

Electronic Supplementary Information
Enhancement of protein production by microalgae *Dunaliella salina* under mixotrophic condition using response surface methodology

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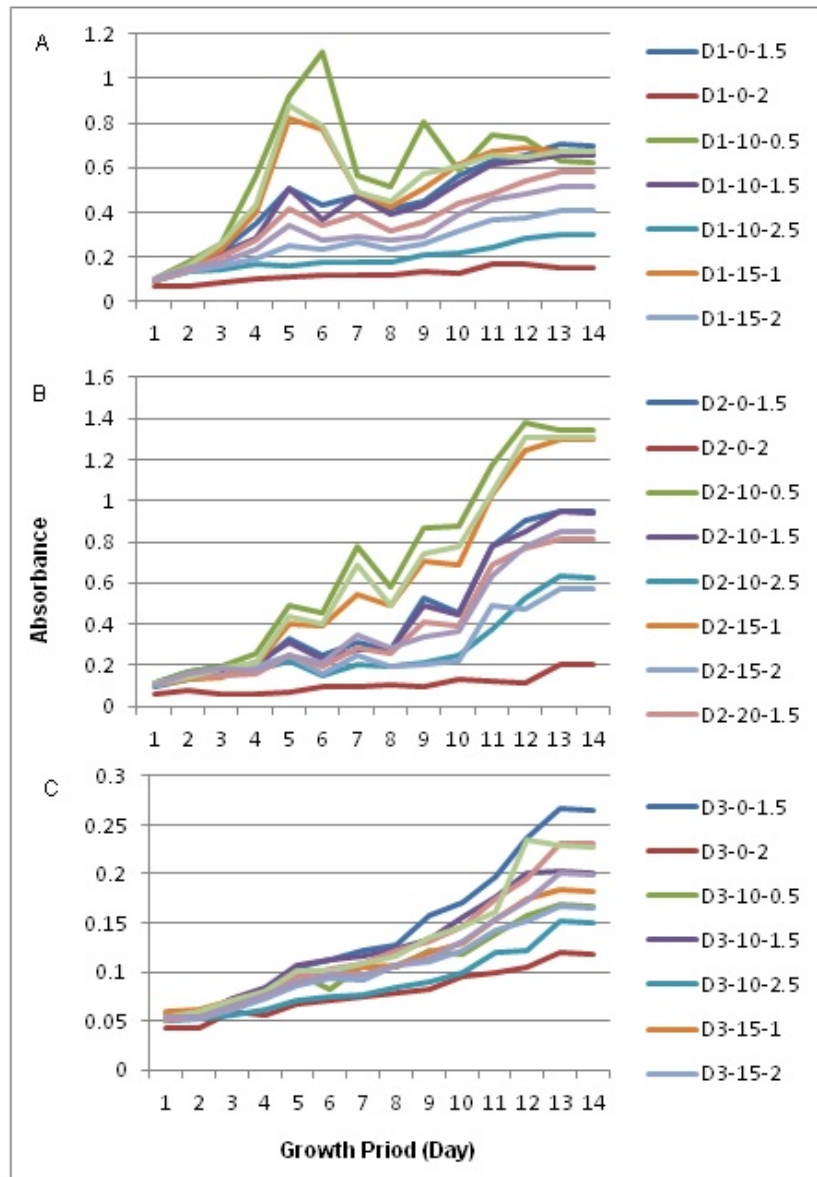
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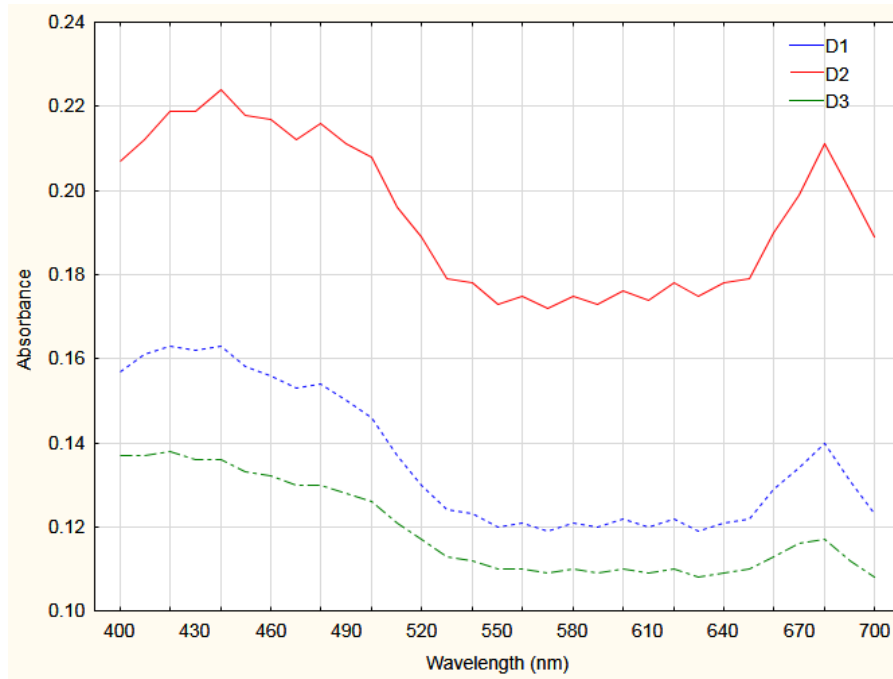
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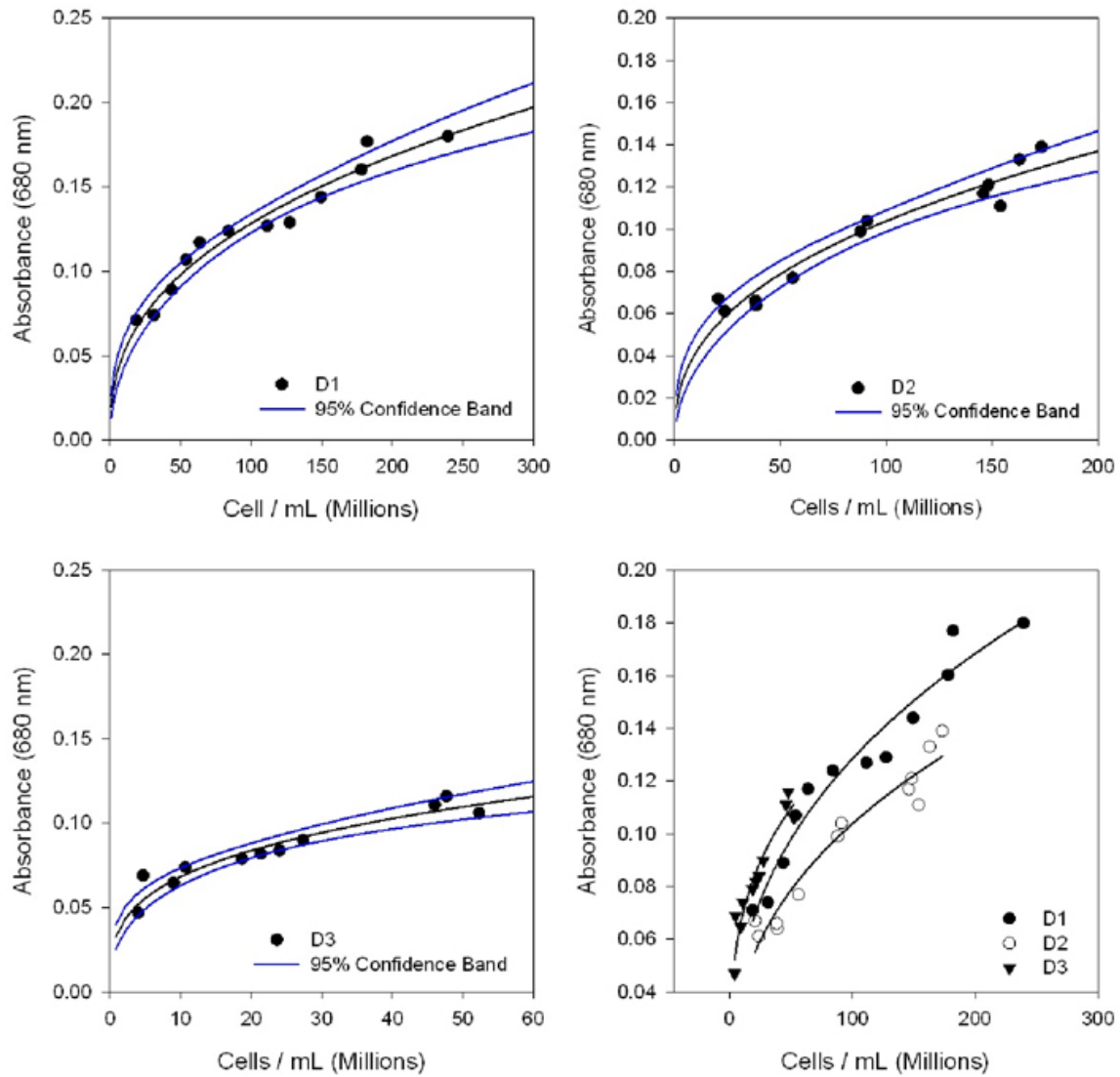
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Supplementary Figure 1. Growth curves of 3 strains in 9 combinational treatments (run) and 1 control based on the absorbance (cellular chlorophyll content). A=D1, B=D2, C=D3.



Supplementary Figure 2. Absorbance spectral curve (400-700 nm) of *D.salina* strains.



Supplementary Figure 3. Relationship between absorbance (680nm) and cell density for *D.salina* strains (D1, D2 and D3). The black lines represent the adjusted absorbance equations. The 5% confidence level has been shown with blue lines. The related correlation fit equations has been provided in Supplementary Table 2.

Supplementary Table 1. Statistical parameters of the model developed for biomass through response surface methodology in *D.salina* strains.

Statistical Parameters	Strain		
	D1	D2	D3
R-Squared	0.9885	0.8608	0.8577
Adj R-Squared	0.9802	0.7614	0.7560
Pred R-Squared	0.9578	0.3421	0.4235
Adeq Precision	37.319	11.012	10.886
Std. Dev.	0.018	0.13	0.014
Mean	0.58	0.92	0.19
C.V. %	3.10	13.69	7.35
PRESS	8.135E-003	0.82	5.538E-003

Supplementary Table 2. Correlation curve fit equations for three *Dunaliella* strains based on optical density versus cell density, expressed using a general power equation.

Strain	Equation	R ²	Adj R ²	StE
D1	$y = 0.0213 x^{0.39}$ $X = e^{\{[\ln(A_{680}) + 3.849]/0.39\}}$	0.9531	0.9485	0.0083
D2	$y = 0.0165 x^{0.3996}$ $X = e^{\{[\ln(A_{680}) + 4.104]/0.3996\}}$	0.9361	0.9298	0.0075
D3	$y = 0.0347 x^{0.2944}$ $X = e^{\{[\ln(A_{680}) + 3.361]/0.3996\}}$	0.9234	0.9157	0.0066

Y: absorbance at 680 nm (A_{680}), X: cell density (cell/mL)

Adj R²: Adjusted R², StE: Standard Error of Estimate