Title: A useful method integrating production and immobilization of recombinant cellulase

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Table S1: Optimization of the assembly condition for AOBs assembled with CelA-Ole and Ole-CelK by the Box-Behnken design.

Trial	Coded levels (X_1 = A/K; X_2 = pH; X_3 = Temperature)			Actual levels			Response: enzyme activity (U/mg)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	0	-1	+1	0.5	4	40	1.13
2	+1	-1	0	0.9	4	22	0.60
3	-1	+1	0	0.1	4	22	1.15
4	+1	+1	0	0.9	10	22	1.71
5	-1	0	+1	0.1	7	40	2.38
6	0	-1	-1	0.5	4	4	1.02
7	0	0	0	0.5	7	22	2.96
8	+1	0	-1	0.9	7	4	2.93
9	-1	0	-1	0.1	7	4	2.53
10	0	0	0	0.5	7	22	2.92
11	0	0	0	0.5	7	22	3.04
12	+1	0	+1	0.9	7	40	2.83
13	0	+1	-1	0.5	10	4	2.15
14	-1	+1	0	0.1	10	22	1.85
15	0	+1	+1	0.5	10	40	1.61

* CelA-Ole plus Ole-CelK in cell pellets was 200 μg in total for assembly of AOBs. The target proteins (50 μg in total) involved in AOBs were utilized to determine the enzyme activity.

Table S2. Result of ANOVA for the Box-Behnken design.

Factor	Parameter estimate	Standard error	*P*-value
X_1	0.021	0.102	0.842
X_2	0.429	0.102	0.008
X_3	-0.086	0.102	0.435
X_1 x X_2	0.104	0.144	0.501
X_1 x X_3	0.011	0.144	0.940
X_2 x X_3	-0.164	0.144	0.306
X_1 x X_1	-0.281	0.150	0.119
X_2 x X_2	-1.470	0.150	0.0002
X_3 x X_3	-0.129	0.150	0.429

Table S3: Optimization of the assembly condition for AOBs assembled with CelA-Ole, Ole-CelK, and Ole-Gls by the Box-Behnken design.

Trial	Coded levels (X_1 = G/AK; X_2 = pH; X_3 = temperature)			Actual levels			Response: enzyme activity (g/l-h)
	X_1	X_2	X_3	X_1	X_2	X_3	
1	+1	0	-1	4	6.5	20	0.24
2	0	+1	+1	2.5	8	40	0.17
3	-1	0	-1	1	6.5	20	0.22
4	0	-1	-1	2.5	5	20	0.24
5	0	-1	+1	2.5	5	40	0.25
6	+1	-1	0	4	5	30	0.22
7	0	+1	-1	2.5	8	20	0.17
8	+1	+1	0	4	8	30	0.16
9	-1	-1	0	1	5	30	0.23
10	0	0	0	2.5	6.5	30	0.26
11	0	0	0	2.5	6.5	30	0.26
12	0	0	0	2.5	6.5	30	0.27
13	-1	0	+1	1	6.5	40	0.23
14	-1	+1	0	1	8	30	0.20
15	+1	0	+1	4	6.5	40	0.24

* CelA-Ole, Ole-CelK, and Ole-Gls in cell pellets was 200 μg in total for assembly of AOBs. The target proteins (50 μg in total) involved in AOBs were utilized to determine the enzyme activity.

Table S4. Result of ANOVA for the Box-Behnken design.

Factor	Parameter estimate	Standard error	*P*-value
X_1	-0.0025	0.0057	0.6812
X_2	-0.0300	0.0057	0.0034
X_3	0.0025	0.0057	0.6812
$X_1 \times X_2$	-0.0075	0.0081	0.3977
$X_1 \times X_3$	-0.0025	0.0081	0.7704
$X_2 \times X_3$	-0.0025	0.0081	0.7704
$X_1 \times X_1$	-0.0179	0.0084	0.0873
$X_2 \times X_2$	-0.0429	0.0084	0.0038
$X_3 \times X_3$	-0.0129	0.0084	0.1867

Table S5. Optimization of the reaction condition for AOBs-bound cellulase using the CCD method.

Trial	Coded levels (Z_1 = pH ; Z_2 = temperature)		Actual levels		Response: enzyme activity (g/l-h)
	Z_1	Z_2	Z_1	Z_2 (^oC)	
1	0	-0.75	6	46	0.10
2	0	0	6	60	0.32
3	-0.75	0	4.6	60	0.015
4	0	0	6	60	0.34
5	+1	+1	7	70	0.29
6	-1	+1	5	70	0.27
7	0	0	6	60	0.34
8	+1	-1	7	50	0.14
9	0	0	6	60	0.34
10	0	0	6	60	0.34
11	0	+1.1	6	74	0.31
12	-1	-1	5	50	0.18
13	+1.1	0	7.4	60	0.22

Table S6. Result of ANOVA for the CCD method.

Factor	Parameter estimate	Standard error	*P*-value
Z_1	0.0337	0.0187	0.1146
Z_2	0.0671	0.0187	0.0089
Z_1 x Z_2	0.0150	0.0265	0.5888
Z_1 x Z_1	-0.0946	0.0201	0.0022
Z_2 x Z_2	-0.0508	0.0201	0.0392