catalysts

Supplementary Material

Table S1. Box-Behnken design and its responses for optimization of immobilization conditions of KDN lipase by adsorption method.

No.	A amount of enzyme（mg/g）	B pH	C temperature（℃）	D time（h）	Enzyme activity（U/g）
1	1	0	-1	0	153.81±2.39
2	0	-1	1	0	184.42±8.66
3	1	-1	0	0	156.20±9.35
4	1	1	0	0	213.03±1.62
5	0	-1	0	-1	112.92±0.03
6	0	0	0	0	179.46±2.74
7	0	1	0	1	126.84±6.70
8	0	0	0	0	206.19±1.97
9	0	0	0	0	216.73±0.25
10	0	0	1	-1	182.66±2.41
11	0	1	0	-1	179.56±12.88
12	0	0	-1	-1	159.71±10.52
13	1	0	0	1	149.27±1.45
14	0	0	0	0	197.67±2.77
15	-1	0	1	0	91.76±4.65
16	0	1	1	0	139.01±1.40
17	-1	0	-1	0	142.67±7.98
18	0	0	-1	1	160.89±9.15
19	1	0	1	0	197.05±3.24
20	-1	1	0	0	93.71±1.71
21	0	0	1	1	115.05±3.24
22	0	-1	0	1	153.26±5.15
23	0	1	-1	0	119.81±0.15
24	1	0	0	-1	138.58±5.76
25	0	-1	-1	0	127.66±0.16
26	-1	0	0	-1	146.88±10.25
27	0	0	0	0	168.38±1.70
28	-1	-1	0	0	124.46±3.85
29	-1	0	0	1	143.59±5.99

Notes: the quadratic polynomial regression equation is as follows:.

$Y= + 193.69 + 1.09*A+3.78*B - 5.95*C +22.07*D - 9.39*A*B - 23.26*A*C +21.90*A*D -17.20*B*C + 23.54*B*D +3.49*C*D - 26.85*A^2 - 21.40* B^2 - 22.06* C^2 - 24.33* D^2.$

Table S2. ANOVA and fit statistics of the quadratic model for optimization of immobilization conditions of KDN lipase by adsorption method.

Model	Sum of Squares	Mean Square	*F*-Value	*P*-Value	significant
	23752.28	1696.59	2.81	0.0313	
A	14.17	14.17	0.023	0.8804	
B	171.76	171.76	0.28	0.6019	
C	424.95	424.95	0.70	0.4153	
D	5846.34	5846.34	9.69	0.0076	very significant
AB	352.69	352.69	0.58	0.4571	
AC	2165.04	2165.04	3.59	0.0790	
AD	1917.56	1917.56	3.18	0.0962	
BC	1183.02	1183.02	1.96	0.1831	
BD	2216.06	2216.06	3.67	0.0759	
CD	48.86	48.86	0.081	0.7801	
A^2	4676.14	4676.14	7.75	0.0146	
B^2	2969.76	2969.76	4.92	0.0435	
C^2	3156.15	3156.15	5.23	0.0382	
D^2	3840.75	3840.75	6.37	0.0243	
Residual	8442.75	603.05			
Lack of Fit	6896.73	689.67	1.78	0.3032	non significant
Pure Error	1546.02	386.51			
Cor Total	32195.02				

The order of influence of the four factors on the immobilization of KDN lipase by macroporous resin adsorption was as follows: adsorption time > adsorption temperature > buffer pH > amount of enzyme, in which the adsorption time had a significant effect on the immobilization of KDN lipase (P<0.01). Use Design Expert V8.0.6 software to analyze the interaction between various factors. The analysis results are shown in Fig.S1.

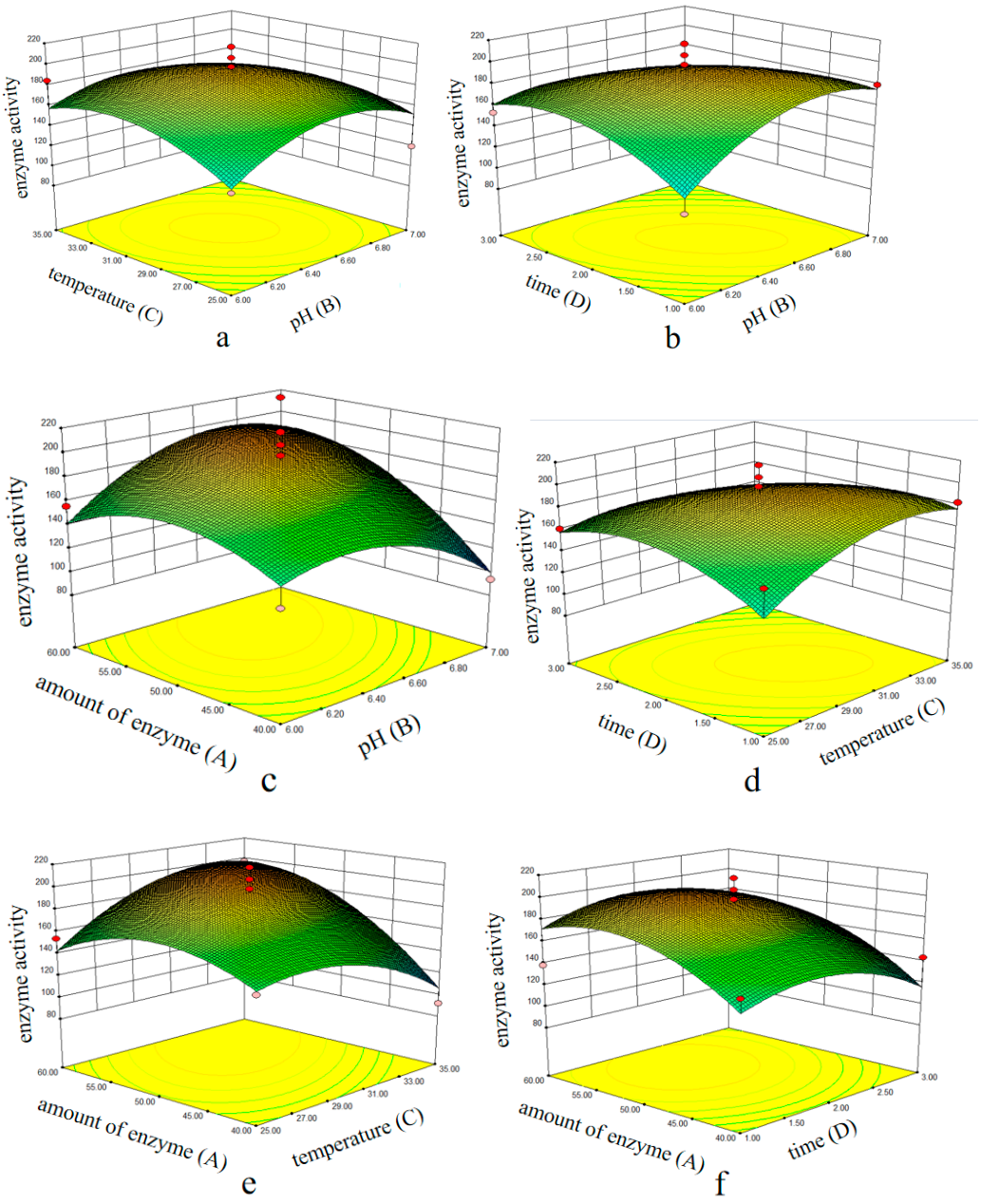

Figure S1. Response surface plot and contour plot for adsorption immobilization showing significant model terms: (a) temperature vs. pH, (b) time vs. pH, (c) amount of enzyme vs. pH, (d) time vs. temperature, (e) amount of enzyme vs. temperature, (f)amount of enzyme vs.time.

Combined with Table S2, it can be seen that the interaction between buffer pH and adsorption time, amount of enzyme and adsorption temperature, amount of enzyme and adsorption time has a greater impact on the adsorption immobilization of KDN lipase.

The extreme point was obtained by derivation of the regression equation, and the theoretical optimal immobilization conditions of the adsorption method for immobilization of KDN lipase were obtained as follows: the amount of enzyme added was 60.00 mg/g, the adsorption temperature was 33.90 ℃, the buffer pH was 6.80, and the adsorption time was 80 min. Through experimental verification, the enzyme activity was 210.58 U/g, and the recovery rate of enzyme activity was 9.62%.

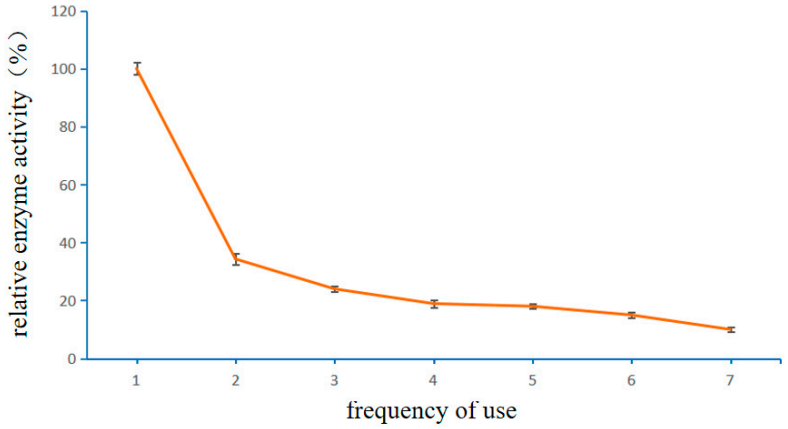

Figure S2. Reusability analysis of immobilization of lipase by adsorption.

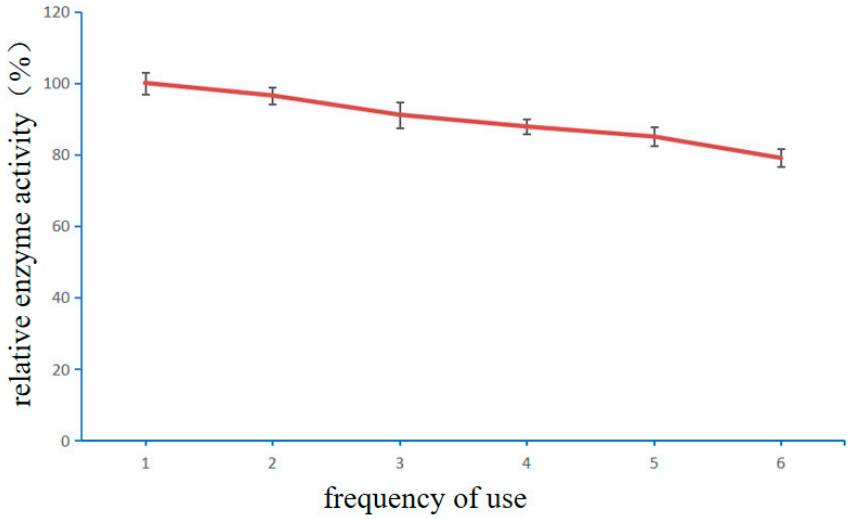

Figure S3. Reusability analysis of immobilized KDN lipase catalyzed synthesis of isopropyl myristate synthesis.