

Supplementary Information

Growth Performance and Estimated CO₂ Fixation of *Chlorella* sp. in Flat Panel and Tubular Photobioreactors under Tropical Outdoor Conditions in Thailand

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Table S1. Jaworski's Medium as the growth medium for *Chlorella* sp. AARL G049

Component	Amount (mg·L ⁻¹)
Ca(NO ₃) ₂ ·4H ₂ O	20
KH ₂ PO ₄	12.4
MgSO ₄ ·7H ₂ O	50
NaHCO ₃	15.9
EDTAF _e Na	2.25
EDTANa ₂	2.25
H ₃ BO ₃	2.48
MnCl ₂ ·4H ₂ O	1.39
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	1
Cyanocobalamin (Vitamin B12)	0.04
Thiamine HCl (Vitamin B1)	0.04
Biotin	0.04
NaNO ₃	80
Na ₂ HPO ₄ ·12H ₂ O	36

Table S2. Meteorological conditions during the three outdoor cultivation periods in Chiang Mai, Thailand

Period	Date	Avg. air temperature (°C)	Daily max temperature (°C)	Daily min temperature (°C)	Avg. RH (%)	Rainfall	Avg. evaporation (mm/day)	Avg. max wind speed (km/h)	Weather condition
1	31 Oct–8 Nov 2025	25.98 ± 0.81	27.0–33.6	21.9–24.0	86.78 ± 4.89	163.0 mm	3.39 ± 1.12	19.67 ± 4.47	Humid and rainy
2	12–20 Dec 2025	24.18 ± 0.63	27.7–31.8	17.2–21.0	77.44 ± 2.83	0.2 mm, with one trace day	3.02 ± 1.49	16.56 ± 2.19	Cooler and mostly dry
3	15–23 Jan 2026	22.97 ± 0.38	30.4–32.4	15.2–18.0	70.67 ± 1.73	0.0 mm	3.23 ± 0.26	15.67 ± 2.65	Cooler and dry

Source: Northern Meteorological Center, Mueang District, Chiang Mai Province

Table S3. Period-specific growth performance, organic carbon content, and biomass-based estimated CO₂ fixation rate.

Period	Start date	Condition	PBR	Specific growth rate (day ⁻¹)	Biomass productivity (g L ⁻¹ day ⁻¹)	Organic carbon (%)	Estimated CO ₂ fixation rate (g CO ₂ L ⁻¹ day ⁻¹)
1	31 Oct 2025	Controlled room	FPPBR	0.251	0.072	53.07	0.141
1	31 Oct 2025	Controlled room	TPBR	0.333	0.084	53.49	0.164
1	31 Oct 2025	Outdoor environmental	FPPBR	0.275	0.020	43.53	0.032
1	31 Oct 2025	Outdoor environmental	TPBR	0.080	0.010	42.55	0.016

2	12 Dec 2025	Controlled room	FPPBR	0.348	0.095	51.61	0.180
2	12 Dec 2025	Controlled room	TPBR	0.340	0.089	54.35	0.177
2	12 Dec 2025	Outdoor environmental	FPPBR	0.207	0.021	38.57	0.030
2	12 Dec 2025	Outdoor environmental	TPBR	0.207	0.021	37.01	0.029
3	15 Jan 2026	Controlled room	FPPBR	0.268	0.056	56.99	0.118
3	15 Jan 2026	Controlled room	TPBR	0.330	0.065	54.37	0.130
3	15 Jan 2026	Outdoor environmental	FPPBR	0.211	0.028	47.23	0.048
3	15 Jan 2026	Outdoor environmental	TPBR	0.188	0.026	42.98	0.041

Table S4. Replicate organic carbon measurements used to calculate treatment-period means.

Period	Condition	PBR	Replicate	Organic carbon (%)
1	Controlled room	FPPBR	1	52.91
1	Controlled room	FPPBR	2	53.23
1	Controlled room	TPBR	1	53.52
1	Controlled room	TPBR	2	53.46
1	Outdoor environmental	FPPBR	1	43.74
1	Outdoor environmental	FPPBR	2	43.32
1	Outdoor environmental	TPBR	1	42.62
1	Outdoor environmental	TPBR	2	42.47
2	Controlled room	FPPBR	1	51.99
2	Controlled room	FPPBR	2	51.23
2	Controlled room	TPBR	1	54.75
2	Controlled room	TPBR	2	53.96
2	Outdoor environmental	FPPBR	1	38.37
2	Outdoor environmental	FPPBR	2	38.77
2	Outdoor environmental	TPBR	1	37.53
2	Outdoor environmental	TPBR	2	36.48
3	Controlled room	FPPBR	1	56.76
3	Controlled room	FPPBR	2	57.23
3	Controlled room	TPBR	1	54.68
3	Controlled room	TPBR	2	54.06

3	Outdoor environmental	FPPBR	1	46.83
3	Outdoor environmental	FPPBR	2	47.64
3	Outdoor environmental	TPBR	1	41.92
3	Outdoor environmental	TPBR	2	44.04

Organic carbon measurements were performed in duplicate for each treatment-period sample. The two analytical replicates were averaged to obtain one treatment-period value, which was subsequently used in UNIANOVA.

Table S5. Observed mean $\pm$ SD for each cultivation condition $\times$ PBR type group across three temporal blocks (n = 3).

Condition	PBR	Specific growth rate (day ⁻¹)	Biomass productivity (g L ⁻¹ day ⁻¹)	Organic carbon (%)	Estimated CO ₂ fixation rate (g CO ₂ L ⁻¹ day ⁻¹)
Controlled room	FPPBR	0.289 $\pm$ 0.052	0.075 $\pm$ 0.019	53.89 $\pm$ 2.78	0.146 $\pm$ 0.031
Controlled room	TPBR	0.334 $\pm$ 0.005	0.079 $\pm$ 0.013	54.07 $\pm$ 0.50	0.157 $\pm$ 0.024
Outdoor environmental	FPPBR	0.231 $\pm$ 0.038	0.023 $\pm$ 0.004	43.11 $\pm$ 4.35	0.037 $\pm$ 0.010
Outdoor environmental	TPBR	0.158 $\pm$ 0.069	0.019 $\pm$ 0.008	40.84 $\pm$ 3.33	0.029 $\pm$ 0.013

Table S6. Factorial UNIANOVA results for cultivation condition, PBR type, temporal block, and Condition $\times$ PBR type interaction.

Response	Effect	df1	df2	F	p	η^2	Interpretation
Specific growth rate	Cultivation condition	1	6	17.192	0.006	0.741	Significant
Specific growth rate	PBR type	1	6	0.229	0.649	0.037	Not significant
Specific growth rate	Condition $\times$ PBR type	1	6	4.386	0.081	0.422	Not significant
Specific growth rate	Period	2	6	0.727	0.522	0.195	Not significant
Biomass productivity	Cultivation condition	1	6	63.844	<0.001	0.914	Significant
Biomass productivity	PBR type	1	6	0.004	0.954	0.001	Not significant
Biomass productivity	Condition $\times$ PBR type	1	6	0.356	0.573	0.056	Not significant
Biomass productivity	Period	2	6	1.238	0.355	0.292	Not significant
Organic carbon	Cultivation condition	1	6	101.225	<0.001	0.944	Significant
Organic carbon	PBR type	1	6	0.766	0.415	0.113	Not significant
Organic carbon	Condition $\times$ PBR type	1	6	1.053	0.344	0.149	Not significant

Organic carbon	Period		2	6	5.900	0.038	0.663	Significant
Estimated CO ₂ fixation rate	Cultivation condition		1	6	90.445	<0.001	0.938	Significant
Estimated CO ₂ fixation rate	PBR type		1	6	0.013	0.913	0.002	Not significant
Estimated CO ₂ fixation rate	Condition × PBR type	×	1	6	0.559	0.483	0.085	Not significant
Estimated CO ₂ fixation rate	Period		2	6	0.933	0.444	0.237	Not significant

Table S7. Bonferroni-adjusted estimated marginal mean comparisons between FPPBR and TPBR within each cultivation condition.

Response	Condition	Comparison	Mean difference	p	95% CI lower	95% CI upper	Interpretation
Specific growth rate	Controlled room	FPPBR – TPBR	-0.046	0.297	-0.143	0.052	Not significant
Specific growth rate	Outdoor environmental	FPPBR – TPBR	0.073	0.119	-0.025	0.170	Not significant
Biomass productivity	Controlled room	FPPBR – TPBR	-0.005	0.659	-0.029	0.020	Not significant
Biomass productivity	Outdoor environmental	FPPBR – TPBR	0.004	0.717	-0.020	0.028	Not significant
Organic carbon	Controlled room	FPPBR – TPBR	-0.180	0.919	-4.309	3.949	Not significant
Organic carbon	Outdoor environmental	FPPBR – TPBR	2.268	0.227	-1.860	6.397	Not significant
Estimated CO ₂ fixation rate	Controlled room	FPPBR – TPBR	-0.011	0.565	-0.054	0.033	Not significant
Estimated CO ₂ fixation rate	Outdoor environmental	FPPBR – TPBR	0.008	0.670	-0.035	0.051	Not significant

Table S8. Bonferroni-adjusted estimated marginal mean comparisons between controlled-room and outdoor conditions within each PBR type.

Response	PBR	Comparison	Mean difference	p	95% CI lower	95% CI upper	Interpretation
Specific growth rate	FPPBR	Controlled room – Outdoor	0.058	0.197	-0.040	0.156	Not significant

Specific growth rate	TPBR	Controlled room – Outdoor	0.176	0.005	0.079	0.274	Significant
Biomass productivity	FPPBR	Controlled room – Outdoor	0.052	0.002	0.027	0.076	Significant
Biomass productivity	TPBR	Controlled room – Outdoor	0.060	0.001	0.036	0.084	Significant
Organic carbon	FPPBR	Controlled room – Outdoor	10.780	0.001	6.651	14.909	Significant
Organic carbon	TPBR	Controlled room – Outdoor	13.228	<0.001	9.100	17.357	Significant
Estimated CO2 fixation rate	FPPBR	Controlled room – Outdoor	0.110	0.001	0.066	0.153	Significant
Estimated CO2 fixation rate	TPBR	Controlled room – Outdoor	0.128	<0.001	0.085	0.172	Significant

Table S9. Tukey HSD comparisons among cultivation periods.

Response	Period comparison	Mean difference	p	95% CI lower	95% CI upper	Interpretation
Specific growth rate	Period 1 – Period 2	-0.0411	0.502	-0.1472	0.0650	Not significant
Specific growth rate	Period 1 – Period 3	-0.0144	0.910	-0.1205	0.0917	Not significant
Specific growth rate	Period 2 – Period 3	0.0267	0.733	-0.0794	0.1328	Not significant
Biomass productivity	Period 1 – Period 2	-0.0100	0.512	-0.0363	0.0163	Not significant
Biomass productivity	Period 1 – Period 3	0.0028	0.943	-0.0234	0.0291	Not significant
Biomass productivity	Period 2 – Period 3	0.0128	0.357	-0.0134	0.0391	Not significant
Organic carbon	Period 1 – Period 2	2.7738	0.219	-1.7099	7.2574	Not significant
Organic carbon	Period 1 – Period 3	-2.2363	0.343	-6.7199	2.2474	Not significant
Organic carbon	Period 2 – Period 3	-5.0100	0.032	-9.4936	-0.5264	Significant
Estimated CO2 fixation rate	Period 1 – Period 2	-0.0157	0.591	-0.0627	0.0313	Not significant
Estimated CO2 fixation rate	Period 1 – Period 3	0.0042	0.960	-0.0428	0.0512	Not significant

Estimated CO2 fixation rate	Period 2 – Period 3	0.0199	0.447	-0.0272	0.0669	Not significant
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Table S10. Shapiro-Wilk tests of residual normality for the four UNIANOVA models.

Response	Shapiro-Wilk W	df	p	Interpretation
Specific growth rate	0.965	12	0.857	No evidence of non-normal residuals
Biomass productivity	0.935	12	0.439	No evidence of non-normal residuals
Organic carbon	0.969	12	0.896	No evidence of non-normal residuals
Estimated CO2 fixation rate	0.907	12	0.196	No evidence of non-normal residuals

All Shapiro-Wilk tests were non-significant ($p > 0.05$), consistent with the residual Q-Q plots shown in Supplementary Figures S1-S4.

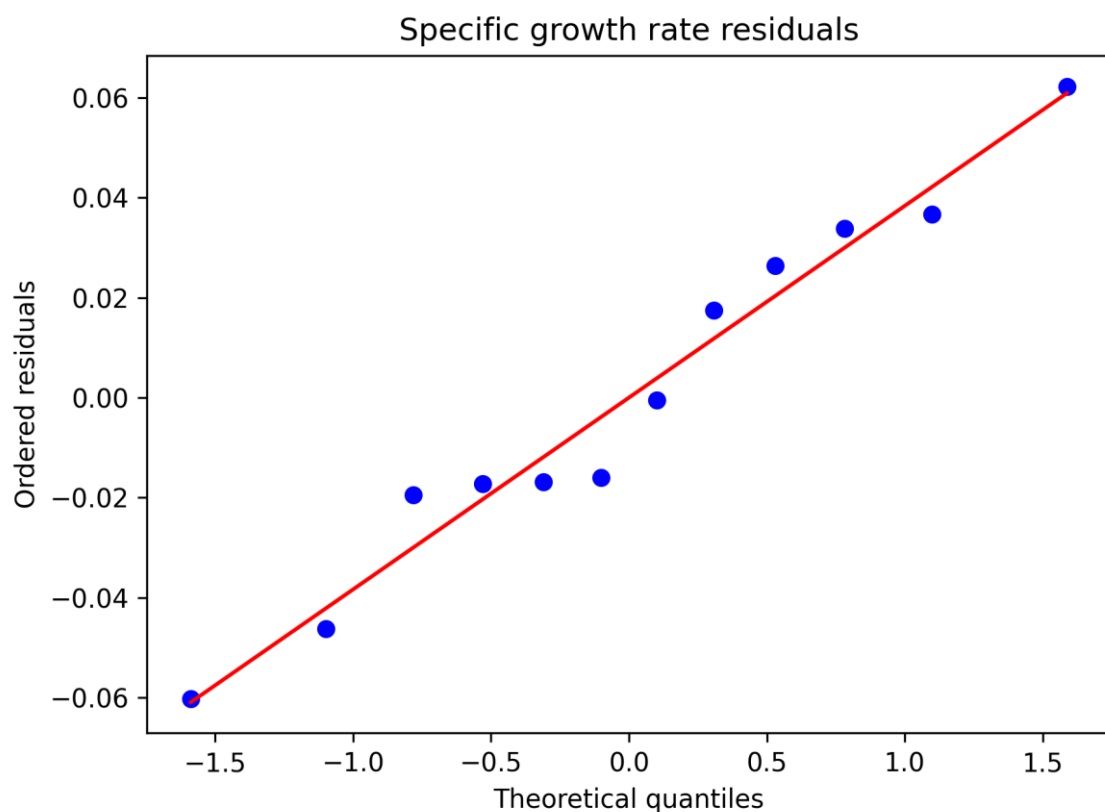


Figure S1. Normal Q-Q plot of residuals for specific growth rate.

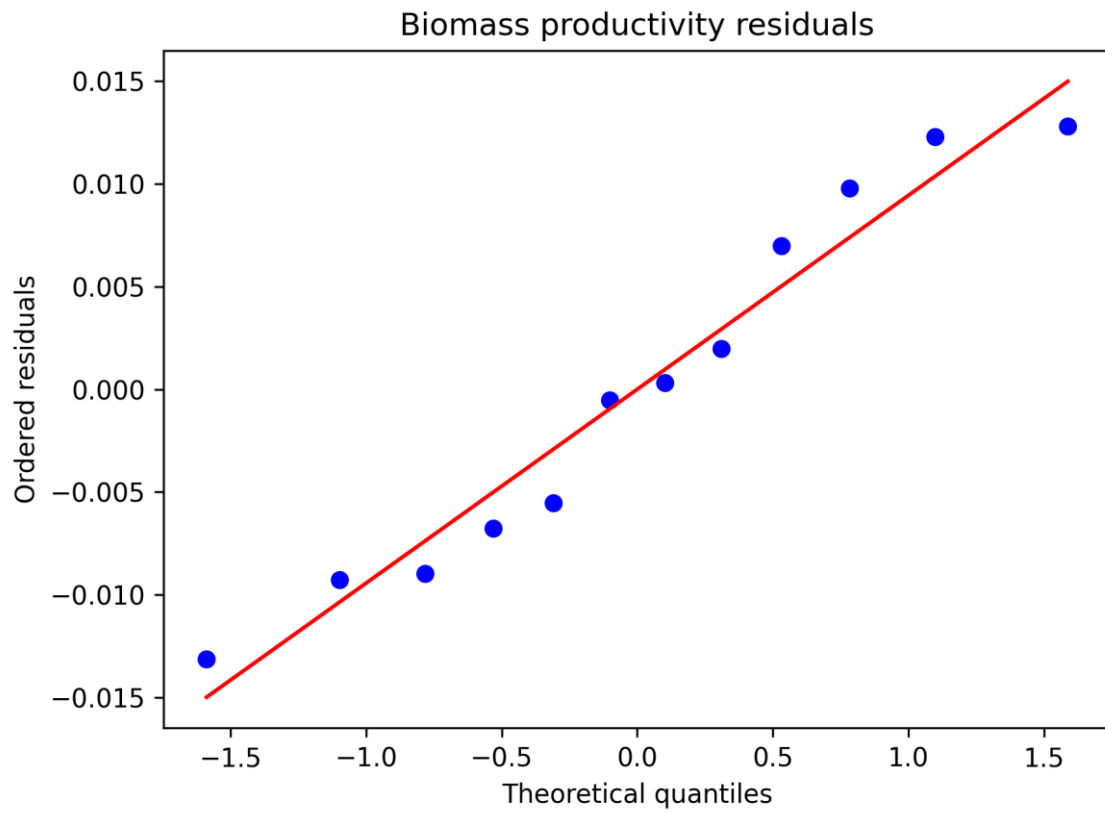


Figure S2. Normal Q-Q plot of residuals for biomass productivity.

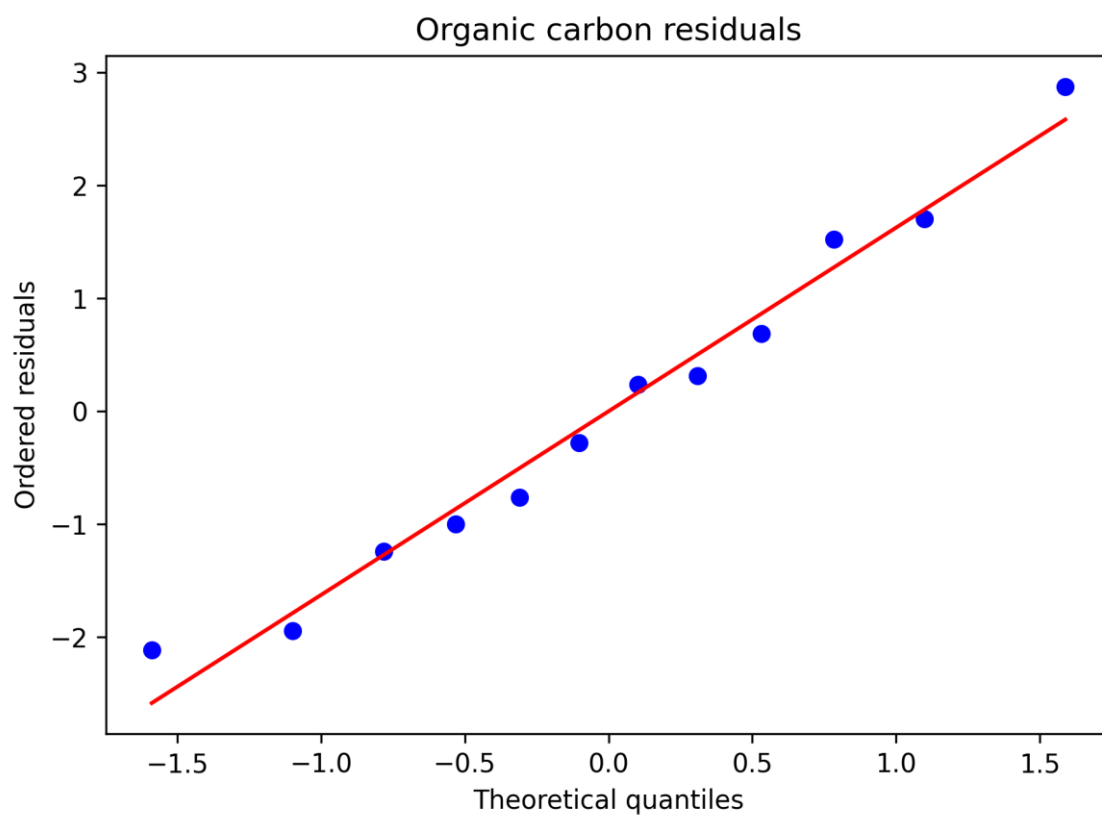


Figure S3. Normal Q-Q plot of residuals for organic carbon content.

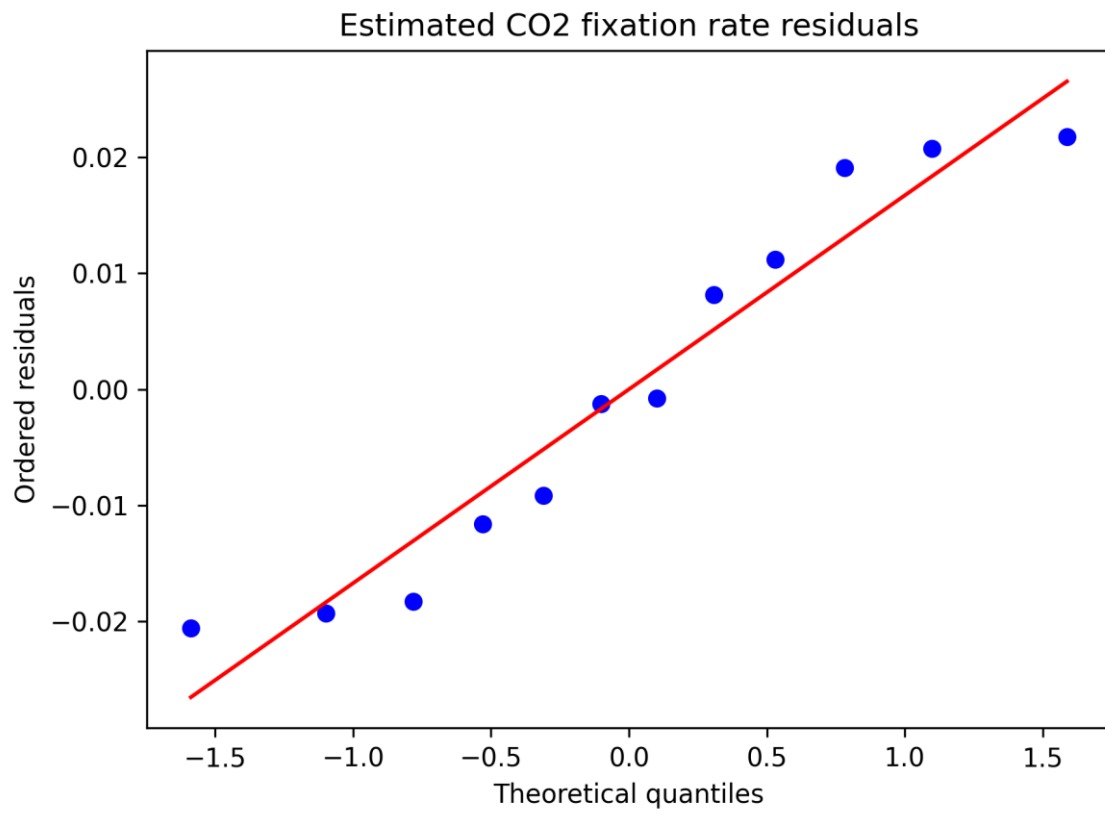


Figure S4. Normal Q-Q plot of residuals for biomass-based estimated CO2 fixation rate.