

Supplementary Information

## Optimization, simultaneous saccharification and fermentation of *Lemna minor* using amyolytic cocktail produce from copra meal

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Table S1 — A three-factor central composite design (CCD) model having coded and non-coded data created with Minitab software

Run	Space type	Coded factor							Non coded factor							Enzyme activity (U/ml)
		pH	temp (°C)	Copra (%)	KH <sub>2</sub> PO <sub>4</sub> (%)	Peptone (%)	Time of incubation (days)	CM C (%)	pH	temp (°C)	Copra (%)	KH <sub>2</sub> PO <sub>4</sub> (%)	Peptone (%)	Time of incubation (days)	CM C (%)	
1	Corner	-1	-1	-1	-1	-1	-1	1	3.5	25	2	0.01	0.125	6	2	842.86
2	Corner	1	-1	-1	-1	-1	-1	-1	4.5	25	2	0.01	0.125	6	0.5	740.71
3	Corner	-1	1	-1	-1	-1	-1	-1	3.5	35	2	0.01	0.125	6	0.5	880.86
4	Corner	1	1	-1	-1	-1	-1	1	4.5	35	2	0.01	0.125	6	2	741.43
5	Corner	-1	-1	1	-1	-1	-1	-1	3.5	25	4	0.01	0.125	6	0.5	903.86
6	Corner	1	-1	1	-1	-1	-1	1	4.5	25	4	0.01	0.125	6	2	764.43
7	Corner	-1	1	1	-1	-1	-1	1	3.5	35	4	0.01	0.125	6	2	809.14
8	Corner	1	1	1	-1	-1	-1	-1	4.5	35	4	0.01	0.125	6	0.5	850.71

9	Corner	-1	-1	-1	1	-1	-1	-1	3.5	25	2	0.03	0.125	6	0.5	920.71
10	Corner	1	-1	-1	1	-1	-1	1	4.5	25	2	0.03	0.125	6	2	826.57
11	Corner	-1	1	-1	1	-1	-1	1	3.5	35	2	0.03	0.125	6	2	826
12	Corner	1	1	-1	1	-1	-1	-1	4.5	35	2	0.03	0.125	6	0.5	867.57
13	Corner	-1	-1	1	1	-1	-1	1	3.5	25	4	0.03	0.125	6	2	849
14	Corner	1	-1	1	1	-1	-1	-1	4.5	25	4	0.03	0.125	6	0.5	890.57
15	Corner	-1	1	1	1	-1	-1	-1	3.5	35	4	0.03	0.125	6	0.5	890
16	Corner	1	1	1	1	-1	-1	1	4.5	35	4	0.03	0.125	6	2	795.86
17	Corner	-1	-1	-1	-1	1	-1	-1	3.5	25	2	0.01	0.5	6	0.5	920
18	Corner	1	-1	-1	-1	1	-1	1	4.5	25	2	0.01	0.5	6	2	825.86
19	Corner	-1	1	-1	-1	1	-1	1	3.5	35	2	0.01	0.5	6	2	825.29
20	Corner	1	1	-1	-1	1	-1	-1	4.5	35	2	0.01	0.5	6	0.5	866.86
21	Corner	-1	-1	1	-1	1	-1	1	3.5	25	4	0.01	0.5	6	2	848.29
22	Corner	1	-1	1	-1	1	-1	-1	4.5	25	4	0.01	0.5	6	0.5	889.86
23	Corner	-1	1	1	-1	1	-1	-1	3.5	35	4	0.01	0.5	6	0.5	889.29
24	Corner	1	1	1	-1	1	-1	1	4.5	35	4	0.01	0.5	6	2	795.14
25	Corner	-1	-1	-1	1	1	-1	1	3.5	25	2	0.03	0.5	6	2	865.14
26	Corner	1	-1	-1	1	1	-1	-1	4.5	25	2	0.03	0.5	6	0.5	906.71
27	Corner	-1	1	-1	1	1	-1	-1	3.5	35	2	0.03	0.5	6	0.5	906.14

28	Corner	1	1	-1	1	1	-1	1	4.5	35	2	0.03	0.5	6	2	812
29	Corner	-1	-1	1	1	1	-1	-1	3.5	25	4	0.03	0.5	6	0.5	929.14
30	Corner	1	-1	1	1	1	-1	1	4.5	25	4	0.03	0.5	6	2	835
31	Corner	-1	1	1	1	1	-1	1	3.5	35	4	0.03	0.5	6	0.5	902.29
32	Corner	1	1	1	1	1	-1	-1	4.5	35	4	0.03	0.5	6	0.5	876
33	Corner	-1	-1	-1	-1	-1	1	-1	3.5	25	2	0.01	0.125	10	0.5	917.43
34	Corner	1	-1	-1	-1	-1	1	1	4.5	25	2	0.01	0.125	10	2	823.29
35	Corner	-1	1	-1	-1	-1	1	1	3.5	35	2	0.01	0.125	10	2	822.71
36	Corner	1	1	-1	-1	-1	1	-1	4.5	35	2	0.01	0.125	10	0.5	864.29
37	Corner	-1	-1	1	-1	-1	1	1	3.5	25	4	0.01	0.125	10	2	845.71
38	Corner	1	-1	1	-1	-1	1	-1	4.5	25	4	0.01	0.125	10	0.5	887.29
39	Corner	-1	1	1	-1	-1	1	-1	3.5	35	4	0.01	0.125	10	0.5	886.71
40	Corner	1	1	1	-1	-1	1	1	4.5	35	4	0.01	0.125	10	2	792.57
41	Corner	-1	-1	-1	1	-1	1	1	3.5	25	2	0.03	0.125	10	2	862.57
42	Corner	1	-1	-1	1	-1	1	-1	4.5	25	2	0.03	0.125	10	0.5	904.14
43	Corner	-1	1	-1	1	-1	1	-1	3.5	35	2	0.03	0.125	10	0.5	903.57
44	Corner	1	1	-1	1	-1	1	1	4.5	35	2	0.03	0.125	10	2	809.43
45	Corner	-1	-1	1	1	-1	1	-1	3.5	25	4	0.03	0.125	10	0.5	926.57
46	Corner	1	-1	1	1	-1	1	1	4.5	25	4	0.03	0.125	10	2	832.43

47	Corner	-1	1	1	1	-1	1	1	3.5	35	4	0.03	0.125	10	2	831.86
48	Corner	1	1	1	1	-1	1	-1	4.5	35	4	0.03	0.125	10	0.5	873.43
49	Corner	-1	-1	-1	-1	1	1	1	3.5	25	2	0.01	0.5	10	2	861.86
50	Corner	1	-1	-1	-1	1	1	-1	4.5	25	2	0.01	0.5	10	0.5	903.43
51	Corner	-1	1	-1	-1	1	1	-1	3.5	35	2	0.01	0.5	10	0.5	902.86
52	Corner	1	1	-1	-1	1	1	1	4.5	35	2	0.01	0.5	10	2	808.71
53	Corner	-1	-1	1	-1	1	1	-1	3.5	25	4	0.01	0.5	10	0.5	925.86
54	Corner	1	-1	1	-1	1	1	1	4.5	25	4	0.01	0.5	10	2	831.71
55	Corner	-1	1	1	-1	1	1	1	3.5	35	4	0.01	0.5	10	2	831.14
56	Corner	1	1	1	-1	1	1	-1	4.5	35	4	0.01	0.5	10	0.5	872.71
57	Corner	-1	-1	-1	1	1	1	-1	3.5	25	2	0.03	0.5	10	0.5	942.71
58	Corner	1	-1	-1	1	1	1	1	4.5	25	2	0.03	0.5	10	2	848.57
59	Corner	-1	1	-1	1	1	1	1	3.5	35	2	0.03	0.5	10	2	848
60	Corner	1	1	-1	1	1	1	-1	4.5	35	2	0.03	0.5	10	0.5	889.57
61	Corner	-1	-1	1	1	1	1	1	3.5	25	4	0.03	0.5	10	2	871
62	Corner	1	-1	1	1	1	1	-1	4.5	25	4	0.03	0.5	10	0.5	912.57
63	Corner	-1	1	1	1	1	1	-1	3.5	35	4	0.03	0.5	10	0.5	912
64	Corner	1	1	1	1	1	1	1	4.5	35	4	0.03	0.5	10	2	817.86
65	Axial	-2.8	0	0	0	0	0	0	3	30	3	0.02	0.25	8	1	1086.86

66	Axial	2.8	0	0	0	0	0	0	5	30	3	0.02	0.25	8	1	907
67	Axial	0	-2.828	0	0	0	0	0	4	20	3	0.02	0.25	8	1	1109.57
68	Axial	0	2.828	0	0	0	0	0	4	40	3	0.02	0.25	8	1	1074.14
69	Axial	0	0	-2.828	0	0	0	0	4	30	1	0.02	0.25	8	1	1053
70	Axial	0	0	2.8284	0	0	0	0	4	30	5	0.02	0.25	8	1	1067.71
71	Axial	0	0	0	-2.82843	0	0	0	4	30	3	0.001	0.25	8	1	1076.71
72	Axial	0	0	0	2.828427	0	0	0	4	30	3	0.04	0.25	8	1	945.57
73	Axial	0	0	0	0	-2.8284271	0	0	4	30	3	0.02	0.0625	8	1	935
74	Axial	0	0	0	0	2.8284271	0	0	4	30	3	0.02	1	8	1	1030
75	Axial	0	0	0	0	0	-2.8284271	0	4	30	3	0.02	0.25	4	1	1049.29
76	Axial	0	0	0	0	0	2.8284271	0	4	30	3	0.02	0.25	12	1	1047.29
77	Axial	0	0	0	0	0	0	-2.828	4	30	3	0.02	0.25	8	0.001	1036.57
78	Axial	0	0	0	0	0	0	2.828	4	30	3	0.02	0.25	8	2	1014.14
79	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005
80	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005
81	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005
82	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005
83	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005
84	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005
85	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005
86	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005
87	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005
88	Centre	0	0	0	0	0	0	0	4	30	3	0.02	0.25	8	1	1005

Table S2 — Model analysis for amylase production from *Aspergillus niger* sp. using ANOVA test

### Analysis of Variance

Source	DF	Seq SS	Contribution	F-Value	P-Value	Remark
Model	14	485036	76.81%	17.27	0.000	Significant
Linear	7	174631	27.66%	3.42	0.003	
pH	1	31375	4.97%	14.14	0.000	
temperature (°C)	1	7295	1.16%	4.41	0.039	
Coconut oil cake concentration	1	173	0.03%	0.01	0.920	
KH <sub>2</sub> PO <sub>4</sub> (%)	1	3047	0.48%	0.31	0.582	
Peptone (%)	1	666	0.11%	0.72	0.400	
Time of incubation (days)	1	3033	0.48%	2.02	0.159	
Carboxy Methyl Cellulose (%)	1	129042	20.44%	2.26	0.137	
Square	7	310405	49.16%	22.11	0.000	
pH*pH	1	97485	15.44%	13.33	0.000	
temperature (°C)*temperature (°C)	1	21646	3.43%	0.35	0.557	
Coconut oil cake concentration*Coconut oil cake conc.	1	29918	4.74%	2.58	0.113	
KH <sub>2</sub> PO <sub>4</sub> (%)*KH <sub>2</sub> PO <sub>4</sub> (%)	1	56411	8.93%	12.00	0.001	
Peptone (%)*Peptone (%)	1	9497	1.50%	4.09	0.047	
Time of incubation (days)*Time of incubation (days)	1	22644	3.59%	3.98	0.050	
Carboxy Methyl Cellulose (%)*Carboxy Methyl Cellulose (%)	1	72802	11.53%	36.30	0.000	
Error	73	146422	23.19%			
Lack-of-Fit	64	146422	23.19%	*	*	
Pure Error	9	0	0.00%			
Total	87	631458	100.00%			

Table S3 — Model summary statistics for optimal amylase production (response Y)

Source	S	R-sq	R-sq(adj)	Press	R-sq(pred)	Remarks
Linear	75.5667	27.66%	21.33%	530208	16.03%	
Linear + Square	44.7859	76.81%	72.37%	2995016	0.00%	Suggested
Linear + Interaction	87.1609	29.02%	0.00%	754047	0.00%	

Full quadratic	51.5231	78.14%	63.43%	3031637	0.00%	
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Table S4: A three-factor central composite design (CCD) model having coded and non-coded data created with Minitab software

Run	Space type	Coded factor				Non coded factor				Ethanol (%)
		pH	Temp (°C)	Lemna minor biomass (%)	Amylase enzyme extract (ml)	pH	Temp (°C)	Lemna minor biomass (%)	Amylase enzyme extract (ml)	
1	Corner	-0.5	-0.5	-0.5	-0.5	4.5	30	0.5	5	9.35
2	Corner	0.5	-0.5	-0.5	-0.5	5.5	30	0.5	5	9.725
3	Corner	-0.5	0.5	-0.5	-0.5	4.5	40	0.5	5	9.975
4	Corner	0.5	0.5	-0.5	-0.5	5.5	40	0.5	5	10.35
5	Corner	-0.5	-0.5	0.5	-0.5	4.5	30	2	5	9.7
6	Corner	0.5	-0.5	0.5	-0.5	5.5	30	2	5	10.075
7	Corner	-0.5	0.5	0.5	-0.5	4.5	40	2	5	10.325
8	Corner	0.5	0.5	0.5	-0.5	5.5	40	2	5	10.7
9	Corner	-0.5	-0.5	-0.5	0.5	4.5	30	0.5	7	9.775
10	Corner	0.5	-0.5	-0.5	0.5	5.5	30	0.5	7	10.15
11	Corner	-0.5	0.5	-0.5	0.5	4.5	40	0.5	7	10.4
12	Corner	0.5	0.5	-0.5	0.5	5.5	40	0.5	7	10.775
13	Corner	-0.5	-0.5	0.5	0.5	4.5	30	2	7	10.125
14	Corner	0.5	-0.5	0.5	0.5	5.5	30	2	7	10.5
15	Corner	-0.5	0.5	0.5	0.5	4.5	40	2	7	10.75
16	Corner	0.5	0.5	0.5	0.5	5.5	40	2	7	11.125
17	Axial	-1	0	0	0	4	35	1	6	10.95
18	Axial	1	0	0	0	6	35	1	6	11.5
19	Axial	0	-1	0	0	5	25	1	6	10.225
20	Axial	0	1	0	0	5	45	1	6	11.025
21	Axial	0	0	-1	0	5	35	0.25	6	10.375

22	Axial	0	0	1	0	5	35	3	6	10.825
23	Axial	0	0	0	-1	5	35	1	4	11.225
24	Axial	0	0	0	1	5	35	1	8	11.4
25	Centre	0	0	0	0	5	35	1	6	11.625
26	Centre	0	0	0	0	5	35	1	6	11.625
27	Centre	0	0	0	0	5	35	1	6	11.625
28	Centre	0	0	0	0	5	35	1	6	11.625
29	Centre	0	0	0	0	5	35	1	6	11.625
30	Centre	0	0	0	0	5	35	1	6	11.625
31	Centre	0	0	0	0	5	35	1	6	11.625

Table S5 – Model summary statistics for optimal ethanol production (response Y)

Source	S	R-sq	R-sq(adj)	Press	R-sq(pred)	Remarks
Linear	0.66542	0.2135	0.0925	15.0852	0	
Linear + Square	0.41735	0.7382	0.643	29.1425	0	Suggested
Linear + Interaction	0.75842	0.2141	0	30.0066	0	
Full quadratic	0.48885	0.7388	0.5102	35.8634	0	

Table S6 – Model analysis for ethanol production from *Lemna minor* sp. using ANOVA test

### Analysis of Variance

Source	DF	Seq SS	Contribution	F-Value	P-Value
Model	8	10.8058	73.82%	7.75	0.000
Linear	4	3.1255	21.35%	5.22	0.004
pH	1	0.7004	4.79%	4.02	0.057
Temperature	1	1.8150	12.40%	10.42	0.004
Lemna minor biomass (%)	1	0.0241	0.16%	3.06	0.094

Amylase enzyme extract (ml)	1	0.5859	4.00%	3.36	0.080
Square	4	7.6803	52.47%	11.02	0.000
pH*pH	1	0.5361	3.66%	7.47	0.012
Temperature*Temperature	1	2.8491	19.46%	21.68	0.000
Lemna minor biomass (%)*Lemna minor biomass (%)	1	3.2477	22.19%	20.64	0.000
Amylase enzyme extract (ml)*Amylase enzyme extract (ml)	1	1.0474	7.16%	6.01	0.023
Error	22	3.8319	26.18%		
Lack-of-Fit	16	3.8319	26.18%	*	*
Pure Error	6	0.0000	0.00%		
Total	30	14.6377	100.00%		

Table S6 — Levels of independent variables considered for amylase and ethanol optimization

Amylase activity (U/mL)							
Description	Variables	Unit	Levels				
			-2	-1	0	1	2
pH	A	-	3	3.5	4	4.5	5
Temperature	B	°C	20	25	30	35	40
Coconut oil cake concentration	C	%(w/v)	1	2	3	4	5
KH <sub>2</sub> PO <sub>4</sub>	D	%(w/v)	0.001	0.01	0.02	0.03	0.04
Peptone	E	%(w/v)	0.0625	0.125	0.25	0.5	1
Time of incubation	F	Days	4	6	8	10	12
Carboxy Methyl Cellulose	G	%(w/v)	0.001	0.5	1	1.5	2
Ethanol production (%)							
pH	A	-	4	4.5	5	5.5	6
Temperature	B	°C	25	30	35	40	45
Lemna minor biomass	C	%	0.25	0.5	1	2	3

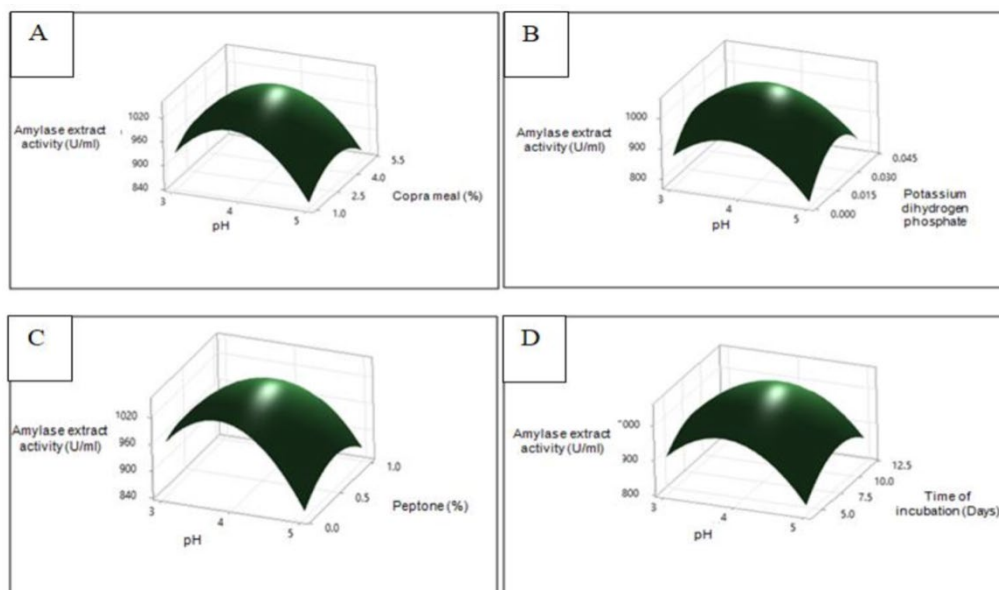
Amylaseenzymeextract	D	ml	4	5	6	7	8
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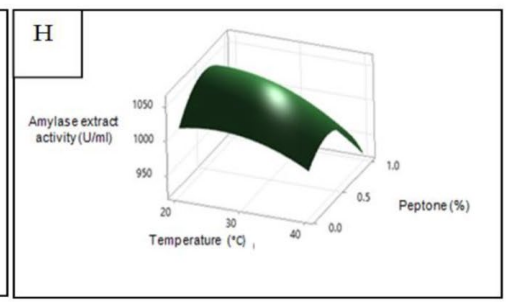
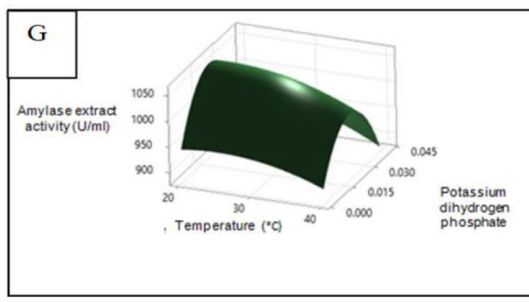
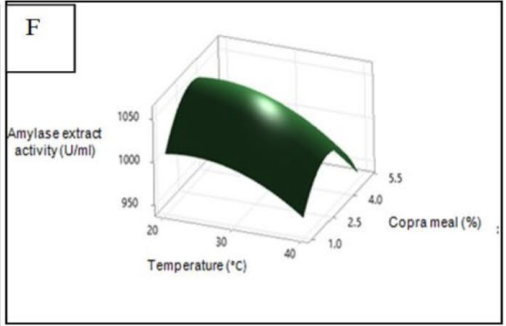
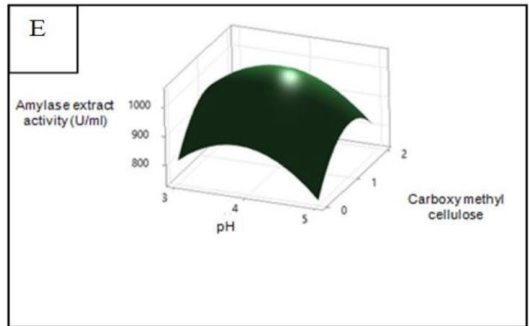
Table S7 — Levels of independent variables considered for amylase and ethanol optimization

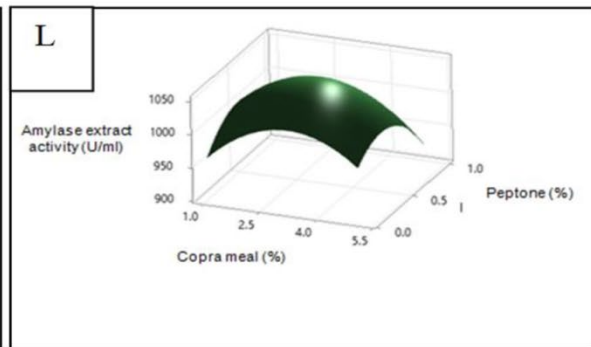
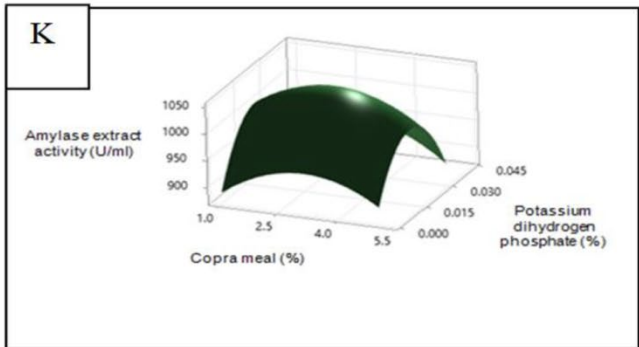
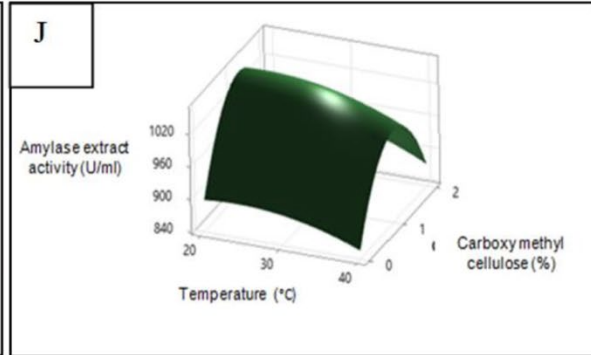
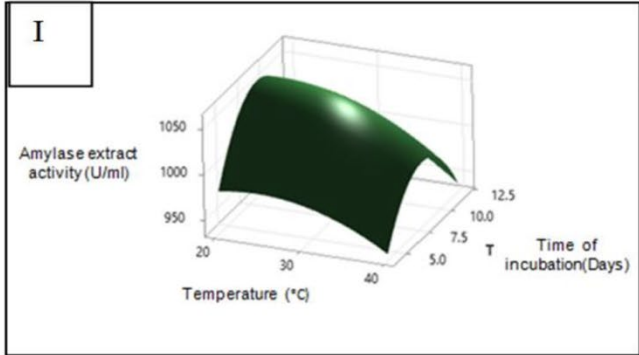
Amylase activity (U/mL)							
Description	Variables	Unit	Levels				
			-2	-1	0	1	2
pH	A	-	3	3.5	4	4.5	5
Temperature	B	°C	20	25	30	35	40
Coconutoilcakeconcentration	C	%(w/v)	1	2	3	4	5
KH <sub>2</sub> PO <sub>4</sub>	D	%(w/v)	0.001	0.01	0.02	0.03	0.04
Peptone	E	%(w/v)	0.0625	0.125	0.25	0.5	1
Timeofincubation	F	Days	4	6	8	10	12
CarboxyMethylCellulose	G	%(w/v)	0.001	0.5	1	1.5	2
Ethanol production (%)							
pH	A	-	4	4.5	5	5.5	6
Temperature	B	°C	25	30	35	40	45
Lemnaminorbiomass	C	%	0.25	0.5	1	2	3
Amylaseenzymeextract	D	ml	4	5	6	7	8

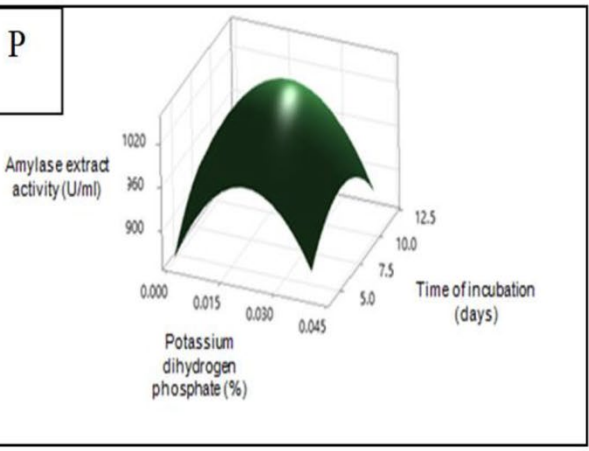
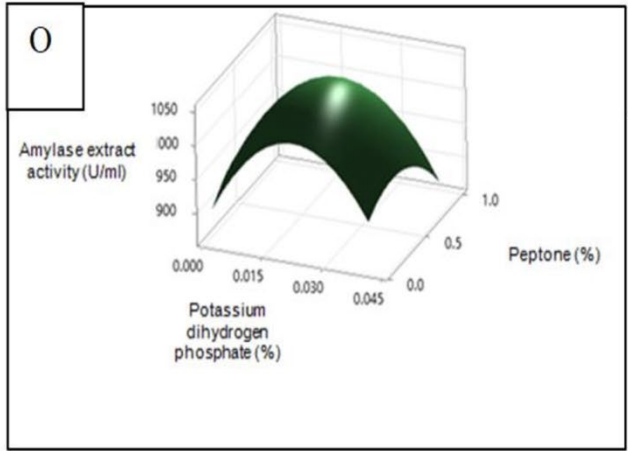
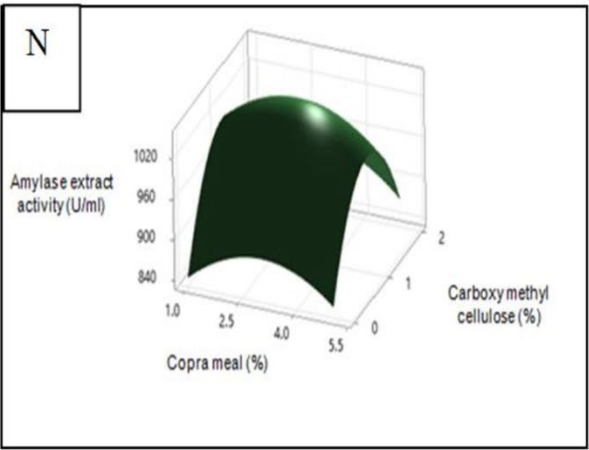
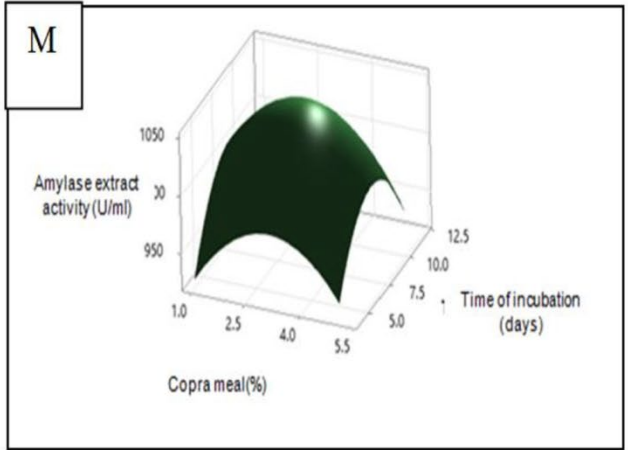
Table S8— Composition of Copra meal and Lemna minor biomass

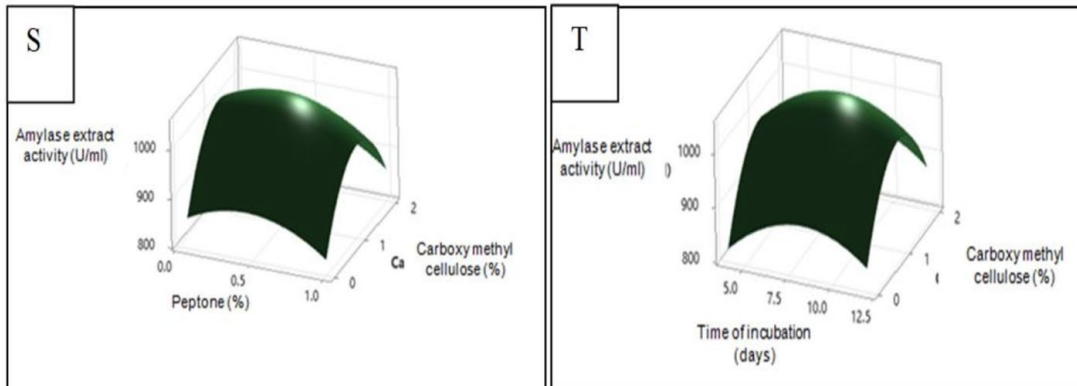
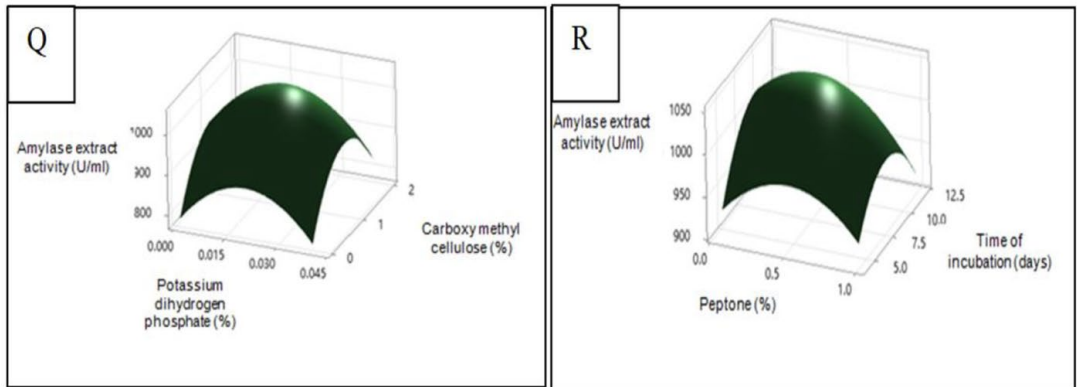
Chemical constituents	Lemna minor (% w/w)	Copra meal (% w/w)
Glucose	2	0.2
Fructose	1	0.3
Starch	26	1.6
Protein	14.60	21





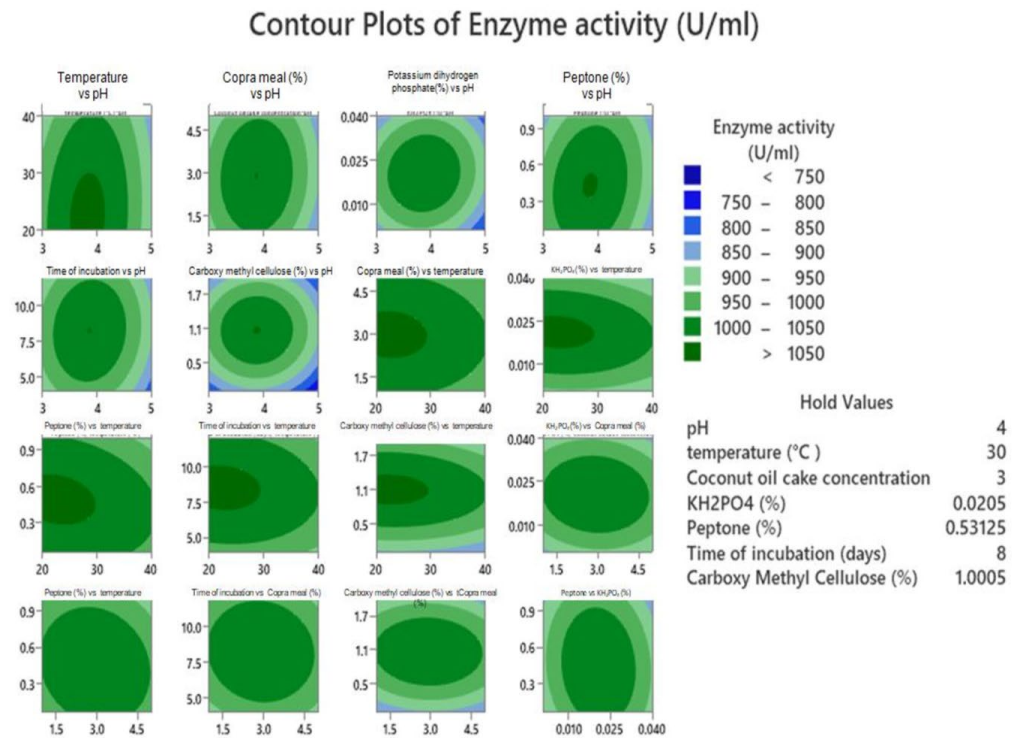






Hold Values	
pH	4
temperature (°C )	30
Coconut oil cake concentration	3
KH <sub>2</sub> PO <sub>4</sub> (%)	0.0205
Peptone (%)	0.53125
Time of incubation (days)	8
Carboxy Methyl Cellulose (%)	1.0005

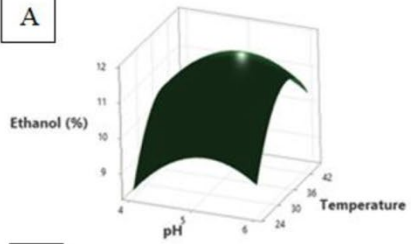
(a)



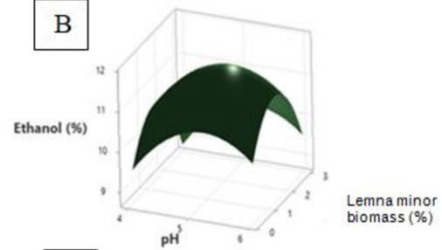
(b)

Fig. S1 — (a): 3D surface plot of various combinations of each factor for Amylase cocktail preparation (A-T) and (b) 3D contour plot of various combinations of each factor

A

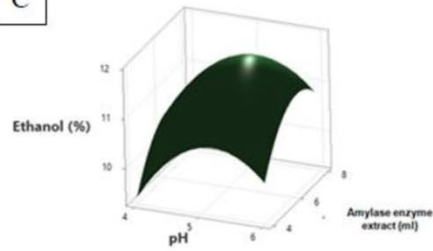


B

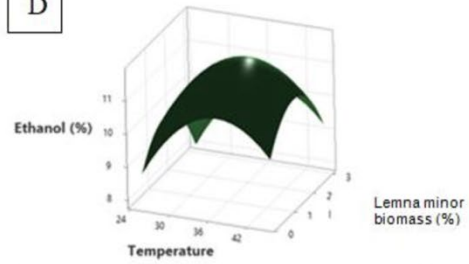


Hold Values  
 pH 5  
 Temperature 35  
 Lemna minor biomass (%) 1.625  
 Amylase enzyme extract (ml) 6

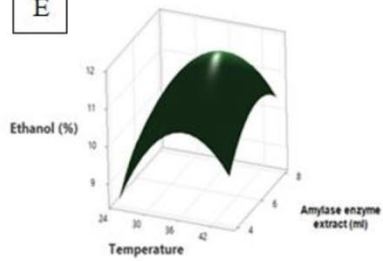
C



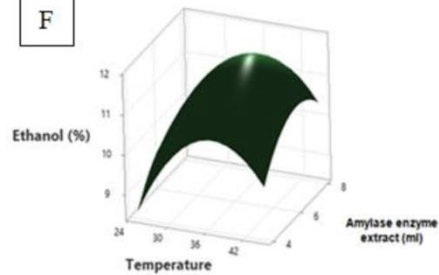
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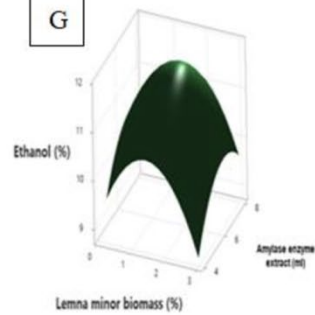
E



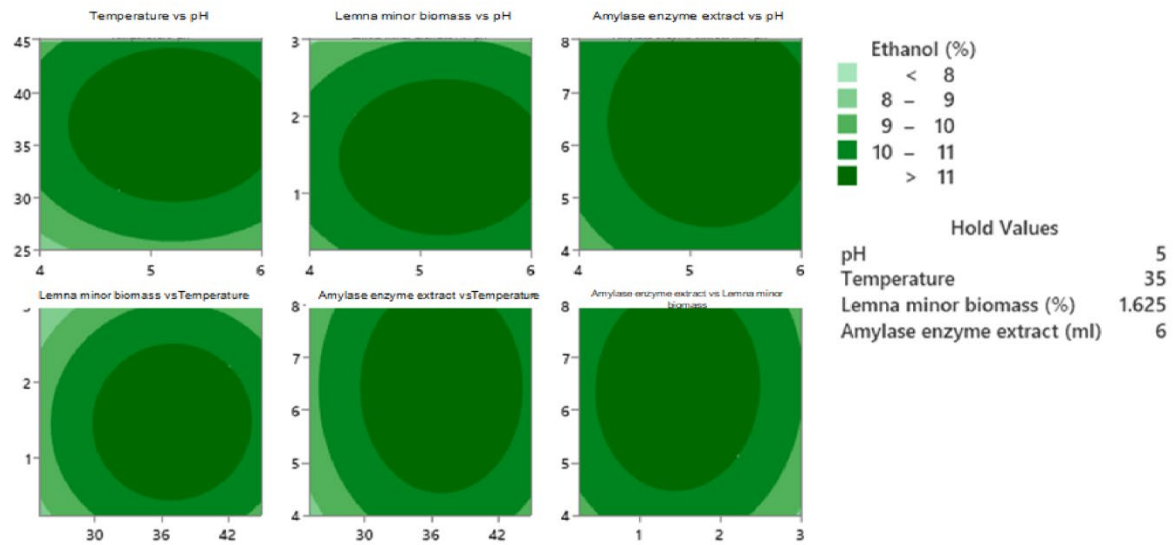
F



G



(a)



(b)

Fig. S2 — 3D surface plot (a) and Contour plot (b) of various combinations of each factor