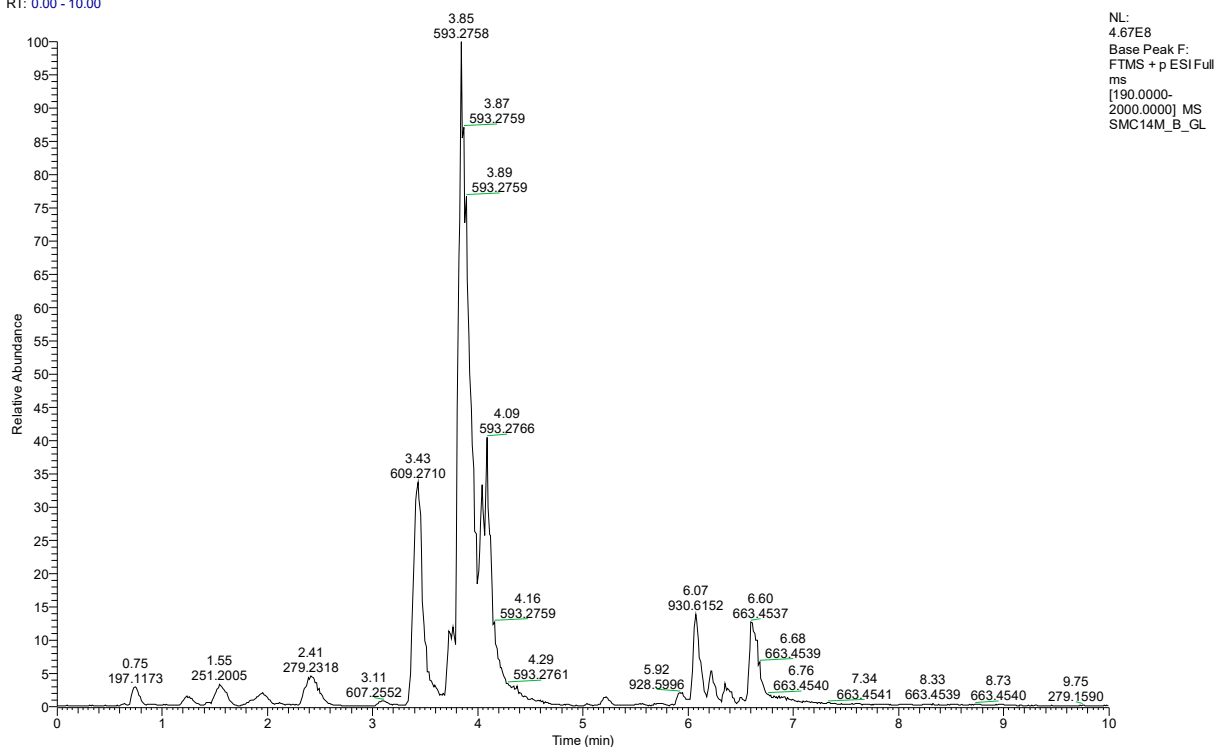


Figure S4. Mass spectrum of metabolites eluted at 3.03 min (top) and fragmentation ions corresponding to neutral loss of TAG (50:2).

RT: 0.00 - 10.00



RT: 0.00 - 10.00

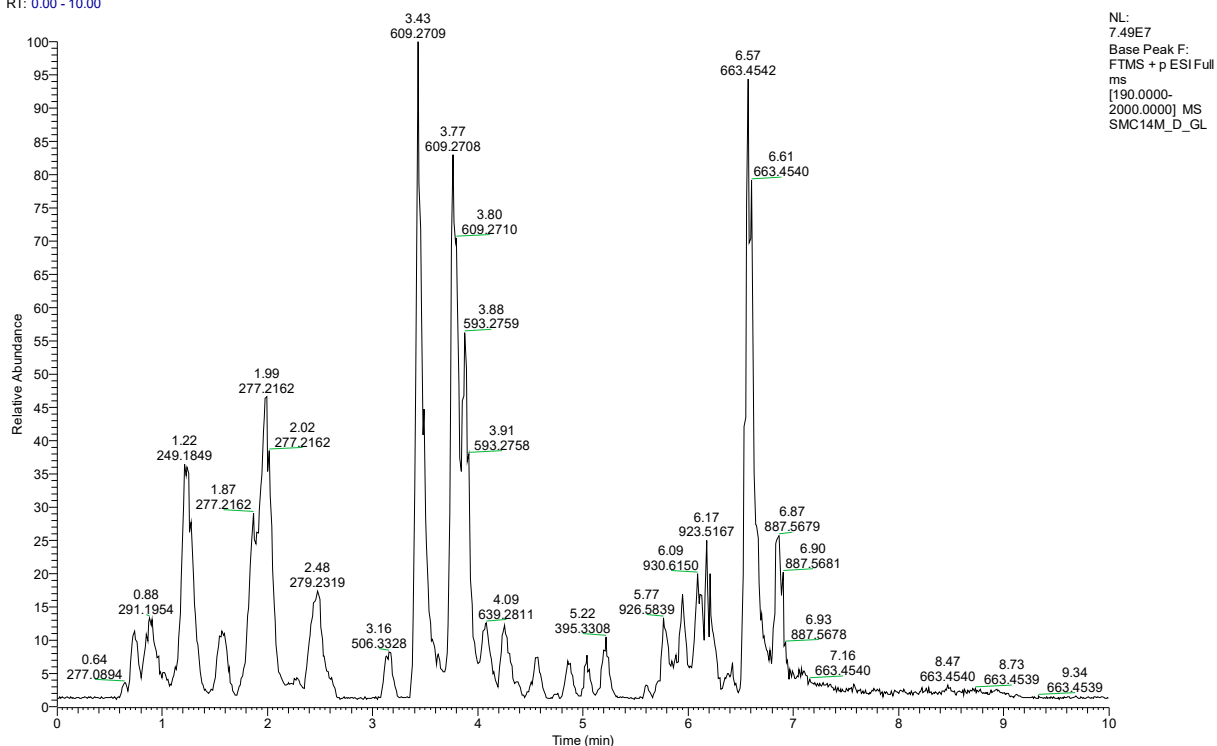
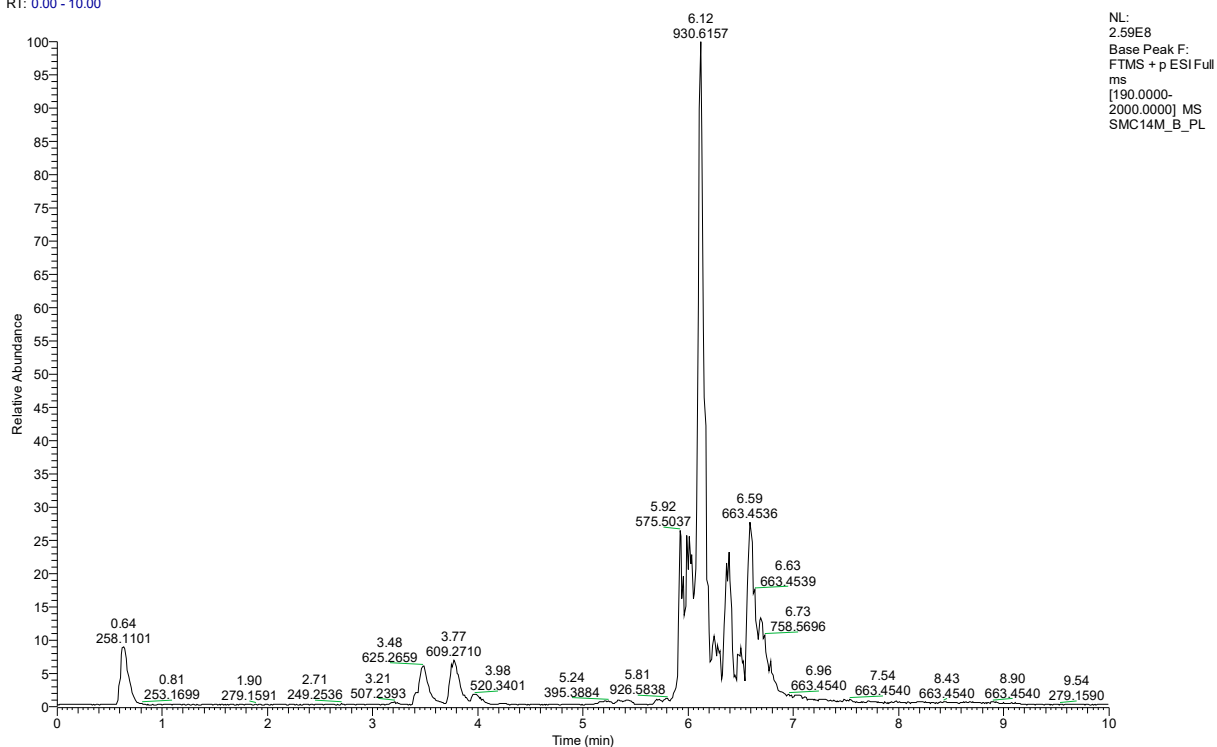


Figure S5. Total ion chromatograms (TICs) of the glycolipid fraction of *C. sorokiniana* biomass cultured in 20 L (top) and 25000 L (bottom) Photobioreactors.

RT: 0.00 - 10.00



RT: 0.00 - 10.00

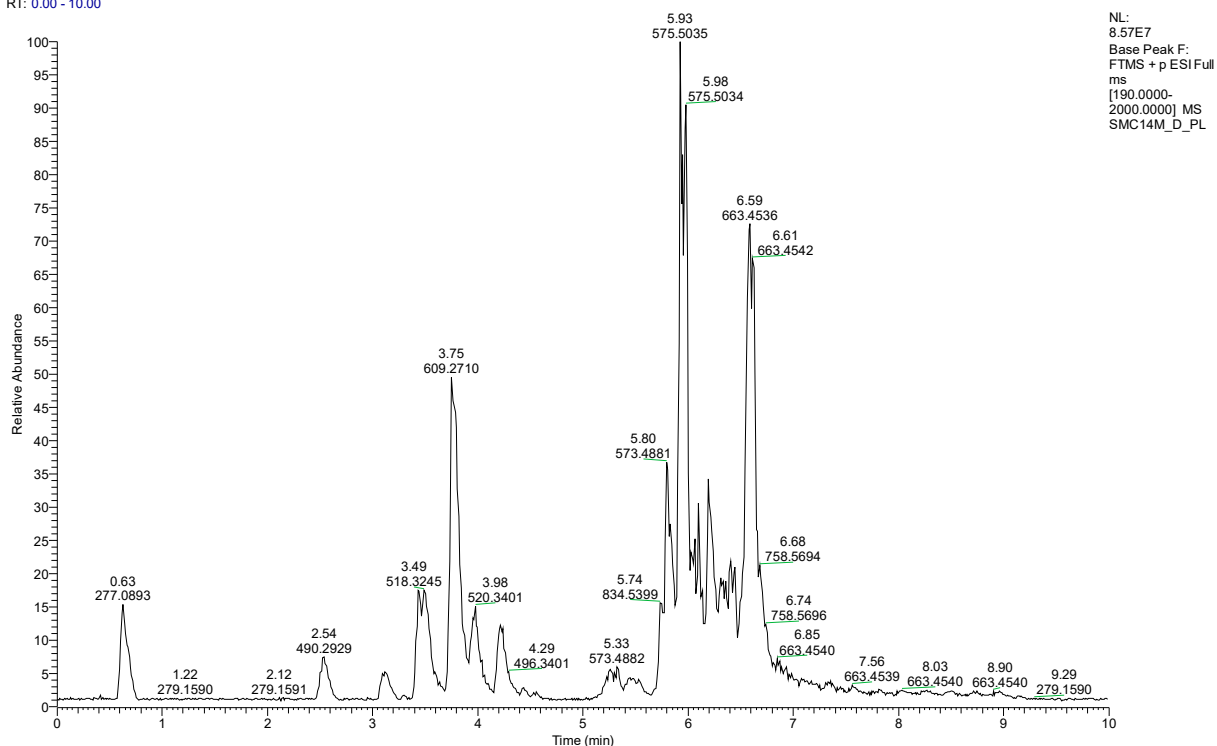
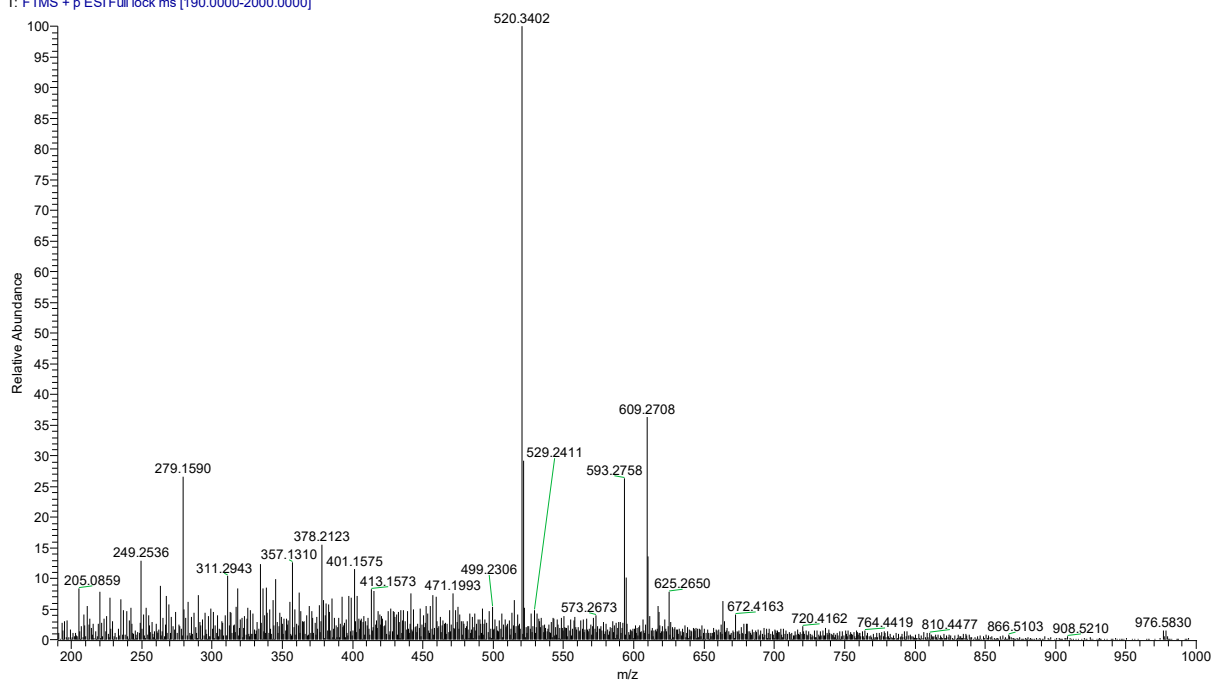


Figure S6. Total ion chromatograms (TICs) of the phospholipid fraction of *C. sorokiniana* biomass cultured in 20 L (top) and 25000 L (bottom) Photobioreactors.

SMC14M_B_PL#1363-1400 RT: 3.92-4.03 AV: 11 NL: 3.71E6
T: FTMS + p ESI Full lock ms [190.0000-2000.0000]



SMC14M_B_PL#218-1481 RT: 0.63-4.26 AV: 13 NL: 1.57E5
T: Average spectrum MS2 520.33 (218-1481)

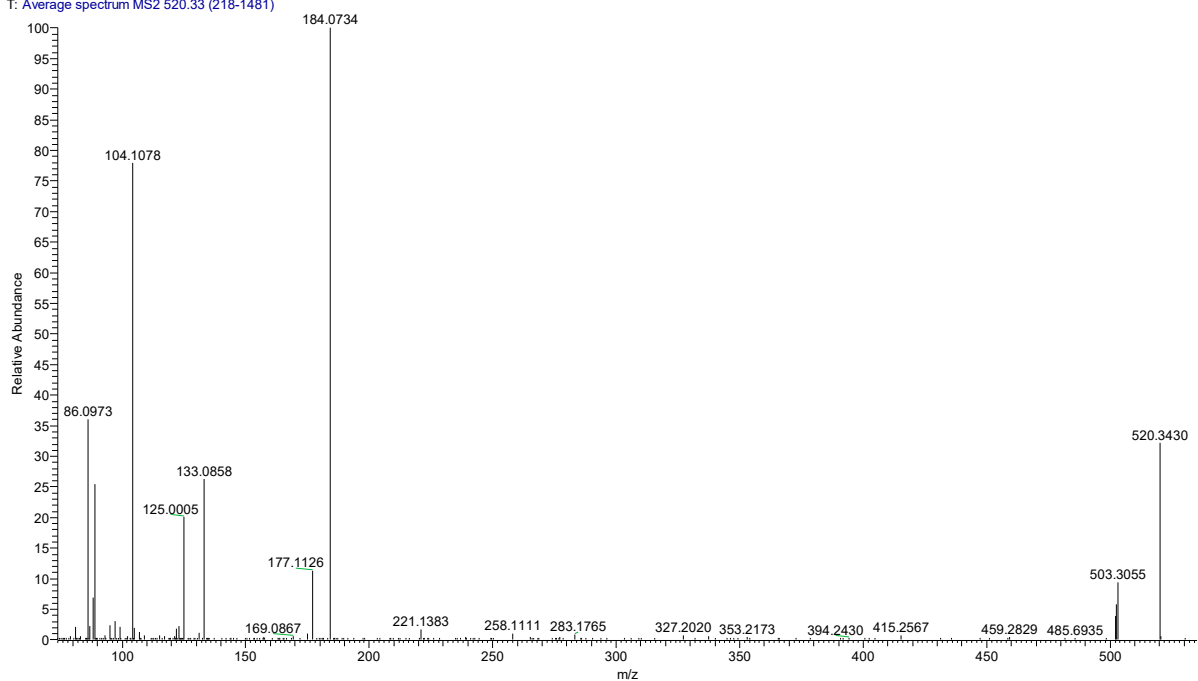
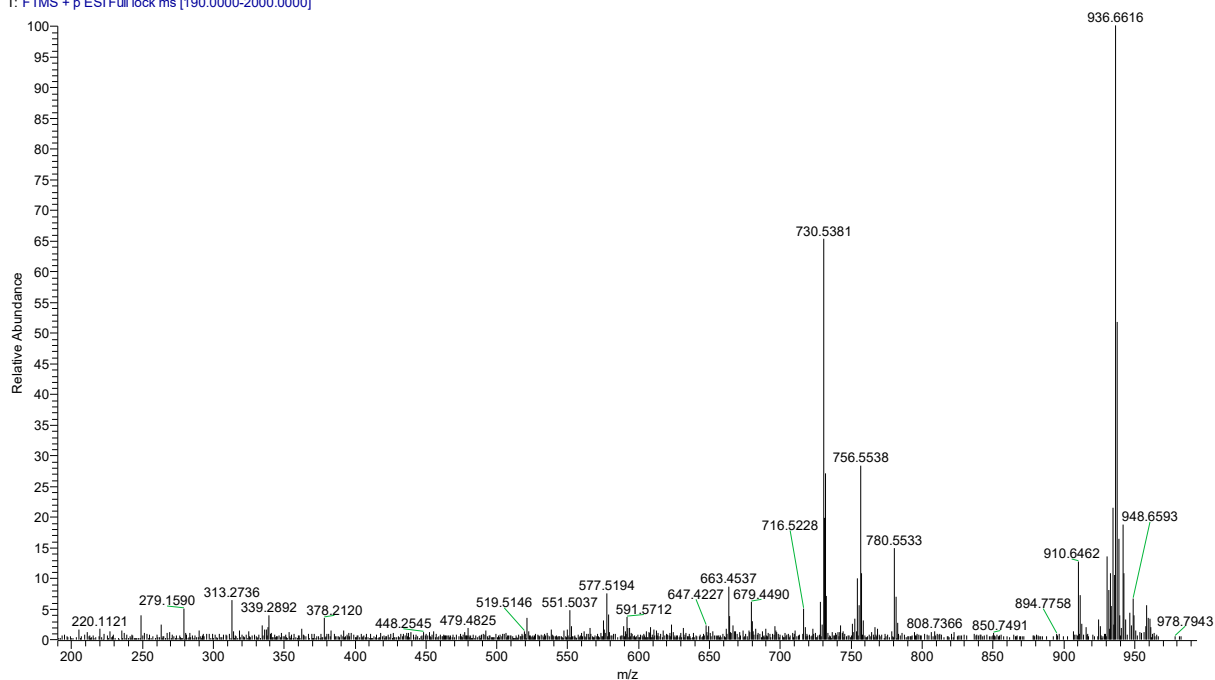


Figure S7. Mass spectrum of metabolites eluted between 3.92-4.00 min (top) and fragmentation ions corresponding to LPC (18:2).

SMC14M_B_PL#2252 RT: 6.49 AV: 1 NL: 1.96E7
T: FTMS + p ESI Full lock ms [190.0000-2000.0000]



SMC14M_B_PL#2254-2446 RT: 6.50-7.06 AV: 9 NL: 6.68E5
T: Average spectrum MS2 756.55 (2254-2446)

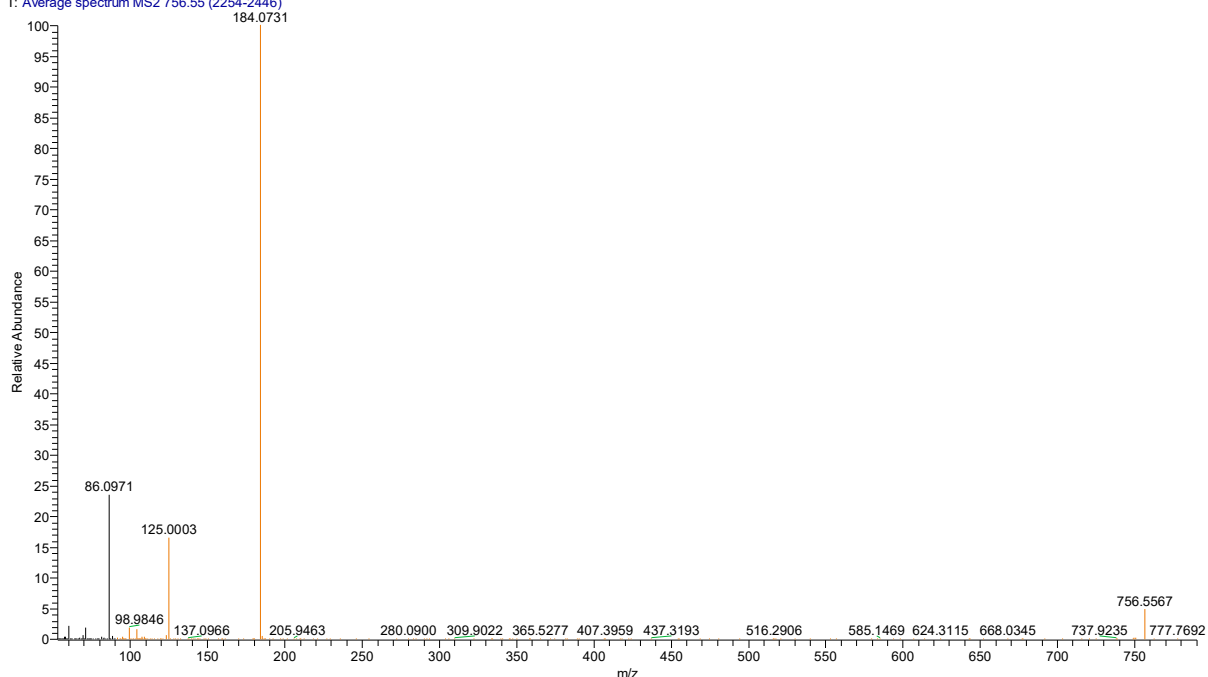
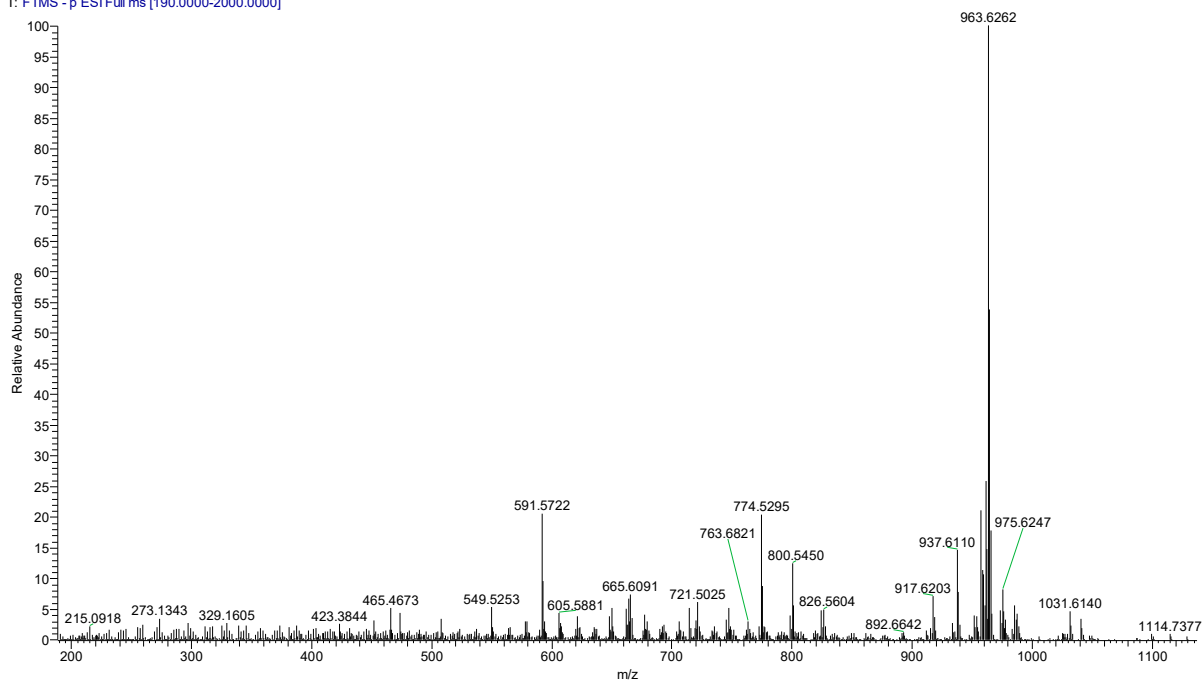


Figure S8. Mass spectrum of metabolites eluted at 6.49 min (top) and fragmentation ions corresponding to PC (34:3) in positive mode (bottom).

SMC14M_B_PL_Nb #1555-1593 RT: 6.45-6.56 AV: 13 NL: 2.99E6
T: FTMS - p ESI Full ms [190.0000-2000.0000]



SMC14M_B_PL_Nb #1578-1601 RT: 6.52-6.59 AV: 4 NL: 2.34E3
T: Average spectrum MS2 800.55 (1578-1601)

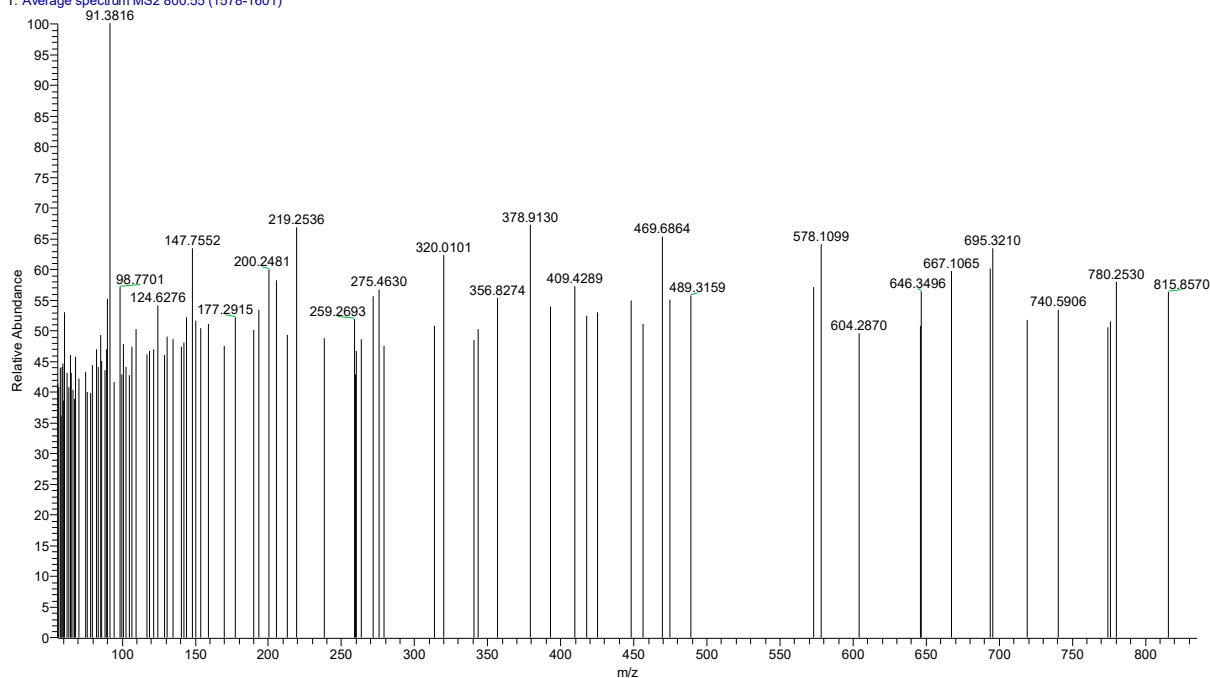


Figure S9. Mass spectrum of metabolites eluted between 6.45-6.56min (top) and fragmentation ions corresponding to PC (34:3) in negative mode (bottom).

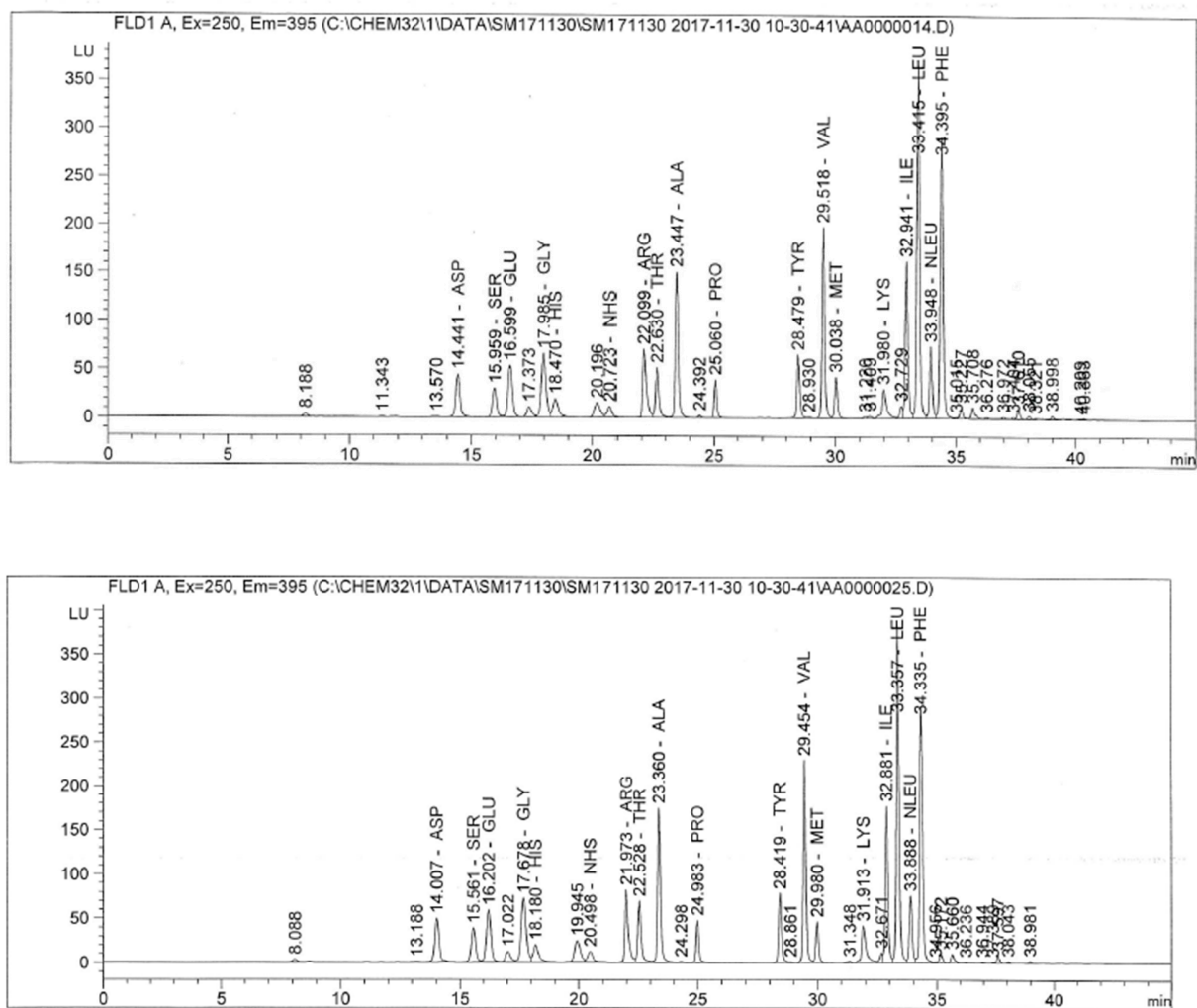


Figure S10. HPLC chromatogram of amino acid analysis of protein isolate extracted from biomasses collected from 20 L (top) and 25000 L photobioreactors (bottom).

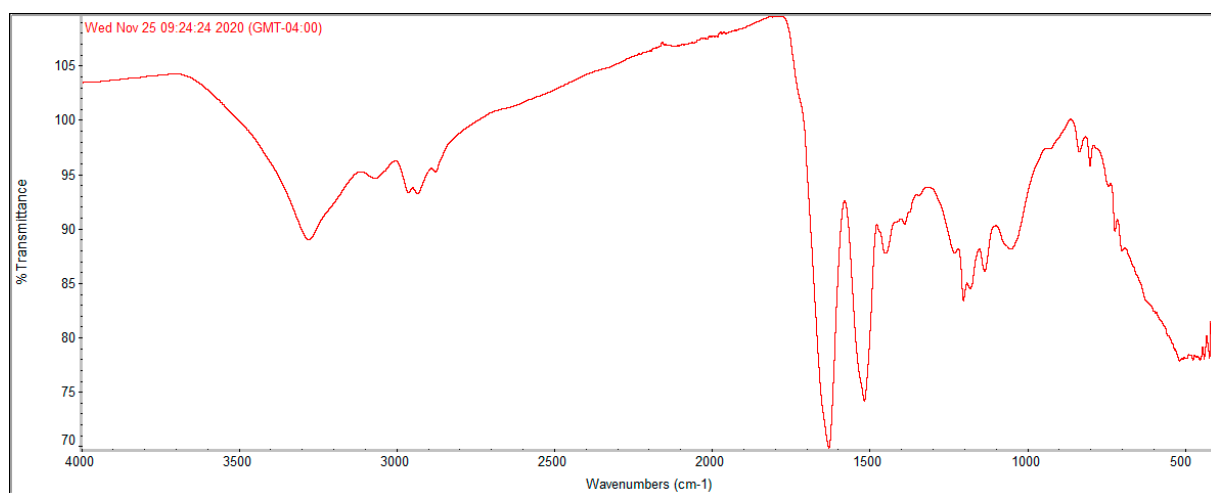
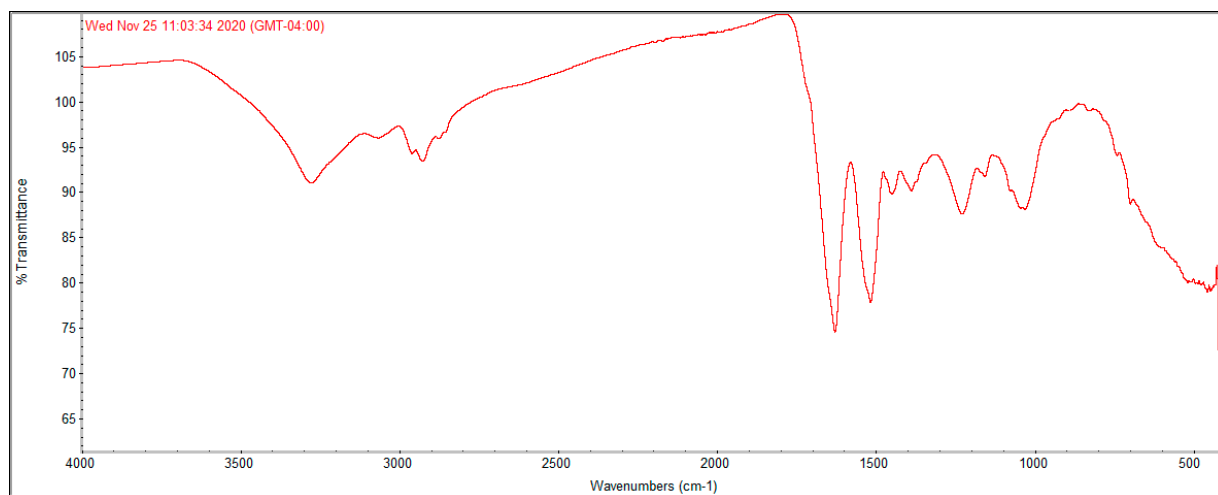


Figure S11. IR spectra of protein isolates extracted from residual biomass of 20 L (top) and 25000 L photobioreactors (bottom).

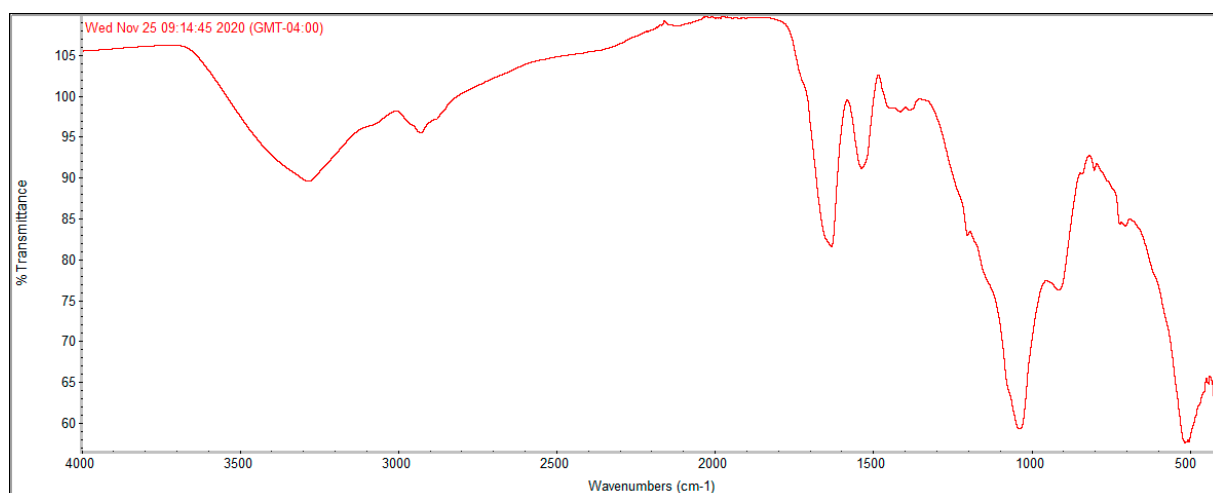
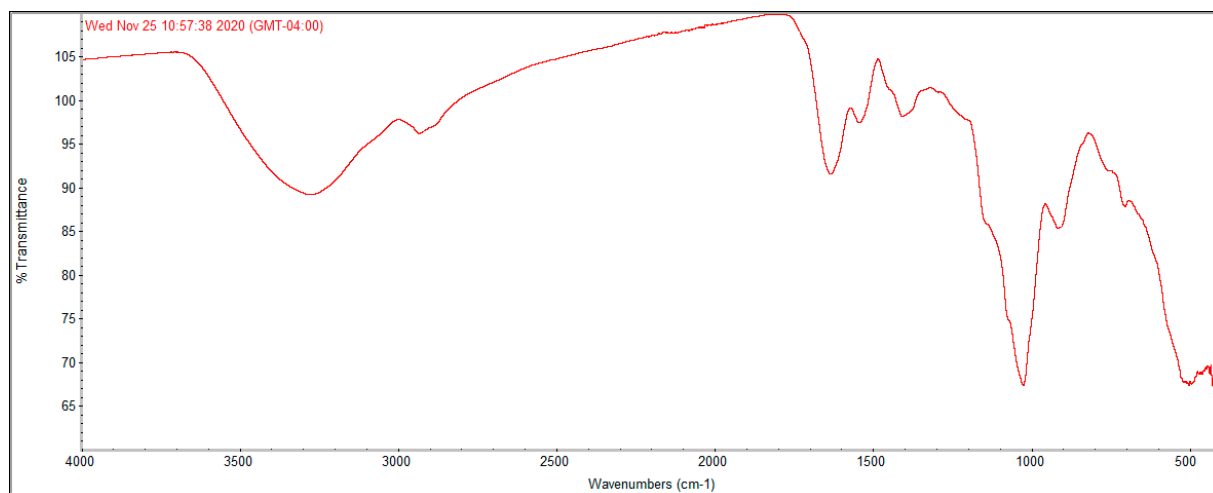


Figure S12. IR spectra of polysaccharides extracted from residual biomass of 20 L (top) and 25000 L photobioreactors (bottom).

Table S1. Fatty acid profile of total lipid extracts. Results are expressed in mg/g of total lipid and the percentage of individual fatty acid in the oil is given in the parenthesis.

<i>Fatty acid</i>	<i>20 L Photobioreactor</i>	<i>25000 L Photobioreactor</i>
<i>Myristic acid</i>	1.2±0.2 (0.4)	-
<i>Myristoleic acid</i>	6.0±1.0 (2.2)	6.5±0.6 (3.0)
<i>Pentadecanoic acid</i>	0.5±0.4 (0.2)	1.4±0.5 (0.6)
<i>cis-10-Pentadecenoic acid</i>	-	-
<i>Palmitic acid</i>	76.5±4.0 (27.5)	55.4±1.7 (25.1)
<i>Palmitoleic acid</i>	3.3±0.1 (1.2)	3.9±0.3 (1.8)
<i>C16:2 n-4</i>	0.5±0.1 (0.2)	0.2±0.3 (0.1)
<i>Heptadecanoic acid</i>	0.7±0.1 (0.2)	1.2±0.2 (0.5)
<i>16:3 n-4</i>	1.3±0.0 (0.5)	1.5±0.2 (0.7)
<i>cis-10-Heptadecenoic acid</i>	-	-
<i>C16:4 n-1</i>	1.1±0.1 (0.4)	1.9±0.1 (0.9)
<i>Stearic acid</i>	2.9±0.1 (1.1)	1.6±0.0 (0.7)
<i>Oleic acid</i>	20.3±0.7 (7.3)	4.4±0.1 (2.0)
<i>C18:1 n-7</i>	3.3±0.1 (1.2)	-
<i>Linoleic acid</i>	124.5±4.2 (44.8)	65.2±1.7 (29.6)
<i>C18:2 n-4</i>	0.3±0.1±0.1 (0.1)	0.2±0.0 (0.1)
<i>γ-linolenic acid</i>	-	0.2±0.0 (0.1)
<i>C18:3 n-4</i>	-	0.1±0.1 (0.0)
<i>α-linolenic acid</i>	24.5±0.8 (8.8)	69.9±1.7 (31.7)
<i>C18:4 n-3</i>	0.3±0.1 (0.1)	0.2±0.0 (0.1)
<i>C18:4 n-1</i>	0.8±0.1 (0.3)	0.7±0.0 (0.3)
<i>Arachidic acid</i>	0.3±0.1 (0.1)	0.2±0.0 (0.1)
<i>cis-11-Eicosenoic acid</i>	1.9±0.1 (0.7)	1.1±0.0 (0.5)
<i>cis-11,14-Eicosadienoic acid</i>	0.5±0.2 (0.2)	0.4±0.0 (0.2)
<i>cis-8,11,14-Eicosatrienoic acid</i>	-	-
<i>Henicosanoic acid</i>	-	-
<i>Arachidonic acid</i>	-	-
<i>cis-8,11,14-Eicosatrienoic acid</i>	-	-
<i>C20:4 n-3</i>	-	-
<i>Eicosapentaenoic acid</i>	1.8±0.1 (0.7)	1.1±0.0 (0.5)
<i>Behenic acid</i>	0.9±0.3 (0.3)	0.4±0.0 (0.2)
<i>Erucic acid</i>	3.4±0.2 (1.2)	2.0±0.1 (0.9)
<i>Docosadienoic acid</i>	-	-
<i>C22:4 n-6</i>	-	-
<i>Lignoceric acid</i>	0.4±0.1 (0.2)	0.4±0.0 (0.2)
<i>Docosahexaenoic acid/C24:1 n-9</i>	0.7±0.1 (0.2)	0.4±0.0 (0.2)
SFA	83.4±5.0 (30.0)	60.6±2.4 (27.5)
MUFA	38.8±2.0 (14.0)	18.4±1.0 (8.3)
PUFA	155.7±5.1 (56.0)	141.5±3.6 (64.2)
Total FA mg/g (%)	277.9±11.9 (100)	220.5±7.0 (100)

- not detected, Fatty acid analysis was done on triplicate.

Table S2. Separation of CHCl₃/MeOH extract obtained from biomass harvested from a 25000 L photobioreactor into fractions and identification of metabolites within each fraction

Fraction	Eluent (mL)	Weight (g)	Metabolites identified by HPLC or LC/MS analysis
1	CHCl ₃ (400)	0.238	β-carotene α-carotene fatty acid-16:2 fatty acid-16:3 fatty acid-18:2 n-6) fatty acid-18:3
2	CHCl ₃ (300)	0.830	pheophytin-a* fatty acid-16:2 fatty acid-16:3 fatty acid-18:2 fatty acid-18:3
3	CHCl ₃ (1600) CHCl ₃ /MeOH (19:1, 100)	0.467	Lutein chlorophyll-a chlorophyll-b fatty acid-16:2 fatty acid-16:3 fatty acid-18:2 fatty acid-18:3
4	CHCl ₃ /MeOH (19:1 – 4:1, 1700)	1.190	MGDG <i>m/z</i> 740.4 MGDG <i>m/z</i> 766.4 MGDG <i>m/z</i> 738.4 MGDG <i>m/z</i> 764.4 MGDG <i>m/z</i> 792.4 MGDG <i>m/z</i> 742.4 MGDG <i>m/z</i> 768.4 MGDG <i>m/z</i> 762.4 MGDG <i>m/z</i> 794.5 DGDG <i>m/z</i> 928.5 DGDG <i>m/z</i> 930.5 DGDG <i>m/z</i> 932.5

* Degraded product of chlorophyll-a

The freeze dry biomass (15.0 g) collected from the pilot-scale photoreactor was extracted with chloroform/MeOH (1:1, 100 ml x 3) by sonicating (15 min) at room temperature. The combined extract was then dried under vacuum yielding chloroform/MeOH extract (3.10 g). The chloroform/MeOH extract was further portioned into five fractions by Flash chromatography using a silica gel column (12 x 4.5 cm) eluting with chloroform/MeOH gradient [Chloroform 400 mL-Fraction 1, 0.238 g; chloroform 300 mL – Fraction 2, 0.830 g; chloroform 1600 mL – Fraction 3, 0.410; chloroform/MeOH (19:1) 100 ml – Fraction 4, 0.057 g; chloroform/MeOH (19:1-5:1) 1700 mL – Fraction 5, 1.191 g]. The major compound present within the fractions were identified either by HPLC and LC/MS with single quadrupole ms detector.