Appendix A. Supplementary Data

Optimization and Comparison of Three Cell Disruption Processes on Lipid Extraction from Microalgae

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The supplementary data presented here show the experimental results obtained for the central composite face centered (CCF) design for ultrasound and microwave processes, carried out for the microalgae *N. gaditana*, *N. oceanica* and *T. suecica*. The response for all experimental models is the Total Lipid Extracted (TLE) of the microalgae suspensions.

Tables S1, S4, S7, S9, S12 and S15 show the design space studied with the coded variables and their value for each process and microalgae. The experimental and the predicted values of TLE for each run are also shown in these tables.

Figures S1, S2, S3, S4, S5 and S6 exhibit centered and scaled coefficients of the regression for the response TLE. A positive coefficient means a positive influence on the TLE, while a negative value means a negative effect on the TLE. The non-significant coefficients at a confidence interval of 95% were removed to obtain reduced models. The significant reduced coefficients for each model (for *N. gaditana* and *N. oceanica*) are shown in Tables S2, S5, S10 and S13 with its corresponding standard error and its confidence interval. A p-value <0.5 means a significant coefficient.

Analyses of variance (ANOVA) for the models are shown in Tables S3, S6, S8, S11, S14 and S16. The p-value of the regression model inferior to 0.05 shows the statistical significance of the polynomial regression. The lack of fit ($p>0.05$) indicates the low replicate errors of the models. The good model accuracy is obtained when the coefficients of determination (R^2, Q^2, model validity and reproducibility) for the reduced models are close to 1. The condition number indicates the good orthogonality of the models if they do not exceed a value of 10. These statistical parameters indicate that the models obtained are validated and can predict the effect of the operating conditions of microwave (Temperature and time) and ultrasound (Amplitude and time) processes on the TLE from the microalgae studied.

Optimization of the microwave process by RSM

Microalgae: ***Nannochloropsis gaditana***

Table S 1 . Overview of performed experimental tests and predicted values of Total Lipid Extracted (TLE) from *N. gaditana* after microwave process.

Run order	Coded variables		Variables		Total Lipid Extracted (% DW)	
	x_1	x_2	Temperature (ºC)	Time (min)	Experimental value	Predicted value
2	-1	-1	50	5	14.61	14.46
9	-1	0	50	15	14.88	14.46
7	-1	+1	50	25	13.88	14.46
6	0	-1	75	5	13.12	14.01
1	0	0	75	15	14.88	14.01
4	0	0	75	15	13.54	14.01
3	0	0	75	15	14.21	14.01
11	0	+1	75	25	14.31	14.01
5	+1	-1	100	5	17.71	17.66
10	+1	0	100	15	17.83	17.66
8	+1	+1	100	25	17.44	17.66

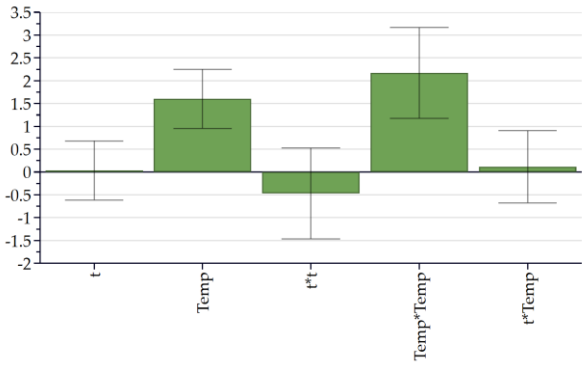

Figure S1. Coefficients of the regression model for Total Lipid Extracted (TLE) from *N. gaditana* after microwave process.

Table S 2 . Coefficient analysis of the regression model for Total Lipid Extracted (TLE) from *N. gaditana* after microwave process.

Model source	Coefficients	Standard Error	P	Confidence interval
Constant	14.012	0.250746	1.16706e-11	0.578221
Temp	1.60167	0.228899	0.000112947	0.527841
Temp*Temp	2.04633	0.339512	0.000313726	0.782915

Table S 3 . ANOVA of the fitted model of Total Lipid Extracted (TLE) from *N. gaditana* after microwave process.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value	p-value	Standard Deviation
Total	11	2546.81	231.528			
Constant	1	2517.48	2517.48			
Total corrected	10	29.3274	2.93274			1.71252
Regression	2	26.8124	13.4062	42.6449	**0.000**	3.66145
Residual (RSD)	8	2.51495	0.314368			0.560686
Lack of Fit (Model error)	6	1.61715	0.269525	0.600411	**0.734**	0.519158
Pure error (Replicate error)	2	0.8978	0.4489			0.67
Summary statistics						
R^2			0.914			
R^2 adjust			0.893			
Q^2			0.852			
Model validity			0.9226			
Reproducibility			0.8469			
Condition number			2.739			
Confidence			0.95			

Microalgae: ***Nannochloropsis oceanica***

Table S 4 . Overview of performed experimental tests and predicted values of Total Lipid Extracted (TLE) from *N. oceanica* after microwave process.

Run order	Coded variables		Variables		Total Lipid Extracted (% DW)	
	x_1	x_2	Temperature (ºC)	Time (min)	Experimental value	Predicted value
2	-1	-1	50	5	43.52	43.39
9	-1	0	50	15	41.34	41.79
7	-1	+1	50	25	42.49	42.17
6	0	-1	75	5	43.33	NR
1	0	0	75	15	46.04	46.41
4	0	0	75	15	46.64	46.35
3	0	0	75	15	46.91	46.41
11	0	+1	75	25	47.23	47.59
5	+1	-1	100	5	46.22	46.35
10	+1	0	100	15	46.44	46.41
8	+1	+1	100	25	48.38	48.33

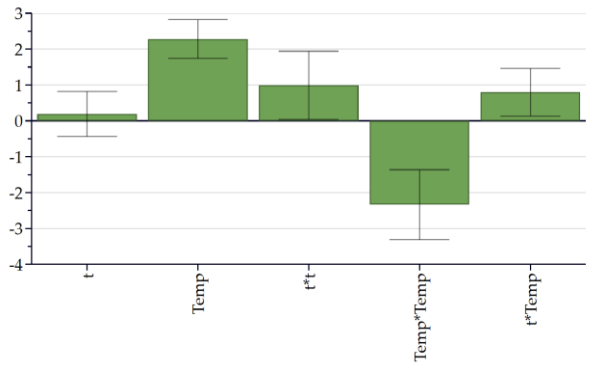

Figure S2. Coefficients of the regression model for Total Lipid Extracted (TLE) from *N. oceanica* after microwave process.

Table S 5 . Coefficient analysis of the regression model for Total Lipid Extracted (TLE) from *N. oceanica* after microwave process.

Model source	Coefficients	Standard Error	P	Confidence interval
Constant	46.41	0.256457	5.56757e-09	0.711208
Temp	2.28	0.195643	0.000309033	0.543194
Temp*Temp	-2.34	0.350757	0.00263114	0.973862
t	0.19	0.226413	0.444272	0.628625
t*t	0.99	0.341875	0.0441881	0.949203
Temp*t	0.80	0.239613	0.0291525	0.665274

Table S 6 . ANOVA of the fitted model of Total Lipid Extracted (TLE) from *N. oceanica* after microwave process.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value	p-value	Standard Deviation
Total	10	20768.2	2076.82			
Constant	1	20721.6	20721.6			
Total corrected	9	46.5503	5.17225			2.27426
Regression	5	45.6317	9.12633	39.739	**0.002**	3.02098
Residual (RSD)	4	0.918626	0.229657			0.479225
Lack of Fit (Model error)	2	0.522028	0.261014	1.31626	**0.432**	0.510895
Pure error (Replicate error)	2	0.396599	0.198299			0.445308
Summary statistics						
R^2			0.980			
R^2 adjust			0.956			
Q^2			0.771			
Model validity			0.790			
Reproducibility			0.962			
Condition number			3.760			
Confidence			0.95			

Microalgae: ***Tetraselmis suecica***

Table S 7 . Overview of performed experimental tests of Total Lipid Extracted (TLE) from *T. suecica a*fter microwave process.

Run order	Coded variables		Variables		Total Lipid Extracted (% DW)
	x_1	x_2	Temperature (ºC)	Time (min)	Experimental value
1	-1	-1	50	5	3.52
3	-1	+1	50	25	2.12
2	0	0	75	15	1.88
4	0	0	75	15	1.83
7	0	0	75	15	1.30
5	+1	-1	100	5	2.93
6	+1	+1	100	25	3.46

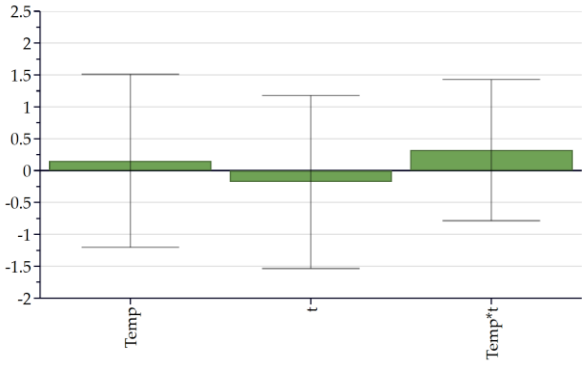

Figure S3. Coefficients of the regression model for Total Lipid Extracted (TLE) from *T. suecica* after microwave process.

Table S 8 . ANOVA of the experimental results of Total Lipid Extracted (TLE) from *T. suecica* after microwave process.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value	p-value	Standard Deviation
Total	7	46.0146	6.57351			
Constant	1	41.4802	41.4802			
Total corrected	6	4.53437	0.755729			0.869327
Regression	3	1.26108	0.420359	0.385262	**0.773**	0.648351
Residual	3	3.2733	1.0911			1.04456
Lack of Fit (Model error)	1	3.0667	3.0667	29.6873	**0.032**	1.7512
Pure error (Replicate error)	2	0.2066	0.1033			0.321403
Summary statistics						
R^2			0.278			
R^2 adjust			-0.444			
Q^2			-0.200			
Model validity			0.138832			
Reproducibility			0.863311			
Condition number			1.2225			
Confidence			0.95			

Optimization of the ultrasound process by RSM

Microalgae: ***Nannochloropsis gaditana***

Table S 9 . Overview of performed experimental tests and predicted values of Total Lipid Extracted (TLE) from *N. gaditana* after ultrasound process.

Run order	Coded variables		Variables		Total Lipid Extracted (% DW)	
	x_1	x_2	Amplitude (%)	Time (min)	Experimental value	Predicted value
3	-1	-1	50	10	16.21	15.8938
6	-1	0	50	20	17.84	17.4338
1	-1	+1	50	30	18.71	18.9738
11	0	-1	65	10	16.18	17.1055
2	0	0	65	20	19.41	18.6455
4	0	0	65	20	18.32	18.6455
7	0	0	65	20	18.98	18.6455
9	0	+1	65	30	19.53	20.1855
8	+1	-1	80	10	18.27	18.3171
5	+1	0	80	20	20.10	19.8571
10	+1	+1	80	30	21.66	21.3971

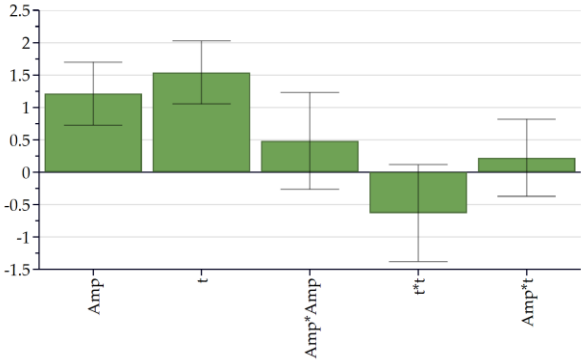

Figure S4. Coefficients of the regression model for Total Lipid Extracted (TLE) from *N. gaditana* after ultrasound process.

Table S 10 . Coefficient analysis of the regression model for Total Lipid Extracted (TLE) from *N. gaditana* after ultrasound process.

Model source	Coefficients	Standard Error	P	Confidence interval
Constant	18.6555	0.170302	5.38885e-14	0.392717
Amp	1.21167	0.230591	0.000769504	0.531742
t	1.54	0.230591	0.000156132	0.531742

Table S 11 . ANOVA of the fitted model of Total Lipid Extracted (TLE) from *N. gaditana* after ultrasound process.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value	p-value	Standard Deviation
Total	11	3853.88	350.352			
Constant	1	3828.29	3828.29			
Total corrected	10	25.9707	2.55907			1.59971
Regression	2	23.0384	11.5192	36.1067	**0.000**	3.394
Residual	8	2.55226	0.319032			0.564829
Lack of Fit (Model error)	6	1.94939	0.324898	1.07784	**0.554**	0.569999
Pure error (Replicate error)	2	0.602867	0.301433			0.54903
Summary statistics						
R^2			0.900			
R^2 adjust			0.875			
Q^2			0.827			
Model validity			0.852332			
Reproducibility			0.88221			
Condition number			1.354			
Confidence			0.95			

Microalgae: ***Nannochloropsis oceanica***

Table S 12 . Overview of performed experimental tests and predicted values of Total Lipid Extracted (TLE) from *N. oceanica* after ultrasound process.

Run order	Coded variables		Variables		Total Lipid Extracted (% DW)	
	x_1	x_2	Amplitude (%)	Time (min)	Experimental value	Predicted value
3	-1	-1	50	10	40.35	40.0748
6	-1	0	50	20	42.01	41.2565
1	-1	+1	50	30	41.83	42.4382
11	0	-1	65	10	40.48	41.3565
2	0	0	65	20	43.19	42.5382
4	0	0	65	20	41.92	42.5382
7	0	0	65	20	42.68	42.5382
9	0	+1	65	30	43.58	43.7198
8	+1	-1	80	10	42.91	42.6382
5	+1	0	80	20	43.55	43.8198
10	+1	+1	80	30	45.42	45.015

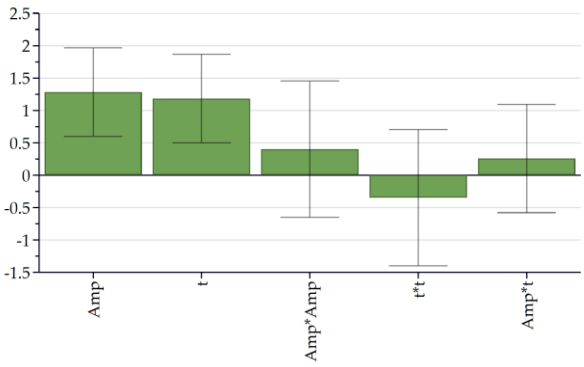

Figure S5. Coefficients of the regression model for Total Lipid Extracted (TLE) from *N. oceanica* after ultrasound process.

Table S 13 . Coefficient analysis of the regression model for Total Lipid Extracted (TLE) from *N. oceanica* after ultrasound process.

Model source	Coefficients	Standard Error	P	Confidence interval
Constant	42.5382	0.183096	1.31883e-16	0.42222
Amp	1.28167	0.247913	0.000853332	0.571688
t	1.18167	0.247913	0.00141502	0.571688

Table S 14 . ANOVA of the fitted model of Total Lipid Extracted (TLE) from *N. oceanica* after ultrasound process.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value	p-value	Standard Deviation
Total	11	19925.7	1811.42			
Constant	1	19904.5	19904.5			
Total corrected	10	21.1842	2.11842			1.45548
Regression	2	18.234	9.11702	24.723	**0.000**	3.01944
Residual	8	2.95013	0.368766			0.607261
Lack of Fit (Model error)	6	21.13326	0.355544	0.870505	**0.622**	0.596275
Pure error (Replicate error)	2	0.816868	0.408434			0.639088
Summary statistics						
R^2			0.861			
R^2 adjust			0.826			
Q^2			0.731514			
Model validity			0.881085			
Reproducibility			0.807199			
Condition number			1.354			
Confidence			0.95			

Microalgae: ***Tetraselmis suecica***

Table S 15 . Overview of performed experimental tests of Total Lipid Extracted (TLE) from *T. suecica* after ultrasound process.

Run order	Coded variables		Variables		Total Lipid Extracted (% DW)
	x_1	x_2	Amplitude (%)	Time (min)	Experimental value
1	-1	-1	50	10	10.93
3	-1	+1	50	30	11.61
4	0	0	65	20	11.39
7	0	0	65	20	11.86
5	0	0	65	20	11.49
6	+1	-1	80	10	12.02
2	+1	+1	80	30	11.63

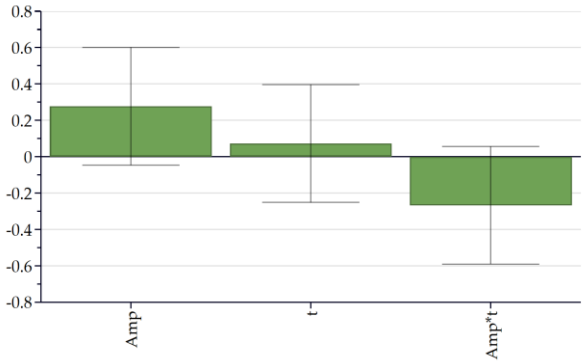

Figure S6. Coefficients of the regression model for Total Lipid Extracted (TLE) from *T. suecica* after ultrasound process.

Table S 16 . ANOVA of the experimental results of Total Lipid Extracted (TLE) from *T. suecica* after ultrasound process.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value	p-value	Standard Deviation
Total	7	936.406	133.772			
Constant	1	935.666	935.666			
Total corrected	6	0.739685	0.123281			0.351114
Regression	3	0.615275	0.205092	4.94552	**0.111**	0.45287
Residual	3	0.12441	0.0414702			0.203642
Lack of Fit (Model error)	1	0.00181076	0.00181076	0.0295394	**0.879**	0.042553
Pure error (Replicate error)	2	0.1226	0.0612999			0.247588
Summary statistics						
R^2			0.832			
R^2 adjust			0.664			
Q^2			0.681			
Model validity			0.967813			
Reproducibility			0.502763			
Condition number			1.323			
Confidence			0.95			