

Temperature effects on growth and biochemical composition of *Chlorella vulgaris* under heterotrophic cultivation

Supplementary materials

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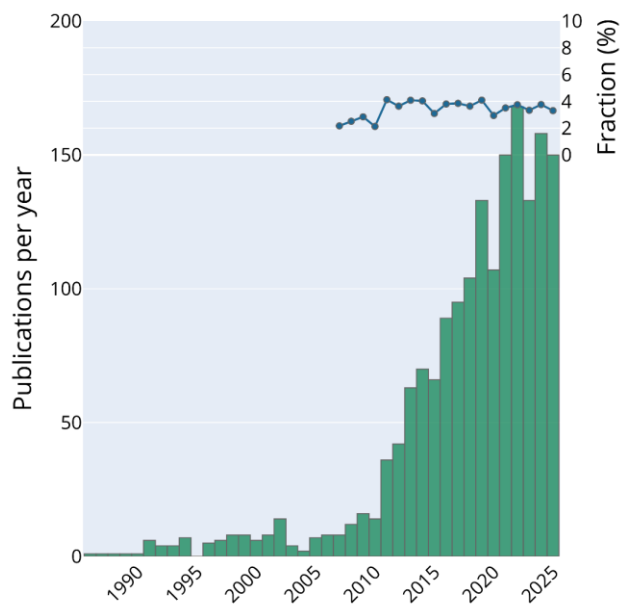


Fig. S1. Articles dealing with heterotrophy published each year and the fraction of the microalgae related publications it represents. The analysis was performed using the Web of Science database on April 29th 2026.

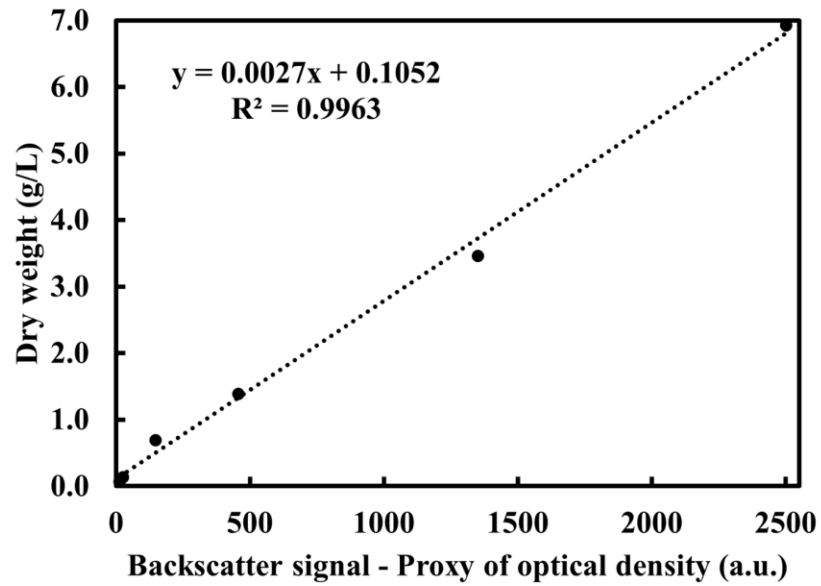


Fig. S2. Calibration curve relating absorbance to dry weight biomass concentration of *Chlorella vulgaris* CV 211-11b. Six calibration points covering 0.06-6.92 g/L were used. Each point was measured five times and averaged, and the linear regression showed a strong correlation ($R^2 = 0.9963$).

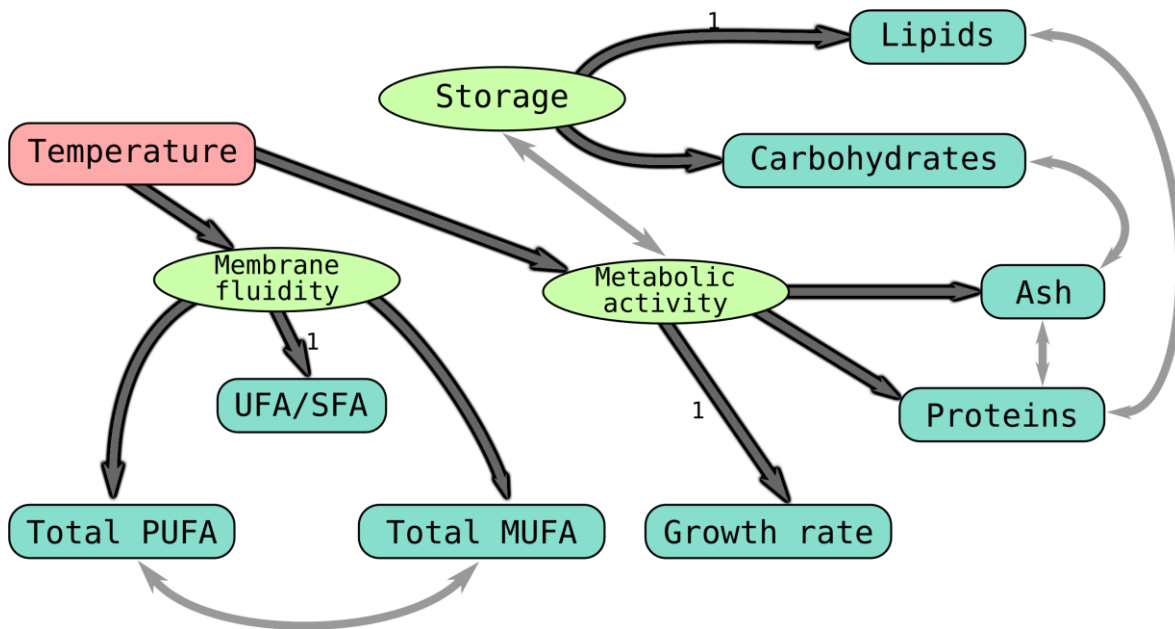


Fig. S3. Initial structural equation modeling framework before parameterization.

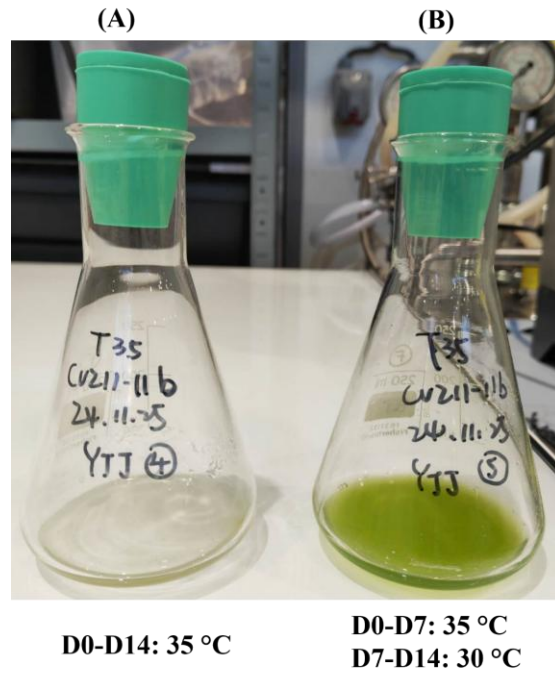


Fig. S4. Representative photo from recovery experiment. (A) Culture maintained at 35 °C. (B) Culture initially incubated at 35 °C for 7 days and then transferred to 30 °C. The transferred culture showed a visible increase in biomass compared with the culture maintained at 35 °C.

Table S1 Composition of the 2× B3N medium used in this study.

Component	Concentration used in 2×B3N (mM)
NaNO ₃	17.64
CaCl ₂ ·2H ₂ O	3.40×10^{-1}
MgSO ₄ ·7H ₂ O	6.08×10^{-1}
K ₂ HPO ₄	8.62×10^{-1}
KH ₂ PO ₄	2.58
NaCl	8.56×10^{-1}
EDTA	3.42×10^{-1}
KOH	11.06×10^{-1}
FeSO ₄ ·7H ₂ O	3.58×10^{-2}
H ₂ SO ₄	3.74×10^{-2}
H ₃ BO ₃	2.70×10^{-1}
ZnSO ₄ ·7H ₂ O	6.14×10^{-2}
MnCl ₂ ·4H ₂ O	14.56×10^{-3}
MoO ₃	9.86×10^{-3}
CuSO ₄ ·5H ₂ O	12.58×10^{-3}
Co(NO ₃) ₂ ·6H ₂ O	3.36×10^{-3}

Table S2 The mean and SD value of biochemical composition of *Chlorella vulgaris* cultivated under different temperatures (4 °C, 15 °C, 20 °C, 25 °C and 30 °C) and the P-value from Tukey's honestly significant difference (HSD) test between each temperature (n = 8).

Parameter	4 °C	15 °C	20 °C	25 °C	30 °C
Growth rate (day ⁻¹)	0.32 ± 0.01	0.44 ± 0.03	0.68 ± 0.09	1.11 ± 0.06	1.14 ± 0.08
<i>P</i> vs 4 °C	-	0.002	< 0.001	< 0.001	< 0.001
<i>P</i> vs 15 °C	-	-	< 0.001	< 0.001	< 0.001
<i>P</i> vs 20 °C	-	-	-	< 0.001	< 0.001
<i>P</i> vs 25 °C	-	-	-	-	0.77
<i>P</i> vs 30 °C	-	-	-	-	-
Lipid content (%)	32.71 ± 1.77	25.49 ± 1.45	31.57 ± 1.53	35.03 ± 3.48	35.49 ± 3.58
<i>P</i> vs 4 °C	-	< 0.001	0.901	0.389	0.218
<i>P</i> vs 15 °C	-	-	< 0.001	< 0.001	< 0.001
<i>P</i> vs 20 °C	-	-	-	0.077	0.034
<i>P</i> vs 25 °C	-	-	-	-	0.996
<i>P</i> vs 30 °C	-	-	-	-	-
Protein content (%)	16.50 ± 1.26	18.71 ± 1.51	14.74 ± 1.23	13.44 ± 0.73	16.83 ± 1.02
<i>P</i> vs 4 °C	-	0.007	0.037	< 0.001	0.978
<i>P</i> vs 15 °C	-	-	< 0.001	< 0.001	0.029
<i>P</i> vs 20 °C	-	-	-	0.192	0.009
<i>P</i> vs 25 °C	-	-	-	-	< 0.001
<i>P</i> vs 30 °C	-	-	-	-	-
Carbohydrate content (%)	39.33 ± 2.29	49.33 ± 1.74	47.58 ± 1.47	46.49 ± 3.81	41.68 ± 3.83
<i>P</i> vs 4 °C	-	< 0.001	< 0.001	< 0.001	0.475
<i>P</i> vs 15 °C	-	-	0.755	0.322	< 0.001
<i>P</i> vs 20 °C	-	-	-	0.94	0.002
<i>P</i> vs 25 °C	-	-	-	-	0.014
<i>P</i> vs 30 °C	-	-	-	-	-
Chl <i>a</i> (mg/g)	10.03 ± 1.90	9.02 ± 1.08	6.86 ± 0.33	5.16 ± 0.30	5.19 ± 0.89
<i>P</i> vs 4 °C	-	0.377	< 0.001	< 0.001	< 0.001
<i>P</i> vs 15 °C	-	-	0.004	< 0.001	< 0.001
<i>P</i> vs 20 °C	-	-	-	0.025	0.029
<i>P</i> vs 25 °C	-	-	-	-	1
<i>P</i> vs 30 °C	-	-	-	-	-
Chl <i>b</i> (mg/g)	4.08 ± 0.73	3.36 ± 0.34	2.93 ± 0.15	1.96 ± 0.10	1.90 ± 0.29
<i>P</i> vs 4 °C	-	0.1	< 0.001	< 0.001	< 0.001
<i>P</i> vs 15 °C	-	-	0.236	< 0.001	< 0.001
<i>P</i> vs 20 °C	-	-	-	< 0.001	< 0.001
<i>P</i> vs 25 °C	-	-	-	-	0.997
<i>P</i> vs 30 °C	-	-	-	-	-
Car <i>c+x</i> (mg/g)	2.22 ± 0.40	2.59 ± 0.29	2.12 ± 0.10	2.25 ± 0.14	2.29 ± 0.34
<i>P</i> vs 4 °C	-	0.1	0.99	0.1	0.021
<i>P</i> vs 15 °C	-	-	0.021	0.151	0.238
<i>P</i> vs 20 °C	-	-	-	0.887	0.765
<i>P</i> vs 25 °C	-	-	-	-	0.999

<i>P</i> vs 30 °C					-
Ash content (%)	9.84 ± 0.87	4.97 ± 0.59	4.92 ± 0.62	4.11 ± 0.42	5.06 ± 0.45
<i>P</i> vs 4 °C	-	< 0.001	< 0.001	< 0.001	< 0.001
<i>P</i> vs 15 °C		-	1	0.07	0.999
<i>P</i> vs 20 °C			-	0.081	0.991
<i>P</i> vs 25 °C				-	0.029
<i>P</i> vs 30 °C					-
