Supplementary Materials

Statistical Optimization for Cost-effective Production of Yeast-bacterium Cell-bound Lipases Using Blended Oily Wastes and Their Potential Applications in Biodiesel Synthesis and Wastewater Bioremediation

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Table S1. Characteristics of POME50, crude glycerol, waste frying oil, and molasses used in the present study.

Parameter	**POME50**	**Crude Glycerol**	**Waste Frying Oil**	**Molasses**
pH	3.80	9.57	6.02	6.10
Glycerol (%)	NA	25.956	5.66	NA
Chemical oxygen demand (COD) (%)	2.977	97.508	NA	0.945
Total Kjeldahl nitrogen (TKN) (%)	0.033	0.125	NA	0.006
Oil and grease (O&G) (%)	0.564	60.098	83.751	0.011
Total solids (TS) (%)	0.647	0.469	1.154	78.742
Suspended Solids (SS) (%)	0.493	0.436	0.258	0.106
Color	Light brown	Dark brown	Dark brown	Black brown

Table S2. ANOVA for the factorial model of **CBLs** obtained from experimental design using PBD.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	5.636E+006	5	1.127E+006	6.22	**0.0229**	significant
A-Waste Frying Oil	1.190E+006	1	1.190E+006	6.57	**0.0427**	significant
B-$(NH_4)_2SO_4$	1.365E+006	1	1.365E+006	7.54	**0.0335**	significant
C-Gum Arabic	43296.45	1	43296.45	0.24	0.6423	
D-Inoculum Size	3.741E+005	1	3.741E+005	2.06	0.2008	
E-Initial pH	2.663E+006	1	2.663E+006	14.70	**0.0086**	significant
Residual	1.087E+006	6	1.812E+005			
Cor Total	6.723E+006	11				

Std. Dev.	425.63	R-Squared	0.8383
Mean	3091.59	Adj R-Squared	0.7036
C.V. %	13.77	Pred R-Squared	0.3533
PRESS	4.348E+006	Adeq Precision	7.864

CBLs = +2161.11475 + -314.88758 * A + -449.73198 * B +120.13389 * C +35.31252 * D +314.07504 * E

Table S3. ANOVA for a factorial model of **CBM** obtained from experimental design using PBD.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	146.52	5	29.30	4.76	**0.0419**	significant
A-Waste Frying Oil	0.70	1	0.70	0.11	0.7472	
B-$(NH_4)_2SO_4$	2.71	1	2.71	0.44	0.5318	
C-Gum Arabic	23.80	1	23.80	3.87	0.0968	
D-Inoculum Size	4.20	1	4.20	0.68	0.4403	
E-Initial pH	115.11	1	115.11	18.71	**0.0050**	significant
Residual	36.92	6	6.15			
Cor Total	183.44	11				

Std. Dev.	2.48	R-Squared	0.7987
Mean	14.05	Adj R-Squared	0.6310
C.V. %	17.66	Pred R-Squared	0.1950
PRESS	147.68	Adeq Precision	6.354

CBM = -3.24259 +0.24167 * A + -0.63333 * B +2.81667 * C +0.11833 * D +2.06481 * E

Table S4. ANOVA for a factorial model of **O&G removal** obtained from experimental design using PBD.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1053.09	5	210.62	8.42	**0.0110**	significant
A-Waste Frying Oil	72.03	1	72.03	2.88	0.1406	
B-$(NH_4)_2SO_4$	380.81	1	380.81	15.23	**0.0080**	significant
C-Gum Arabic	0.083	1	0.083	3.332E-003	0.9558	
D-Inoculum Size	80.08	1	80.08	3.20	0.1238	
E-Initial pH	520.08	1	520.08	20.79	**0.0039**	significant
Residual	150.07	6	25.01			
Cor Total	1203.17	11				

Std. Dev.	5.00	R-Squared	0.8753
Mean	44.23	Adj R-Squared	0.7713
C.V. %	11.31	Pred R-Squared	0.5011
PRESS	600.29	Adeq Precision	8.370

O&G removal = +29.33333 + -2.45000 * A + -7.51111 * B + -0.16667 * C +0.51667 * D + 4.38889 * E

Table S5. ANOVA for a factorial model of **CBLs** obtained from experimental design using RSM-CCD.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.363E+07	9	1.515E+06	13.47	**0.0002**	significant
A-Waste Frying Oil	26004.21	1	26004.21	0.2313	0.6409	
B-Ammonium Sulfate	5.649E+05	1	5.649E+05	5.02	**0.0489**	significant
C-pH	6.958E+06	1	6.958E+06	61.88	**< 0.0001**	significant
AB	10813.98	1	10813.98	0.0962	0.7628	
AC	6.139E+05	1	6.139E+05	5.46	**0.0416**	significant
BC	8.482E+05	1	8.482E+05	7.54	**0.0206**	significant
A^2	99376.60	1	99376.60	0.8838	0.3693	
B^2	1.105E+06	1	1.105E+06	9.83	**0.0106**	significant
C^2	3.871E+06	1	3.871E+06	34.42	**0.0002**	significant
Residual	1.124E+06	10	1.124E+05			
Lack of Fit	8.590E+05	5	1.718E+05	3.24	0.1117	not significant
Pure Error	2.655E+05	5	53101.28			
Cor Total	1.476E+07	19				

Std. Dev.	335.33	R-Squared	0.9238
Mean	3782.25	Adj R-Squared	0.8552
C.V. %	8.87	Pred R-Squared	0.5319
PRESS	6.908E+06	Adeq Precision	13.9218

CBLs = -8430.42 + 1618.87 * A + 56.8879 * B + 3201.78 * C + -49.0215 * AB + -184.676 * AC + 289.442 * BC + -83.0406 * A^2 + -492.227 * B^2 + -230.336 * C^2

Table S6. ANOVA for a factorial model of **CBM** obtained from experimental design using RSM-CCD.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	629.53	9	69.95	10.85	**0.0004**	significant
A-Waste Frying Oil	2.14	1	2.14	0.3322	0.5771	
B-$(NH_4)_2SO_4$	40.79	1	40.79	6.33	**0.0306**	significant
C-pH	347.86	1	347.86	53.94	**< 0.0001**	significant
AB	2.40	1	2.40	0.3721	0.5555	
AC	29.37	1	29.37	4.55	0.0586	
BC	34.48	1	34.48	5.35	**0.0433**	significant
A^2	0.0536	1	0.0536	0.0083	0.9292	
B^2	63.55	1	63.55	9.85	**0.0105**	significant
C^2	120.82	1	120.82	18.74	**0.0015**	significant
Residual	64.49	10	6.45			
Lack of Fit	49.89	5	9.98	3.42	0.1017	not significant
Pure Error	14.59	5	2.92			
Cor Total	694.01	19				

Std. Dev.	2.54	R-Squared	0.9071
Mean	17.00	Adj R-Squared	0.8235
C.V. %	14.94	Pred R-Squared	0.4209
PRESS	401.93	Adeq Precision	12.6208

CBM = -57.5457 + 7.96846 * A + 3.67754 * B + 19.0455 * C + -0.73024 * AB + -1.27738 * AC + 1.8455 * BC + 0.0609913 * A^2 + -3.73308 * B^2 + -1.28688 * C^2

Table S7. ANOVA for a factorial model of **O&G removal** obtained from experimental design using RSM-CCD.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	439.06	9	48.78	10.77	**0.0005**	significant
A-Waste Frying Oil	5.63	1	5.63	1.24	0.2910	
B-$(NH_4)_2SO_4$	30.58	1	30.58	6.75	**0.0265**	significant
C-pH	289.05	1	289.05	63.83	**< 0.0001**	significant
AB	0.31	1	0.31	0.069	0.7977	
AC	32.02	1	32.02	7.07	**0.0239**	significant
BC	22.58	1	22.58	4.99	**0.0496**	significant
A^2	0.33	1	0.33	0.072	0.7942	
B^2	27.00	1	27.00	5.96	**0.0347**	significant
C^2	37.41	1	37.41	8.26	**0.0166**	significant
Residual	45.28	10	4.53			
Lack of Fit	34.68	5	6.94	3.27	0.1097	not significant
Pure Error	10.60	5	2.12			
Cor Total	484.34	19				

Std. Dev.	2.13	R-Squared	0.9065
Mean	52.55	Adj R-Squared	0.8224
C.V. %	4.05	Pred R-Squared	0.4156
PRESS	283.04	Adeq Precision	12.294

O&G removal = -6.14215 + 9.20921 * A + 0.65642 * B + 13.07802 * C + -0.26409 * AB + -1.33380 * AC + 1.49348 * BC + -0.15020 * A^2 + -2.43320 * B^2 + -0.71604 * C^2

Table S8. Fatty acid composition of palm oil, waste frying oil, and oil extracted from POME.

Fatty acids	**Refined palm oil (%)**	**Waste frying oil (%)**	**Extracted oil from POME (%)**
Myristic acid (C14:0)	0.63	0.41	0.24
Palmitic acid (C16:0)	40.61	29.69	33.01
Palmitoleic acid (C16:1)	-	0.28	0.15
Stearic acid (C18:0)	4.87	3.08	1.53
Oleic acid (C18:1)	34.88	28.56	18.35
Linoleic acid (C18:2)	10.14	20.98	5.93
Linolenic acid (C18:3)	0.42	1.65	0.31
Eicosanoic acid (C20:0)	0.12	13.16	3.84
Paulinic acid (C20:1)	-	-	2.93
Docosanoic acid (C22:0)	-	11.83	0.07
Total SFA	46.23	46.34	38.69
Total MUFA	34.88	28.84	21.43
Total PUFA	10.56	22.63	6.24

SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA= polyunsaturated fatty acids