Supplementary Material

Optimization of Cultivation Conditions for *Tetraselmis striata* and Biomass

Quality Evaluation for Fish Feed Production

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Table S1. Physicochemical characterization of the drilling waters.

Parameter	Drill 1	Drill 2
pH	7.74 ± 0.1	7.79 ± 0.11
Electrical Conductivity (ms cm^{-1})	63.2 ± 4.8	44 ± 4.9
Total salinity (%)	3.9 ± 0.1	2.8 ± 0.1
Total Suspended Solids (TSS) (g L^{-1})	0.46 ± 0.05	0.27 ± 0.03
Total Dissolved Solids (TDS) (g L^{-1})	39.44 ± 2.4	24.36 ± 2.8

BOD (mg O_2 L^{-1})	1.0 ± 0.0	0.0 ± 0.0
d-COD (mg O_2 L^{-1})	6.0 ± 4.2	5.3 ± 1.2
NH_4^+-N (mg L^{-1})	0.04 ± 0.02	0.10 ± 0.05
NO_3^--N (mg L^{-1})	0.21 ± 0.10	0.50 ± 0.21
NO_2-N (mg L^{-1})	0.00 ± 0.00	0.00 ± 0.00
PO_4^{3-} (mg L^{-1})	0.34 ± 0.10	0.43 ± 0.20

Table S2. Nutrient composition of all tested growth substrates.

Growth substrate	Medium components	Quantity (g L^{-1})
Experimental set A Salinity 3.9 ± 0.1% N[1]: P[2] ≈5	$(NH_4)_2SO_4$	0.0944
	KNO_3	0.0722
	K_2HPO_4	0.0225
	KH_2PO_4	0.0090
Experimental set B Salinity 3.9 ± 0.1% N: P ≈12	$(NH_4)_2SO_4$	0.2832
	KNO_3	0.0750
	K_2HPO_4	0.0262
	KH_2PO4	0.0102
Experimental set C Salinity 2.8 ± 0.1% N: P ≈12	$(NH_4)_2SO_4$	0.2832
	KNO_3	0.0750
	K_2HPO_4	0.0262
	KH_2PO4	0.0102
Experimental set D Salinity 2.8 ± 0.1% Modified F/2 N: P ≈10	$(NH_4)_2SO_4$	0.2120
	$NaH_2PO_4.H_2O$	0.0240
	Mixed solution (Solution B and trace element stocks) Details for mixed solution: In solution B we add 1 mL of each trace element stock Solution B contains in g L^{-1}: Na_2EDTA 4.36 $FeCl_3.6H_2O$ 3.15 Trace element stock of $CuSO_4·5H_2O$ contains: 10 g L^{-1} Trace element stock of $ZnSO_4·7H_2O$ contains: 22 g L^{-1} Trace element stock of $CoCl_2·6H_2O$ contains: 10 g L^{-1}	1 mL L^{-1}

	Trace element stock of $MnCl_2 \cdot 4H_2O$ contains: 180 g L^{-1} Trace element stock of $Na_2MoO_4 \cdot 2H_2O$ contains: 6 g L^{-1}	
Experimental set E Salinity 2.8 ± 0.1% Fertilizer Nutri-Leaf without $NaHCO_3$ N: P ≈7	Nutri-Leaf 30-10-10 Composition: Total nitrogen 30% of which: Nitrate nitrogen 3% Ammonium nitrogen 3.7% Urea 23.3% P_2O_5 10% K_2P 10% Mg 0.0251% B 0.02% Cu 0.05% Fe 0.1% Mn 0.05% Mo 0.001% Zn 0.05%	0.05
Experimental set F Salinity 2.8 ± 0.1% Fertilizer Nutri-Leaf with $NaHCO_3$ N: P ≈7	Nutri-Leaf 30-10-10	0.05
	$NaHCO_3$	0.18

[1]N=Nitrogen, [2]P= Phosphorus.

Table S3. Fatty acid composition (%) of total lipids (TL), neutral lipids (NL), glycolipids (GL) and phospholipids (PL) synthesized by *T. striata* growing under different pH conditions.

pH value	Lipid fraction	Lipid fraction (%, w/w) in TL	Fatty acid composition (%, w/w)										
			C14:0	C16:0	C16:1	C18:0	C18:1 n-9	C18:2	C20:1 n-9	C20:5 n-3	ΣPUFAs	ΣMUFAs	ΣSFAs
pH 8.0	TL		4.8 ± 0.1	18.5 ± 0.1	24.7 ± 2.0	0.8 ± 0.3	8.3 ± 0.9	5.1 ± 0.7	3.1 ± 0.5	27.6 ± 1.6	33.2 ± 1.3	36.2 ± 1.5	24.1 ± 0.2
	NL	37.1 ± 1.0	2.8 ± 0.3	22.1 ± 0.2	27.0 ± 0.6	2.4 ± 0.5	21.3 ± 0.0	5.5 ± 0.3	3.7 ± 0.3	10.1 ± 0.6	15.6 ± 0.8	52.0 ± 1.3	27.2 ± 0.1
	GL	47.9 ± 0.7	5.8 ± 0.0	16.8 ± 0.3	15.7 ± 0.8	0.8 ± 0.1	7.0 ± 0.9	1.6 ± 0.8	2.3 ± 0.2	35.1 ± 1.4	36.6 ± 1.1	24.9 ± 0.6	23.4 ± 0.1
	PL	15.0 ± 1.1	3.7 ± 0.7	23.6 ± 1.1	16.2 ± 1.3	1.6 ± 0.2	20.1 ± 1.5	12.6 ± 0.8	3.6 ± 0.1	11.4 ± 0.7	24.0 ± 0.6	39.9 ± 0.9	28.9 ± 1.3
pH 7.0	TL		5.0 ± 0.3	29.8 ± 2.1	27.3 ± 1.7	0.5 ± 0.3	9.9 ± 0.3	5.2 ± 0.1	3.3 ± 0.3	14.0 ± 1.3	19.2 ± 1.1	40.5 ± 0.9	35.4 ± 1.5
	NL	48.0 ± 0.9	4.8 ± 0.1	35.4 ± 1.9	33.4 ± 0.9	0.9 ± 0.0	11.1 ± 0.9	4.9 ± 0.3	2.6 ± 0.2	4.4 ± 0.3	9.3 ± 0.1	47.1 ± 1.3	41.1 ± 1.3
	GL	41.8 ± 1.5	6.2 ± 0.1	24.5 ± 2.2	18.2 ± 1.5	0.7 ± 0.2	5.8 ± 0.7	3.0 ± 0.2	2.6 ± 0.1	18.8 ± 1.5	21.8 ± 1.1	26.6 ± 0.8	31.4 ± 1.5
	PL	10.2 ± 0.9	2.6 ± 0.4	17.5 ± 1.7	16.9 ± 0.8	2.4 ± 1.2	27.5 ± 2.3	15.9 ± 0.7	4.0 ± 0.6	9.1 ± 0.9	25.0 ± 0.8	48.4 ± 0.5	22.5 ± 1.1

Table S4. Fatty acid composition (%) of total lipids (TL), neutral lipids (NL), glycolipids (GL) and phospholipids (PL) synthesized by *T. striata* growing under different temperature conditions.

Temperature	Lipid class	Lipid fraction (%, w/w) in TL	Fatty acid composition (%, w/w)										
			C14:0	C16:0	C16:1	C18:0	C18:1 n-9	C18:2	C20:1 n-9	C20:5 n-3	ΣPUFAs	ΣMUFAs	ΣSFAs
19 ± 1 ºC	TL		6.0	26.0	24.2	0.6	9.2	4.4	3.1	20.4	25.1	36.5	32.6
			± 0.3	± 2.1	± 0.8	± 0.1	± 0.7	± 0.9	± 0.5	± 1.3	± 1.1	± 0.4	± 1.5
	NL	42.4 ± 0.8	7.7	24.2	29.0	0.6	8.8	5.7	3.6	16.4	22.6	41.5	32.5
			± 0.6	± 1.0	± 1.1	± 0.0	± 0.2	± 0.8	± 0.3	± 1.5	± 1.4	± 0.8	± 0.9
	GL	38.8 ± 1.8	5.5	23.0	17.5	0.4	4.9	3.0		26.5	29.5	22.5	28.9
			± 0.1	± 1.8	± 0.9	± 0.2	± 0.1	± 0.4	-*	± 0.9	± 0.8	± 0.7	± 1.7
	PL	18.8 ± 1.7	2.5	15.5	20.2		20.4	16.1	6.2	17.2	34.2	46.9	17.9
			± 0.4	± 0.4	± 0.7	-*	± 1.3	± 1.4	± 0.3	± 0.8	± 1.0	± 1.1	± 0.4
25 ± 1 ºC	TL		5.2	34.6	24.9	0.9	8.2	4.0	2.4	16.6	20.7	35.5	40.7
			± 0.2	± 1.6	± 1.9	± 0.4	± 0.7	± 0.1	± 0.6	± 1.1	± 1.4	± 0.9	± 1.4
	NL	44.2 ± 0.9	4.7	39.1	31.8	1.4	10.0	4.1	1.9	6.2	10.2	43.7	43.8
			± 0.1	± 1.4	± 2.2	± 0.8	± 1.7	± 1.1	± 0.7	± 0.3	± 0.8	± 1.2	± 1.1
	GL	45.9 ± 2.0	5.9	37.4	25.6	1.0	6.7	3.0		11.0	14.0	32.3	44.3
			± 0.8	± 1.5	± 1.7	± 0.0	± 0.9	± 0.9	-*	± 0.8	± 1.3	± 0.7	± 0.8
	PL	9.9 ± 0.7	3.3	19.6	11.9	1.0	19.2	12.9	10.3	16.5	31.1	41.3	23.8

			C14:0	C16:0	C16:1	C18:0	C18:1 n-9	C18:2	C20:1 n-9	C20:5 n-3	ΣPUFAs	ΣMUFAs	ΣSFAs
			± 0.2	± 0.8	± 0.1	± 0.2	± 1.8	± 1.2	± 0.9	± 1.3	± 1.5	± 1.1	± 0.7
	TL	ND¹	4.8	35.4	22.3	1.4	11.6	5.9	4.3	10.2	16.1	38.2	41.7
			± 0.8	± 1.1	± 1.8	± 0.3	± 1.4	± 0.4	± 0.7	± 0.8	± 1.1	± 1.5	± 0.8
28 ± 1 ºC	NL	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹
	GL	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹
	PL	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹	ND¹

ND¹ = Not Determined, -* = Not Detected.

Table S5. Fatty acid composition (%) of total lipids (TL), neutral lipids (NL), glycolipids (GL) and phospholipids (PL) synthesized by *T. striata* growing under different photoperiods.

Photoperiod (Light:Dark)	Lipid class	Lipid fraction (%, w/w) in TL	Fatty acid composition (%, w/w)										
			C14:0	C16:0	C16:1	C18:0	C18:1 n-9	C18:2	C20:1 n-9	C20:5 n-3	ΣPUFAs	ΣMUFAs	ΣSFAs
Control set 24:0 h L:D	TL		5.2	34.6	24.9	0.9	8.2	4.0	2.4	16.6	20.7	35.5	40.7
			± 0.2	± 1.6	± 1.9	± 0.4	± 0.7	± 0.1	± 0.6	± 1.1	± 1.4	± 0.9	± 1.4
	NL	44.2 ± 0.9	4.7	39.1	31.8	1.4	10.0	4.1	1.9	6.2	10.2	43.7	43.8
			± 0.1	± 1.4	± 2.2	± 0.8	± 1.7	± 1.1	± 0.7	± 0.3	± 0.8	± 1.2	± 1.1
	GL	45.9 ± 2.0	5.9	37.4	25.6	1.0	6.7	3.0		11.0	14.0	32.3	44.3
			± 0.8	± 1.5	± 1.7	± 0.0	± 0.9	± 0.9	-*	± 0.8	± 1.3	± 0.7	± 0.8
	PL	9.9 ± 0.7	3.3	19.6	11.9	1.0	19.2	12.9	10.3	16.5	31.1	41.3	23.8

Regime	Lipid	Content	1	2	3	4	5	6	7	8	9	10	11
			± 0.2	± 0.8	± 0.1	± 0.2	± 1.8	± 1.2	± 0.9	± 1.3	± 1.5	± 1.1	± 0.7
20:4 h L:D	TL		5.1 ± 0.8	20.7 ± 1.6	20.6 ± 0.5	0.3 ± 0.2	4.4 ± 1.6	6.0 ± 2.1	3.1 ± 1.1	31.5 ± 2.1	37.9 ± 1.1	28.1 ± 0.3	26.1 ± 1.9
	NL	32.2 ± 1.3	4.3 ± 0.2	28.8 ± 2.0	30.1 ± 1.2	0.9 ± 0.3	6.8 ± 1.8	6.5 ± 1.8	3.1 ± 0.7	11.0 ± 0.9	17.9 ± 0.8	40.0 ± 2.1	33.9 ± 1.9
	GL	47.3 ± 2.0	6.7 ± 1.4	19.8 ± 0.8	17.2 ± 0.9	0.2 ± 0.1	1.9 ± 0.4	6.0 ± 1.4	1.3 ± 0.5	32.8 ± 1.4	39.3 ± 0.3	20.4 ± 0.7	26.7 ± 1.1
	PL	20.5 ± 0.9	2.5 ± 0.4	20.0 ± 0.2	21.4 ± 1.5	0.3 ± 0.0	12.2 ± 2.1	17.9 ± 0.8	4.7 ± 1.7	17.3 ± 1.2	35.6 ± 2.0	38.2 ± 1.5	22.8 ± 0.3
18:6 h L:D	TL		5.2 ± 0.9	20.9 ± 1.4	21.0 ± 1.7	1.4 ± 0.4	9.1 ± 1.8	5.5 ± 1.3	2.7 ± 1.2	26.4 ± 0.2	31.9 ± 1.0	32.8 ± 1.9	27.6 ± 1.2
	NL	39.6 ± 1.5	5.4 ± 0.6	24.5 ± 1.5	30.0 ± 2.0	1.4 ± 0.2	8.3 ± 0.7	6.4 ± 0.8	2.9 ± 0.8	14.1 ± 0.4	21.2 ± 0.7	41.2 ± 1.4	31.3 ± 1.0
	GL	47.6 ± 1.9	4.5 ± 1.0	18.3 ± 0.9	14.3 ± 1.9	0.9 ± 0.1	11.8 ± 0.9	5.6 ± 0.7	1.3 ± 0.7	31.8 ± 1.2	37.5 ± 0.7	27.5 ± 0.9	23.7 ± 0.7
	PL	13.5 ± 0.9	2.2 ± 0.5	20.9 ± 1.2	20.9 ± 1.4	-*	19.1 ± 1.5	21.5 ± 0.8	3.7 ± 1.5	10.5 ± 0.8	32.5 ± 0.7	43.7 ± 1.1	23.1 ± 0.8
12:12 h L:D	TL		3.9 ± 1.1	18.6 ± 0.7	18.4 ± 1.3	0.1 ± 0.2	3.9 ± 0.2	6.9 ± 1.5	2.4 ± 0.2	34.3 ± 1.3	41.6 ± 1.2	24.8 ± 1.0	22.7 ± 0.7

	Lipid fraction (%, w/w) in TL	C14:0	C16:0	C16:1	C18:0	C18:1 n-9	C18:2	C20:1 n-9	C20:5 n-3	ΣPUFAs	ΣMUFAs	ΣSFAs
NL	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
GL	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
PL	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]

ND[1] = Not Determined, -* = Not Detected.

Table S6. Fatty acid composition (%) of total lipids (TL), neutral lipids (NL), glycolipids (GL) and phospholipids (PL) synthesized by *T. striata* supplied with CO_2 at different flow rates.

CO_2 flow rate	Lipid class	Lipid fraction (%, w/w) in TL	Fatty acid composition (%, w/w)										
			C14:0	C16:0	C16:1	C18:0	C18:1 n-9	C18:2	C20:1 n-9	C20:5 n-3	ΣPUFAs	ΣMUFAs	ΣSFAs
No supply (Control set)	TL		5.2 ± 0.2	34.6 ± 1.6	24.9 ± 1.9	0.9 ± 0.4	8.2 ± 0.7	4.0 ± 0.1	2.4 ± 0.6	16.6 ± 1.1	20.7 ± 1.4	35.5 ± 0.9	40.7 ± 1.4
	NL	44.2 ± 0.9	4.7 ± 0.1	39.1 ± 1.4	31.8 ± 2.2	1.4 ± 0.8	10.0 ± 1.7	4.1 ± 1.1	1.9 ± 0.7	6.2 ± 0.3	10.2 ± 0.8	43.7 ± 1.2	43.8 ± 1.1
	GL	45.9 ± 2.0	5.9 ± 0.8	37.4 ± 1.5	25.6 ± 1.7	1.0 ± 0.0	6.7 ± 0.9	3.0 ± 0.9	-*	11.0 ± 0.8	14.0 ± 1.3	32.3 ± 0.7	44.3 ± 0.8
	PL	9.9 ± 0.7	3.3 ± 0.2	19.6 ± 0.8	11.9 ± 0.1	1.0 ± 0.2	19.2 ± 1.8	12.9 ± 1.2	10.3 ± 0.9	16.5 ± 1.3	31.1 ± 1.5	41.3 ± 1.1	23.8 ± 0.7
10 mL min⁻¹	TL		5.9 ± 1.1	25.7 ± 2.0	23.0 ± 0.6	0.2 ± 0.1	5.2 ± 0.8	6.4 ± 0.9	3.8 ± 0.7	23.0 ± 1.6	29.8 ± 1.4	32.0 0.7	31.8 ± 1.6

	NL	30.8 ± 2.1	4.8 ± 0.8	36.1 ± 1.1	33.2 ± 1.2	0.4 ± 0.2	6.9 ± 1.3	4.6 ± 1.0	2.9 ± 0.7	6.8 ± 0.8	11.7 ± 1.5	43.0 ± 0.9	41.4 ± 1.0
	GL	55.1 ± 1.7	8.0 ± 1.7	21.8 ± 0.8	18.5 ± 1.8	-*	1.9 ± 0.4	5.7 ± 0.7	1.9 ± 0.3	29.7 ± 2.1	35.4 ± 1.8	22.3 ± 1.3	29.8 ± 1.1
	PL	15.1 ± 2.0	2.0 ± 0.5	20.2 ± 0.8	17.4 ± 0.2	0.3 ± 0.0	13.7 ± 1.2	18.9 ± 0.1	6.8 ± 1.3	17.0 ± 1.6	35.9 ± 1.5	37.9 ± 1.9	22.4 ± 0.7
	TL		5.9 ± 1.1	31.4 ± 2.2	21.0 ± 0.8	0.6 ± 0.3	11.5 ± 1.2	6.3 ± 1.1	2.8 ± 0.8	14.7 ± 1.2	21.0 ± 1.1	35.3 ± 1.6	37.9 ± 1.6
20 mL min^{-1}	NL	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	GL	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	PL	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]

ND[1] = Not Determined, -* = Not Detected.

Carotenoid Analysis

The wet microalgae biomass was centrifuged while a clean-up protocol was applied to remove seawater salts (Mohamed et al., 2014). In detail, 50 mL of wet microalgae biomass was centrifuged using a refrigerated centrifuge at 7000 rpm for 5 minutes at 5°C and the upper layer was discarded. Then 20 mL of ammonium formate 0.5 M was added. Again, the samples were vortexed and centrifuged, and the upper layer was discarded. A further two washing steps followed using 50 mL of deionized water each time, following the same procedure After the last centrifuge, the pellet was transferred into a clean tube with as little deionized water as possible and was placed in a freezer at -20°C. The next day all samples were lyophilized in a freeze-dryer. The dry microalgae biomass that was produced was then homogenized with a mortar and pestle and was kept at -20°C for further carotenoid analysis. All samples were processed under the same conditions in all steps followed. A carotenoid solid-liquid extraction (SLE) was developed based on methods already published in the literature with some modifications.

Determination and quantification of the targeted carotenoids (astaxanthin, lutein, zeaxanthin, canthaxanthin, b-cryptoxanthin, echinenone, lycopene and b-carotene) were performed using a UPLC H-Class -QTOF-MS system (Waters Corp., Millford, MA, USA). The chromatographic separation was carried out on a C18 BEH column (50 mm × 2.1 mm, 1.7 m, Waters). Mobile phases consisted of 0.1% aqueous FA (solvent A) and acetonitrile with 0.1% FA (solvent B). A gradient flow rate and elution program were selected for carotenoids separation as follows: 85-79% A from 0 to 2 min; 79-75% from 2 to 5 min; 75-15% from 5 to 7 min; 15–85 from 7 to 8 min; equilibrate at 85% from 8 to 10 min. Column temperature was maintained at 32°C and the sample's temperature was 15°C. The injection volume was 5 μL. Data acquisition and analysis were executed on MassLynx 4.1 software. Standard stock solutions (1000 μg/ml) of all carotenoids and internal standard (trans- 8'- apo- β- caroten- 8'-al) were prepared in dichloromethane and stored at -20°C.

The qTOF-MS detector was operated using an orthogonal-V electrospray ionization interface (ESI) in positive mode. The electrospray voltage was 3.5 kV and the sample cone voltage was 20 V. The extraction cone voltage was 5 V and the MCP plates were operated at 1800 V. The source temperature was 100°C and the desolvation temperature was set at 300°C. Nitrogen was used as the desolvation and cone gas and was set at 600 and 50 L h-1, respectively. The analyzer was operated in the V optics mode at a resolution (FWHM) of 9000 ± 500. The collision gas used was argon. Ion acquisition was performed at a rate of 10 spectrums per second in continuum mode from m/z 50–600, using multiple SIM functions on a narrow time window specific for each carotenoid according to their retention times monitoring their precursor ion [M+H]+.

Leucine encephalin was used as a reference material for mass spectrometer tuning and calibration compound (m/z 556.2771) that was infused at 4 L min-1 at a concentration of 200 ng/mL in order to maintain mass accuracy avoiding shifting due to temperature changes. For the lock mass spectrum, the scan time was set to 1 s with a frequency of 10 s. For mass calibration, a solution of sodium formate (10% FA/0.1 M NaOH/ACN) at a ratio of (1/1/8) was used.

References

1. do Nascimento, T.; Nass, P.; Fernandes, A.; Vieira, K.; Wagner, R.; Jacob-Lopes, E.; Zepka, L. Exploratory data of the microalgae compounds for food purposes. *Data Br.,* **2020**, *29*, 105182.
2. Vendruscolo, R.; Fernandes, A.; Fagundes, M.; Zepka, L.; de Menezes, C.; Jacob–Lopes, E.; Wagner, R. Development of a new method for simultaneous extraction of chlorophylls and carotenoids from microalgal biomass. *J. Appl. Phycol.* **2021**, *33*, 1987-1997.
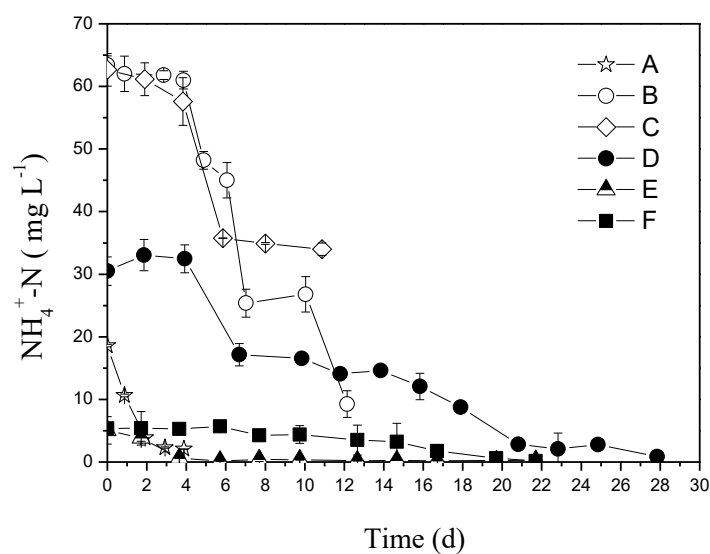
Figure S1. NH4+-N removal over time from the different growth substrates. Experimental sets: A (Salinity 3.9 ± 0.1%, N:P≈5), B (Salinity 3.9 ± 0.1%, N:P≈12), C (Salinity 2.8 ± 0.1%, N:P≈12), D: (Salinity 2.8 ± 0.1%, Modified F/2), E (Salinity 2.8 ± 0.1%, Nutri-Leaf 30-10-10 without $NaHCO_3$), and F (Salinity 2.8 ± 0.1%, Nutri-Leaf 30-10-10 with $NaHCO_3$).
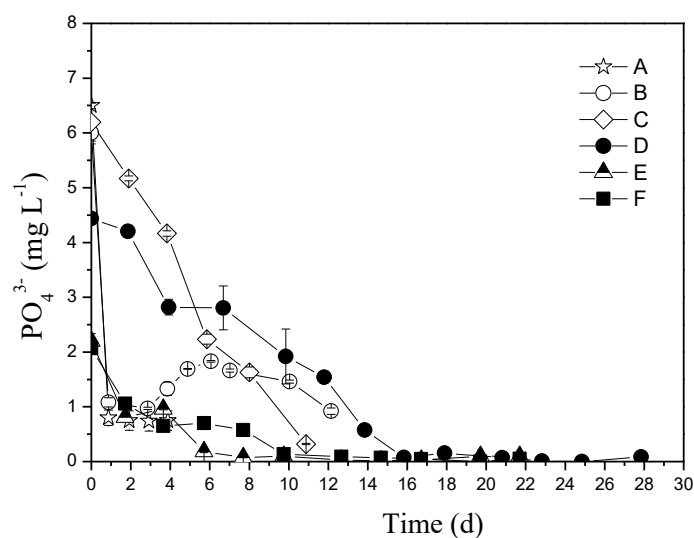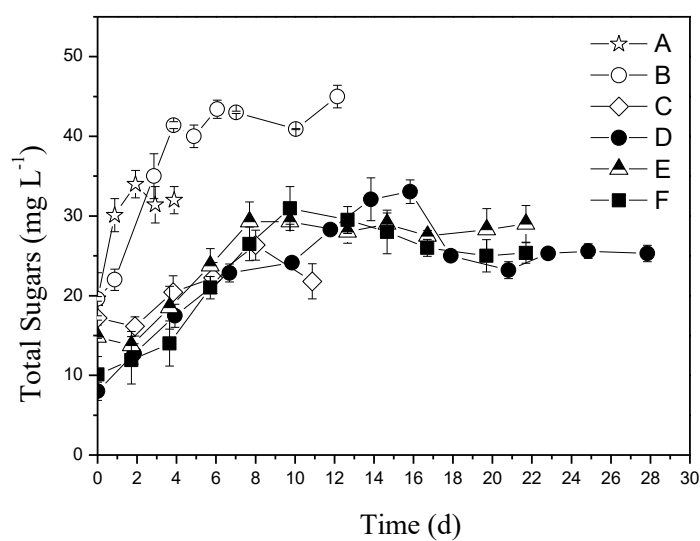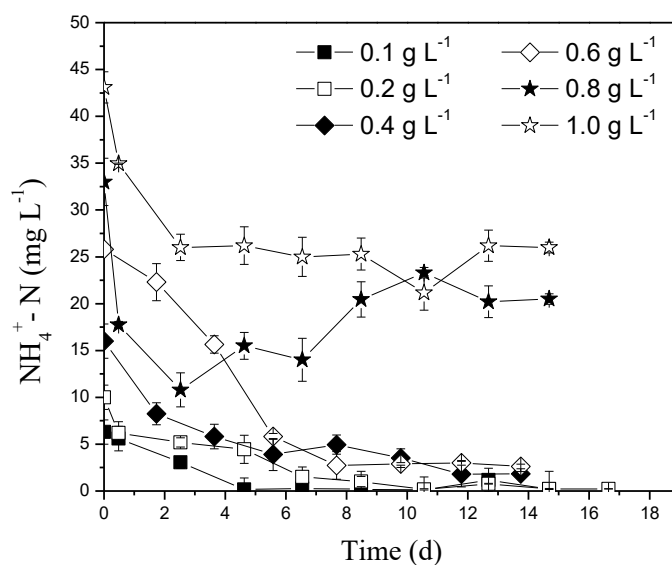

Figure S2. PO4³⁻ removal over time from the different growth substrates. Experimental sets: A (Salinity 3.9 ± 0.1%, N:P≈5), B (Salinity 3.9 ± 0.1%, N:P≈12), C (Salinity 2.8 ± 0.1%, N:P≈12), D: (Salinity 2.8 ± 0.1%, Modified F/2), E (Salinity 2.8 ± 0.1%, Nutri-Leaf 30-10-10 without $NaHCO_3$), and F (Salinity 2.8 ± 0.1%, Nutri-Leaf 30-10-10 with $NaHCO_3$).

Figure S3. Total sugar production over time from the different growth substrates. Experimental sets: A (Salinity 3.9 ± 0.1%, N:P≈5), B (Salinity 3.9 ± 0.1%, N:P≈12), C (Salinity 2.8 ± 0.1%, N:P≈12), D: (Salinity 2.8 ± 0.1%, Modified F/2), E (Salinity 2.8 ± 0.1%, Nutri-Leaf 30-10-10 without NaHCO₃), and F (Salinity 2.8 ± 0.1%, Nutri-Leaf 30-10-10 with NaHCO₃).

Figure S4. NH₄⁺-N removal over time applying different initial fertilizer quantities.
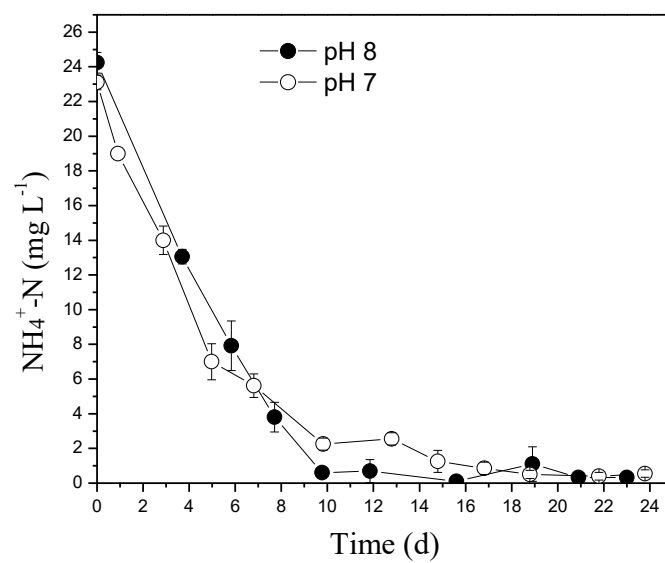
Figure S5. NH$_4^+$-N removal over time during the pH experiments.
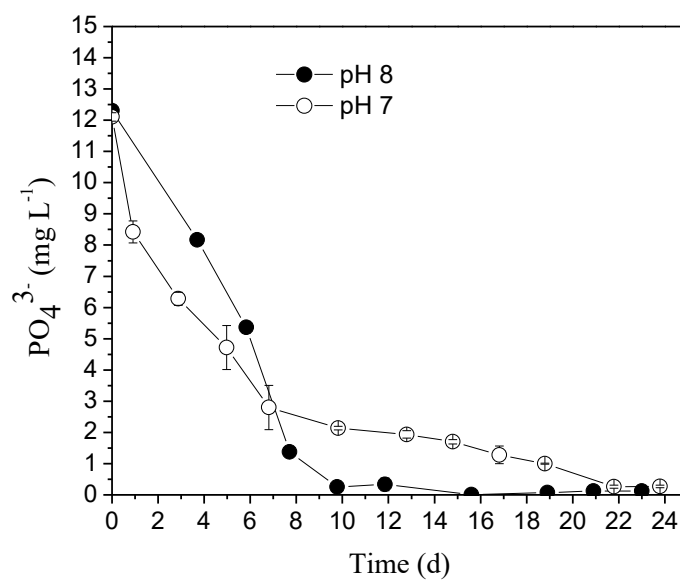
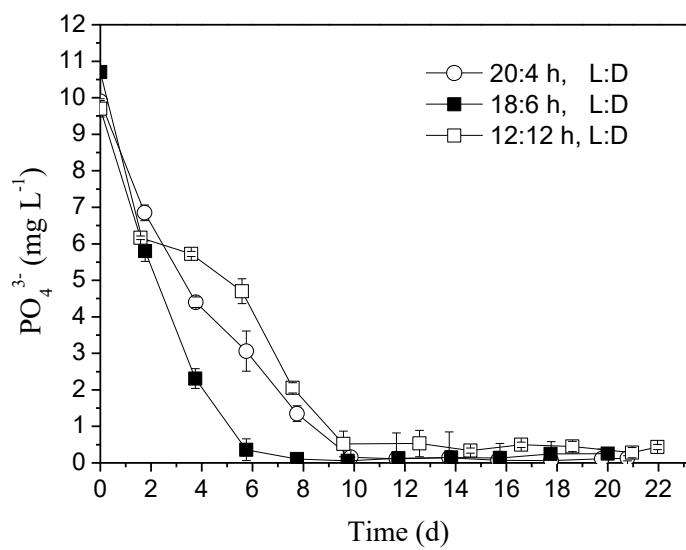**Figure S6.** PO$_4^{3-}$ removal over time during the pH experiments.
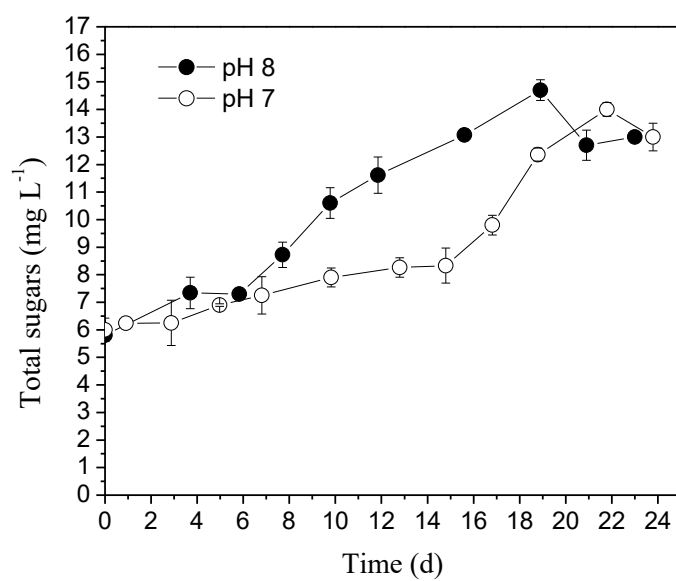
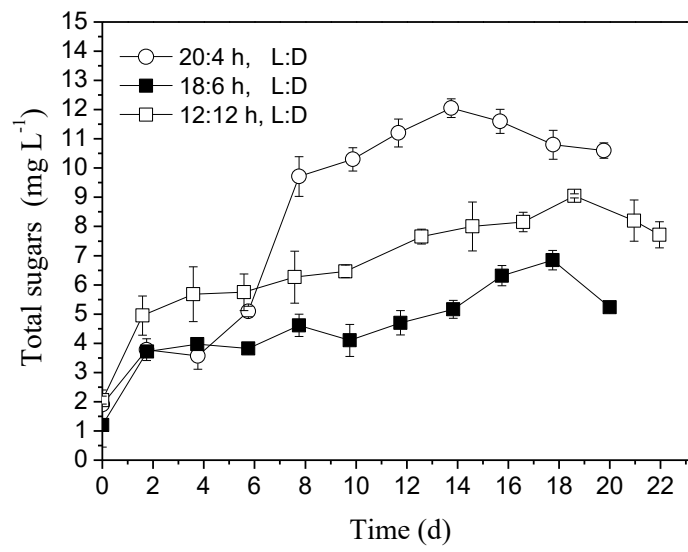**Figure S7.** Total sugar production over time during the pH experiments.
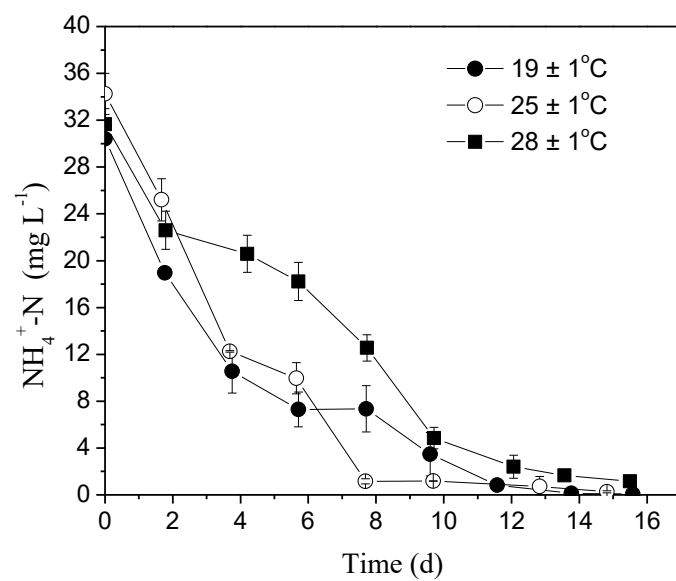
Figure S8. NH₄⁺-N removal over time during the temperature experiments.
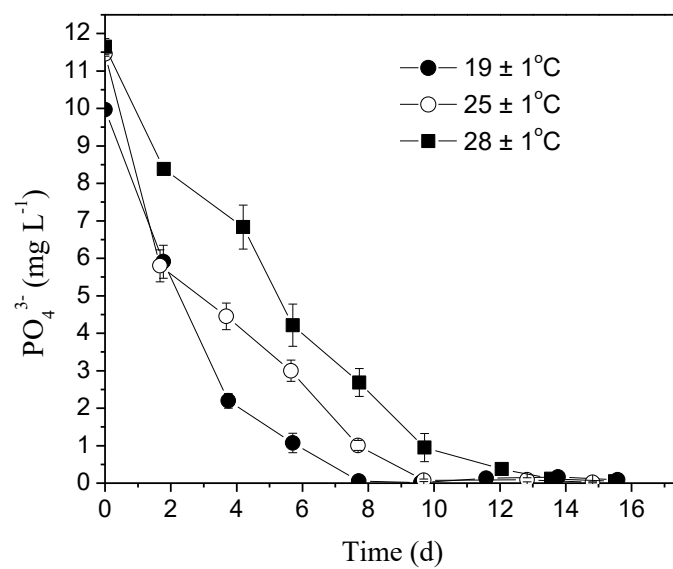
Figure S9. PO_4^{3-} removal over time during the temperature experiments.
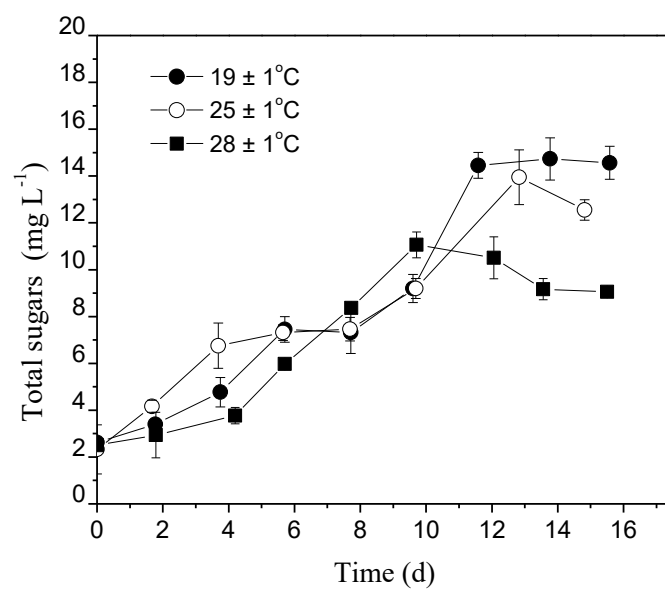
Figure S10. Total sugar production over time during the temperature experiments.
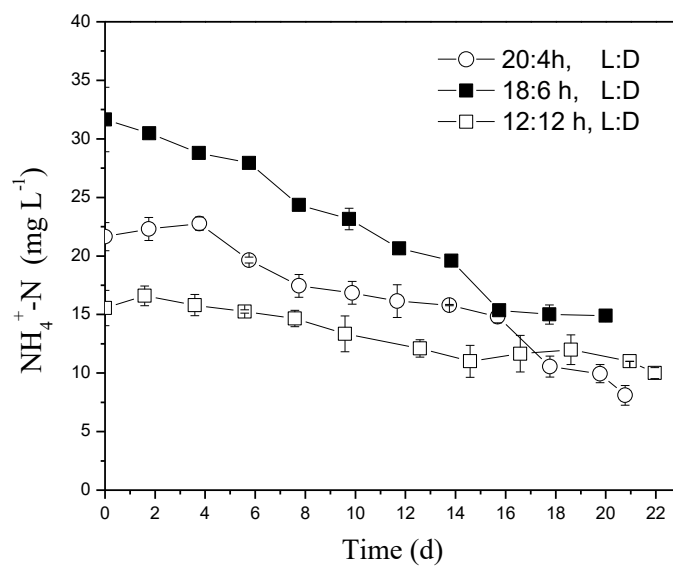
Figure S11. NH_4^+-N removal over time during the photoperiod experiments.

Figure S12. PO_4^{3-} removal over time during the photoperiod experiments.

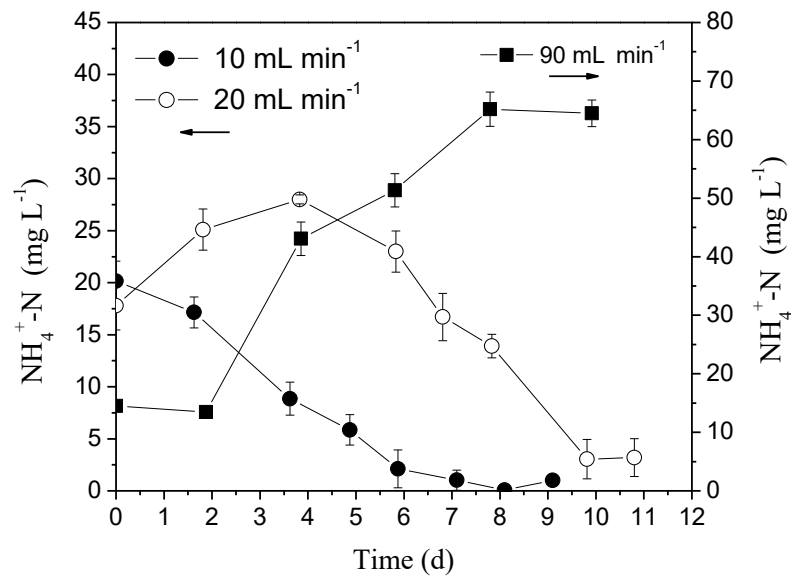**Figure S13.** Total sugar production over time during the photoperiod experiments.

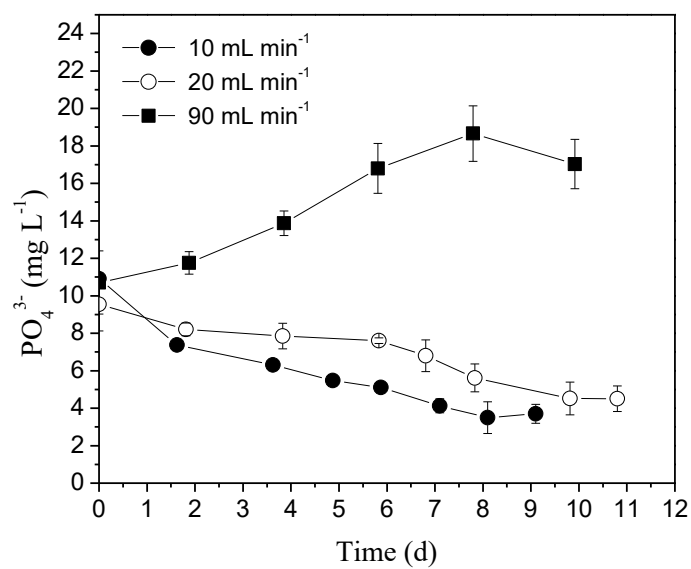**Figure S14.** NH_4^+-N removal over time during the CO_2 experiments.

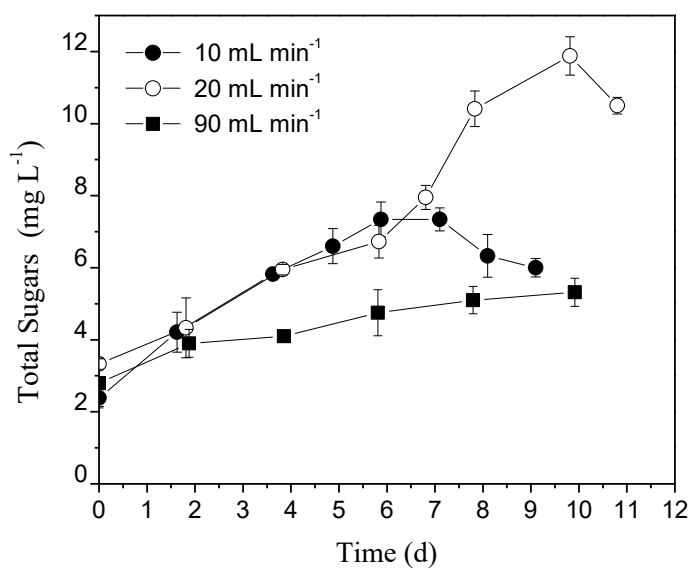**Figure S15.** PO_4^{3-} removal over time during the CO_2 experiments.

Figure S16. Total sugar production over time during the CO_2 experiments.