

Supplementary Materials:

Article

Production of Lipids and Carotenoids in *Coccomyxa onubensis* Under Acidic Conditions in Raceway Ponds

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Supplementary Material

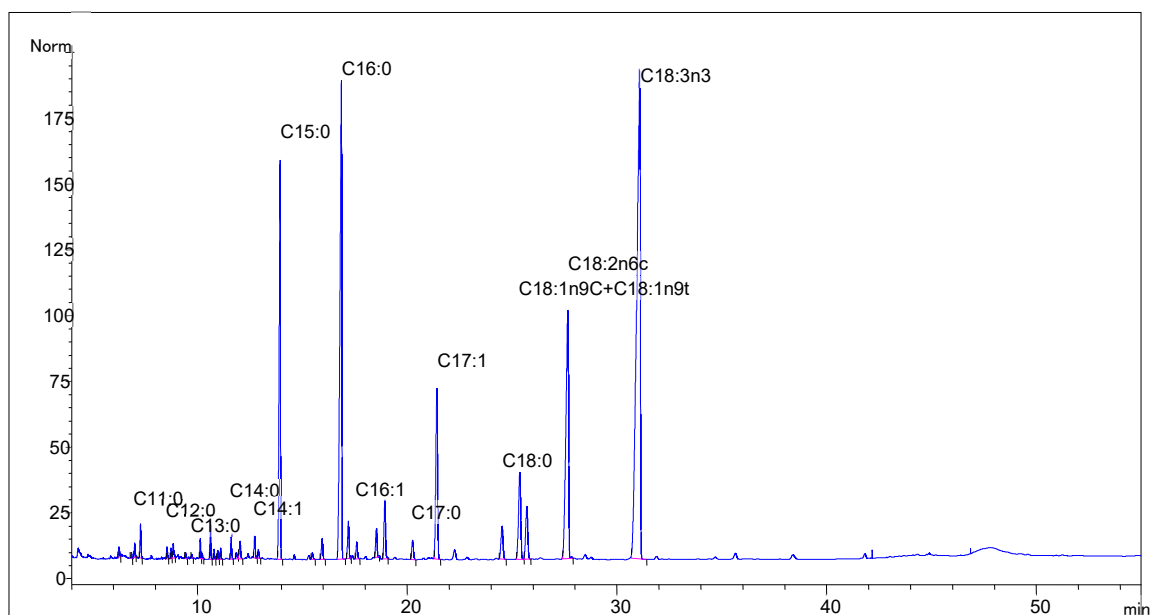


Figure S1. Typical GC chromatogram of *C. onubensis* grown under standard conditions in the culture room.

Table S1. Most abundant fatty acids of *C. onubensis* biomass from laboratory control cultures, classified by the saturation level. 100% corresponds to 125 mg FA·g⁻¹ dw of biomass.

Fatty acid group	Fatty acid name and formula	Approximate abundance (% w/w dw biomass)
Saturated fatty acids (SFA)	Palmitic acid, C16:0	2.11
	Stearic acid, C18:0	0.16
Monounsaturated fatty acids (MUFA)	Palmitoleic acid, C16:1	0.28
	Oleic acid, C18:1	0.25
Polyunsaturated fatty acids (PUFA)	Linoleic acid, C18:2	2.84
	Linolenic acid, C18:3	1.12

Statistical analysis Figure 3

Table S2. Time-course evolution of growth, measured as dry weight (dw), throughout the experiment in cultures of *C. onubensis*. Abbreviation: raceway (RW).

Time	RW pond 1	RW pond 2	RW pond 3
0	0.49 ^a	0.42 ^b	0.45 ^{ab}
4	0.5 ^a	0.43 ^b	0.48 ^b
6	0.525 ^a	0.44 ^b	0.49 ^{ab}
8	0.65 ^a	0.637 ^a	0.607 ^a
11	0.72 ^a	0.66 ^a	0.71 ^a
13	0.826 ^a	0.7 ^b	0.731 ^a
15	0.925 ^a	0.73 ^b	0.75 ^b
18	0.998 ^a	0.81 ^b	0.75 ^b
20	1.1 ^a	0.93 ^b	0.82 ^b
23	1.18 ^a	1.01 ^b	0.87 ^c

^{a, b, c} Different superscript letters indicate differences in treatments with a significance of $p < 0.05$.

Table S3. Growth rate of *C. onubensis* throughout the experiment as a function of the growth stage. Abbreviation: raceway (RW).

	RW pond 1	RW pond 2	RW pond 3
Early growth	0.011 ^a	0.007 ^b	0.011 ^a
Late growth	0.041 ^a	0.032 ^b	0.021 ^c

^{a, b, c} Different superscript letters indicate differences in treatments with a significance of $p < 0.05$.

Statistical analysis Figure 4

Table S4. Time-course evolution of photosynthetic efficiency, measured as Quantum yield (Qy), throughout the experiment in cultures of *C. onubensis*. Abbreviation: raceway (RW).

Time	RW pond 1	RW pond 2	RW pond 3
0	0.58 ^a	0.59 ^a	0.6 ^a
4	0.63 ^a	0.62 ^a	0.62 ^a
6	0.64 ^a	0.61 ^b	0.61 ^b
8	0.61 ^a	0.63 ^a	0.62 ^a
11	0.62 ^a	0.6 ^a	0.61 ^a
13	0.61 ^a	0.63 ^b	0.62 ^a
15	0.68 ^a	0.67 ^a	0.65 ^a
18	0.61 ^a	0.62 ^a	0.59 ^a
20	0.6 ^a	0.59 ^a	0.57 ^a
23	0.6 ^a	0.59 ^a	0.5 ^b

^{a, b, c} Different superscript letters indicate differences in treatments with a significance of $p < 0.05$.

Table S5. Time-course evolution of pH throughout the experiment in cultures of *C. onubensis*. Abbreviation: raceway (RW).

Time	RW pond 1	RW pond 2	RW pond 3
0	2.61 ^{ab}	2.35 ^a	2.71 ^b
4	2.62 ^{ab}	2.38 ^a	2.89 ^b
6	2.64 ^{ab}	2.46 ^a	2.94 ^b
8	2.75 ^{ab}	2.51 ^a	2.89 ^b
11	2.98 ^a	2.48 ^b	3.39 ^c
13	3.12 ^a	2.6 ^b	4.52 ^c
15	2.5 ^a	2.62 ^a	2.68 ^a
18	2.8 ^a	2.73 ^a	2.77 ^a
20	2.87 ^a	2.75 ^a	2.87 ^a
23	2.93 ^a	2.75 ^a	2.95 ^a

^{a, b, c} Different superscript letters indicate differences in treatments with a significance of $p < 0.05$.

Statistical analysis Figure 6

Table S6. Time-course evolution of fatty acids (FAs) expressed as % biomass, throughout the experiment in cultures of *C. onubensis*. Abbreviation: raceway (RW).

Time	RW pond 1	RW pond 2	RW pond 3
0	9.31 ^a	9.45 ^a	8.98 ^a
4	6.6 ^a	9.84 ^b	6.09 ^a
6	7.2 ^a	6.66 ^{ab}	6.37 ^b
8	6.82 ^a	6.66 ^a	7.25 ^a
11	5.87 ^a	7.17 ^b	6.48 ^{ab}
13	7.97 ^a	7.75 ^a	5.08 ^b
15	7.34 ^a	6.23 ^b	6.02 ^b
18	8.45 ^a	6.84 ^b	6.51 ^b
20	9.01 ^a	7.32 ^b	6.23 ^c
23	9.87 ^a	8.02 ^b	5.87 ^c

^{a, b, c} Different superscript letters indicate differences in treatments with a significance of $p < 0.05$.

Table S7. Time-course evolution of fatty acids profile expressed as % biomass, throughout the experiment in cultures of *C. onubensis*.

Time	SFA			MUFA			PUFA		
	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3
0	68 ^a	49 ^b	53 ^b	12 ^a	24 ^b	18 ^c	20 ^a	27 ^b	29 ^b
11	35 ^a	61 ^b	48 ^c	25 ^a	9 ^b	6 ^c	40 ^a	30 ^b	46 ^c
20	32 ^a	43 ^b	37 ^c	19 ^a	14 ^b	16 ^b	49 ^a	44 ^a	47 ^a

^{a, b, c} Different superscript letters indicate differences in treatments with a significance of $p < 0.05$.

Statistical analysis Figure 7

Table S8. Time-course evolution of chlorophyll (Chl) expressed as mg·g⁻¹ biomass, throughout the experiment in cultures of *C. onubensis*.

Time	Chl a			Chl b			Total Chl		
	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3
0	4.8 ^a	2.12 ^b	2.53 ^b	2.16 ^a	1.4 ^b	0.86 ^c	4.3 ^a	3.52 ^b	3.39 ^b
11	4.24 ^a	2.47 ^b	1.66 ^c	1.87 ^a	1.54 ^a	0.91 ^b	6.12 ^a	4.01 ^b	2.58 ^c
20	4.77 ^a	4.56 ^a	2.97 ^b	1.67 ^a	1.75 ^a	1.58 ^a	6.44 ^a	6.31 ^a	4.46 ^b

^{a, b, c} Different superscript letters indicate differences in treatments with a significance of $p < 0.05$.

Table S9. Time-course evolution of carotenoids expressed as mg·g⁻¹ biomass, throughout the experiment in cultures of *C. onubensis*.

Time	B-carot.			Lutein			Total carot.		
	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3
0	1.77 ^{ab}	1.56 ^a	1.92 ^b	3.52 ^a	3.34 ^a	2.21 ^b	4.57 ^a	4.9 ^b	4.75 ^{ab}
11	1.93 ^a	1.65 ^b	1.23 ^c	4.55 ^a	4.39 ^a	3.14 ^b	7.61 ^a	4.76 ^b	4.32 ^b
20	2.12 ^a	1.94 ^a	1.57 ^b	4.64 ^a	4.57 ^a	4.34 ^a	9.04 ^a	6.81 ^b	6.35 ^b

^{a, b, c} Different superscript letters indicate differences in treatments with a significance of $p < 0.05$.