

Supplementary Materials

Table S1. FCC design matrix for Isop/Hex and observed response.

Run	Factor X _A polar/non polar ratio	Factor X _B Time (Vortex) (h)	Factor X _C dry biomass/solvent ratio (mg/mL)	Response Y ₁ Lipids/Dry Biomass (L/DB) (g/g)
1	1	0.5	10	0.06
2	1	0.5	70	0.06
3	0	0.5	70	0.03
4	1	3	10	0.1
5	0.5	3	40	0.05
6	1	0.5	10	0.06
7	0	3	10	0.07
8	0	3	10	0.08
9	0.5	0.5	40	0.05
10	0.5	1.75	70	0.04
11	1	1.75	40	0.05
12	0	1.75	40	0.04
13	1	0.5	10	0.06
14	0.5	1.75	10	0.06
15	1	3	70	0.05
16	0	1.75	40	0.04
17	0.5	1.75	10	0.07
18	1	0.5	70	0.06
19	0	0.5	70	0.03
20	0.5	0.5	40	0.05
21	0.5	1.75	40	0.07
22	0.5	3	40	0.05
23	0	0.5	10	0.04
24	0.5	1.75	40	0.05
25	0	0.5	70	0.02
26	1	1.75	40	0.05
27	1	3	70	0.06
28	1	1.75	40	0.06
29	0	1.75	40	0.05
30	0.5	1.75	40	0.05
31	0.5	3	40	0.04
32	0	0.5	10	0.04
33	0	3	70	0.03
34	0	3	70	0.03
35	1	3	70	0.06
36	1	3	10	0.1
37	1	0.5	70	0.04
38	0	3	70	0.02
39	0	3	10	0.07
40	0.5	1.75	70	0.04
41	1	3	10	0.1
42	0.5	0.5	40	0.04
43	0	0.5	10	0.03

44	0.5	1.75	10	0.07
45	0.5	1.75	70	0.04

Table S2. FCC design matrix for MeOH/AcOEt and observed response.

Run	Factor X _A polar/non polar ratio	Factor X _B Time (Vortex) (h)	Factor X _C dry biomass/solvent ratio (mg/mL)	Response Y ₁ L/DB (g/g)
1	0.5	1.75	70	0.07
2	1	0.5	70	0.05
3	0.5	3	40	0.09
4	1	3	70	0.06
5	0.5	1.75	70	0.08
6	1	0.5	70	0.05
7	0	0.5	70	0.05
8	0	0.5	10	0.05
9	0.5	1.75	40	0.09
10	0	3	10	0.06
11	0	1.75	40	0.07
12	0.5	1.75	10	0.11
13	1	1.75	40	0.07
14	1	3	70	0.05
15	1	3	10	0.09
16	0.5	0.5	40	0.07
17	0	0.5	70	0.04
18	1	0.5	70	0.05
19	0	0.5	70	0.05
20	1	3	10	0.09
21	0	3	10	0.07
22	0.5	0.5	40	0.07
23	1	1.75	40	0.08
24	1	3	10	0.09
25	0.5	3	40	0.09
26	1	0.5	10	0.08
27	0.5	1.75	70	0.08
28	0.5	3	40	0.07
29	0	3	70	0.04
30	0.5	1.75	10	0.11
31	0	1.75	40	0.07
32	0	3	70	0.05
33	0	3	70	0.05
34	1	0.5	10	0.08
35	0.5	1.75	40	0.1
36	0	0.5	10	0.06
37	0	3	10	0.06
38	0.5	0.5	40	0.07
39	0	0.5	10	0.06
40	1	1.75	40	0.06
41	0.5	1.75	10	0.11

42	1	3	70	0.05
43	0.5	1.75	40	0.09
44	0	1.75	40	0.06
45	1	0.5	10	0.09

Table S3. ANOVA results for the applied FCC experimental design for Isop/Hex.

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value
Model	0.0134	6	0.0022	34.68	< 0.0001
X _A -Ratio polar/non polar	0.0041	1	0.0041	63.48	< 0.0001
X _B -Time (Vortex)	0.0019	1	0.0019	29.85	< 0.0001
X _C -Ratio solid/liquid	0.0053	1	0.0053	82.91	< 0.0001
X _A X _B	0.0000	1	0.0000	0.2591	0.6137
X _A X _C	0.0000	1	0.0000	0.2591	0.6137
X _A X _C	0.0020	1	0.0020	31.35	< 0.0001
Residual	0.0024	38	0.0001		
Lack of Fit	0.0012	8	0.0002	3.89	0.0030
Pure Error	0.0012	30	0.0000		
Cor Total	0.0158	44			

SD = 0.0080, R² = 0.8456, R²_{adj} = 0.8212, Adequate Precision = 22.1288.

$$L/DB = + 0.032522 + (0.018778 \cdot X_A) + (0.015511 \cdot X_B) - (0.000044 \cdot X_C) + (0.001333 \cdot X_A \cdot X_B) + (0.000056 \cdot X_A \cdot X_C) - (0.000244 \cdot X_B \cdot X_C)$$

Table S4. ANOVA results for the applied FCC experimental design for MeOH/AcOEt.

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value
Model	0.0144	9	0.0016	37.04	< 0.0001
X _A -Ratio polar/non polar	0.0013	1	0.0013	30.93	< 0.0001
X _B -Time (Vortex)	0.0003	1	0.0003	6.26	0.0172
X _C -Ratio solid/liquid	0.0051	1	0.0051	117.60	< 0.0001
X _A X _B	4.167E-06	1	4.167E-06	0.0966	0.7577
X _A X _C	0.0007	1	0.0007	16.33	0.0003
X _B X _C	0.0000	1	0.0000	0.8698	0.3574
X _A ²	0.0033	1	0.0033	76.97	< 0.0001
X _B ²	0.0012	1	0.0012	27.54	< 0.0001
X _C ²	0.0001	1	0.0001	3.25	0.0802
Residual	0.0015	35	0.0000		
Lack of Fit	0.0004	5	0.0001	2.49	0.0533
Pure Error	0.0011	30	0.0000		
Cor Total	0.0159	44			

SD = 0.0066, R² = 0.9050, R²_{adj} = 0.8805, Adequate Precision = 19.9437.

$$L/DB = + 0.049933 + (0.109574 \cdot X_A) + (0.031193 \cdot X_B) - (0.000573 \cdot X_C) + (0.000667 \cdot X_A \cdot X_B) - (0.000361 \cdot X_A \cdot X_C) - (0.000033 \cdot X_B \cdot X_C) - (0.082963 \cdot X_A^2) - (0.007941 \cdot X_B^2) + (4.73251 \cdot 10^{-6} \cdot X_C^2)$$

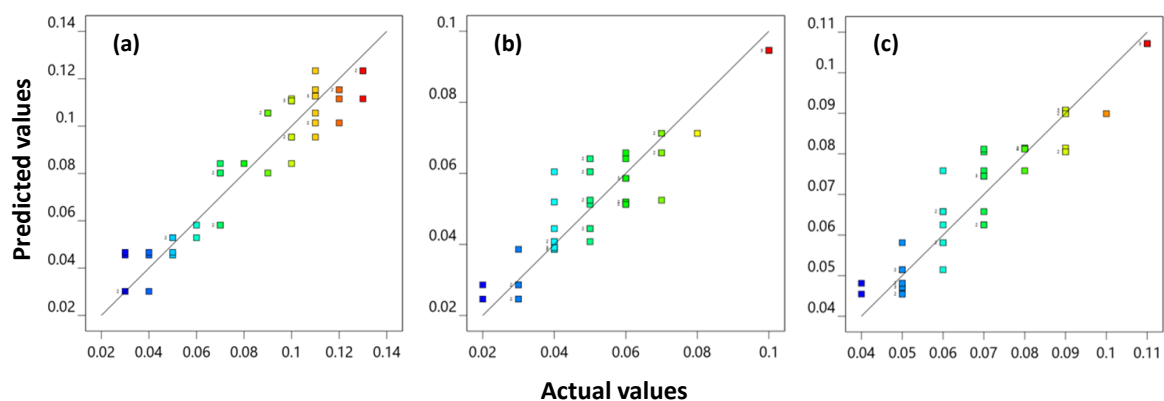


Figure S1. Plot of predicted vs. actual values of lipid yield (L/DB, g/g) response for (a) MeOH/Hex; (b) Isop.Hex; and (c) MeOH/AcOEt.

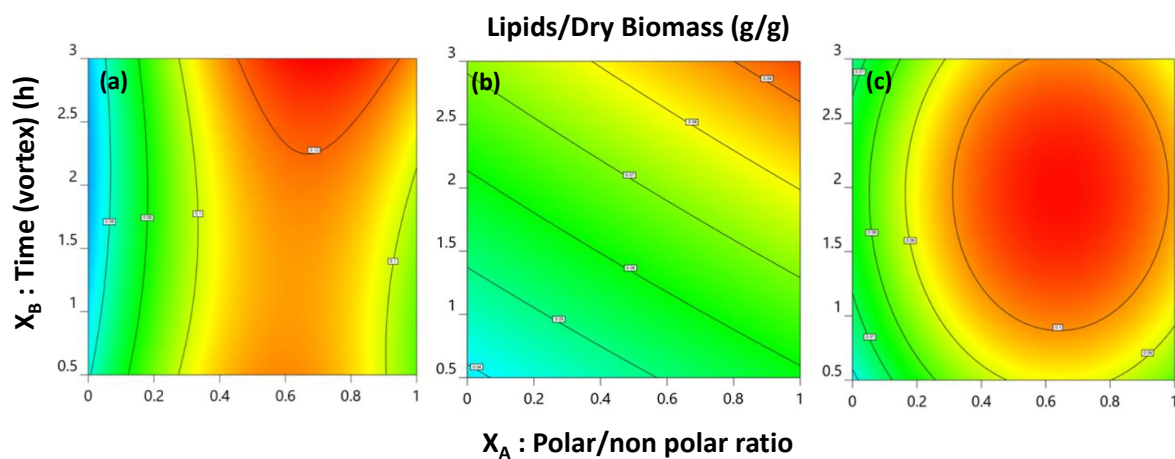


Figure S2. Contour plots for lipid yield (L/DB, g/g) response for all types of solvents, (a) MeOH/Hex (dry biomass/solvent ratio = 40); (b) Isop/Hex (dry biomass/solvent ratio = 10); and (c) MeOH/AcOEt (dry biomass/solvent ratio = 10), as a function of polar/non polar ratio (X_A) and time of vortex (X_B).

Table S5. Lipid extraction conditions of validation experiments.

Type of solvent	Dry biomass/ solvent ratio (mg/mL)	Vortex time (h)	Polar/non-polar ratio
MeOH/Hex	50	0.75	0.33
Isop/Hex	30	1.50	1.00
MeOH/AcOEt	30	1.25	0.60