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Optimization of Washing Conditions with Lipase by Response Surface Methodology

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Abstract: Response surface methodology (RSM) was employed to optimize the washing conditions. A four-factorial three-level Box-Behnken partial orthogonal design was chosen to explain the washing process based on four critical factors, detergent concentration, lipase concentration, washing temperature and buffer pH. According to the responses from the experimental model, the effects of each variable were assessed and the interactions between variables were also determined. The experiment suggested that the optimal conditions for removal of olive oil from the cotton fabrics were 0.60g/L detergent, 4.37g/L lipase, washing temperature of 25.06 °C and buffer pH of 7.29. By actual washing confirmation, the wetting time of the washed fabric under the optimal condition was 0.51s. Home laundry could be conducted at low temperature with addition of lipase.

1 Introduction

Detergent enzymes account for about 32% of the total world-wide enzyme production [1]. Lipases are added to laundry detergents to enhance removal of lipid soils from fabric by catalyzing the breakdown of oils with subsequent hydrophilic release of fatty acids, diacylglycerols, monoglycerols, and glycerol [2].

Response surface methodology (RSM) is a collection of mathematical and statistical techniques widely used to optimize different biotechnological processes [3]. Factorial design of a limited set of variables is advantageous compared to the conventional method, which handles single parameter per trial and as an approach; this frequently fails to consider the effect of possible interactions between factors [4]. Application of lipase using response surface methodology has been reported for esterification, transesterification, interesterification and biodiesel production [5].

In this paper, an attempt was made to use Box-Behnken partial orthogonal design, a tool of RSM, for optimizing the washing conditions, which aimed at removing olive oil from the soiled cotton fabrics efficiently with the addition of lipase.

2 Experimental

2.1 Materials

The cotton fabrics used in this study were supplied by Dyeing and Finishing Professional Laboratory, Zhejiang Sci-Tech University. The detergent used in laundry was supported by P&G , branch in Beijing. Olive oil (CP, Sinopharm Chemical Reagent Co., Ltd., China); Lipase (LJP30031-B, Novozymes Biotechnology Co., Ltd., China). All the other solvents and reagents were of analytical grade.

2.2 Preparation of soiled fabric and washing solutions

The cotton fabric (12cm×12cm) was soiled by spotting with 0.05mL of olive oil in six different sections. After ten minutes, the olive oil penetrated into the fabric adequately and mark the oil outline in the cotton fabric.

The washing solutions were prepared as shown in Table 1.

Table 1 Composition of the washing solutions

Constituents	Volume (ml)
0.05M Tris-Hcl buffer	40
Detergent solution (g/L)	50
Lipase solution (g/L)	10

2.3 Washing procedure

The soiled fabrics were washed with shaking at 100 rev/min using the prepared washing solutions. At the end of 20 min, the fabrics were removed and rinsed thrice with 100 ml of water, each for a period of 2 min and then dried at 40℃ in oven.

2.4 Determination of wetting time

According to AATCC 79-2007 standard, dripping method was quoted to estimate the wetting ability of the washed fabrics. Fix the fabric in the frame, adjust the height from the fabric surface to the burette was 1mm. Distilled water was dropped and recorded the time until no reflectance was observed in the cotton surface. Each fabric was measured five times in different sections and took average finally.

2.5 Factorial design

A four-factorial three-level Box-Behnken partial orthogonal design was chosen to explain the washing process based on four critical factors, viz. detergent concentration(X_1), lipase concentration(X_2), washing temperature(X_3) and buffer pH(X_4), as shown in Table 2.

Table 2 Range of variables for the factorial design

Variables	Symbols	Levels		
		-1.0	0	+1.0
Detergent solution (g/L)	X_1	0.6	0.9	1.2
Lipase solution (g/L)	X_2	1.0	4.0	8.0
Washing temperature (℃)	X_3	25	37	55
Buffer pH	X_4	7.2	8.1	9.0

The relationship between the variation of the response, T (wetting time) and the variation of factors X_1, X_2, X_3 and X_4, was represented by Box-Behnken model using the following equation:

$$T=\beta_0+\sum_{i=1}^{n}\beta_i x_i+\sum_{i=j=1}^{n}\beta_{ij}x_i x_j \quad (1)$$

When the n was equal to four, the above equation was unfolded as followed:

$$T = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 \\ + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 \quad (2)$$

To know the exact coefficients, twenty-nine groups of data were needed. Statistical design and analysis of the model was performed using the 'Design Expert' software package (Version 8.05b, 2011).

3 Results and Discussion

3.1 Wetting time of the fabrics after washing

The experimental results of wetting time using Box-Behnken partial orthogonal design were shown in Table 3.

Table 3 Composition of the various runs of the experimental design and the wetting time using lipase

Runs	Detergent concentration (g/L)	Lipase concentration (g/L)	Washing temperature (℃)	Buffer pH	Wetting time (s)	
					observed	predicted
1	0.9	4.5	55	9.0	2.41	2.22
2	1.2	8.0	40	8.1	5.79	5.51
3	1.2	4.5	40	7.2	4.87	4.65
4	0.9	8.0	40	7.2	4.38	4.56
5	1.2	4.5	55	8.1	2.05	1.66
6	0.9	8.0	40	9.0	5.59	5.16
7	0.9	4.5	40	8.1	4.52	4.59
8	0.9	1.0	40	9.0	4.53	4.33
9	0.9	4.5	40	8.1	4.44	4.59
10	0.6	4.5	55	8.1	2.59	2.48
11	0.6	1.0	40	8.1	5.30	4.95
12	0.9	1.0	55	8.1	2.17	2.16
13	0.9	4.5	40	8.1	4.56	4.59
14	0.9	1.0	25	8.1	1.76	1.57
15	0.9	4.5	40	8.1	4.55	4.59
16	0.6	8.0	40	8.1	5.51	4.55
17	0.6	4.5	40	9.0	5.22	6.09
18	0.9	1.0	40	7.2	2.76	3.17
19	0.9	4.5	40	8.1	4.89	4.59
20	0.9	8.0	55	8.1	1.93	2.77
21	1.2	4.5	40	9.0	2.15	2.61
22	0.6	4.5	40	7.2	2.10	2.33
23	0.9	8.0	25	8.1	2.53	3.19
24	0.6	4.5	25	8.1	1.76	2.13
25	0.9	4.5	25	9.0	2.88	2.38
26	0.9	4.5	25	7.2	1.71	1.58
27	1.2	4.5	25	8.1	1.74	1.84
28	0.9	4.5	25	7.2	1.69	1.26
29	1.2	1.0	40	8.1	2.54	2.88

3.2 Variance analysis of the regression model

Depend on the data in Table 3, a specific quadratic was given by the software as followed:

$$T=4.59-0.28X_1+0.56X_2+0.042X_3+0.44X_4-0.26X_1^2+0.14X_2^2-2.31X_3^2-0.42X_4^2+0.76 X_1X_2-0.13 X_1X_3-1.46 X_1X_4-0.25 X_2X_3-0.14 X_2X_4-0.12 X_3X_4 \quad (3)$$

The adequacy of the model was evaluated by ANOVA, as shown in Table 4.

Table 4 ANOVA for quadratic model

Source	Sum of squares	Degrees of freedom	Mean square	F value	P>F
Model	55.17	14	3.94	11.47	<0.0001
Residual	4.81	14	0.34		
Lack of fit	4.69	10	0.47	15.65	0.0087
Pure error	0.12	4	0.030		
Total	59.98	28			
R^2=0.9198	AdjR^2=0.8396				

The ANOVA for the quadratic model was highly significant with an F value of 11.47 as shown by Fisher's test, along with a very low probability value (P_{model}>F=0.0001), which was significant at 95% confidence interval. The adjust determination coefficient (AdjR^2) of the model was 0.8396 indicating that 83.96% of variability in the response could be accounted by the model and it showed that the model was suitable to represent the real relationship among the selected factors. The insignificant lack of fit test also indicated that the model was reasonable.

3.3 Optimization of washing conditions using RSM

Compared with conventional orthogonal design, RSM can not only show the single effect of the selected factors, but also illustrate the interactive influence between different variables as shown in Figure 1 to 3.

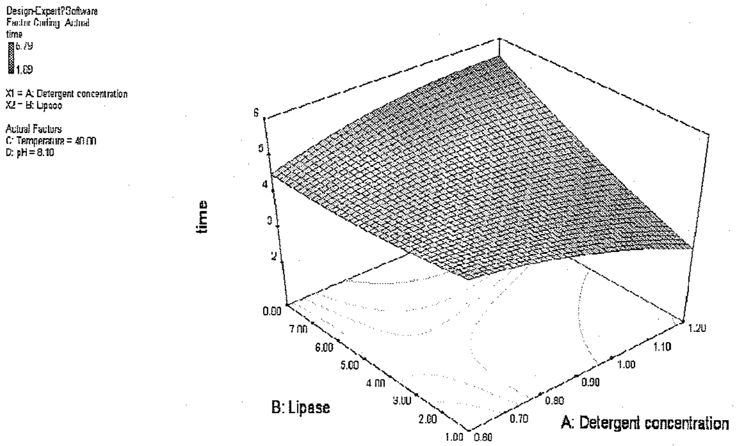

Figure 1 Response surface showing the effect of detergent concentration and lipase concentration on the wetting time of the washed cotton fabrics

The effect of detergent concentration and lipase concentration on the wetting time of the washed cotton fabrics at 40℃ and pH of 8.10 was shown in response surface of Figure 1. The wetting time was optimum when the detergent concentration was 1.10-1.20g/L and the lipase concentration was 1.00-2.00g/L. When the detergent concentration was reduced from 1.20g/L to 0.95g/L, decreasing the concentration of lipase had a significant effect on wetting time, because the detergent and lipase had mutual suppression when both were of high concentration. However, when the detergent concentration was 0.60g/L to 0.95g/L, the change of detergent concentration had remarkable influence on the washing effect, because the ability of lipase was not the largest since the detergent concentration was in the middle level and the interaction between detergent and lipase was not so obvious.

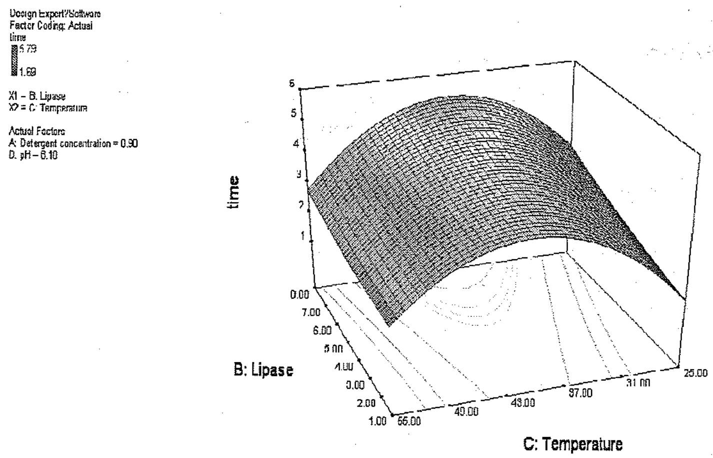

Figure 2 Response surface showing the effect of washing temperature and lipase concentration on the wetting time of the washed cotton fabrics

The effect of washing temperature and lipase concentration on the wetting time of the washed cotton fabrics at detergent concentration of 0.90g/L and pH of 8.10 was shown in response surface of Figure 2. The wetting time was optimum when the washing temperature was 25-31°C and the lipase concentration was 1.00-2.00g/L. When the washing temperature was increased from 25°C to 55°C, the wetting time increased firstly, then decreased and had a minimum when the temperature were 25°C and 55°C, so the ideal washing temperature should be 25°C in order to save energy and had a better lipase ability.

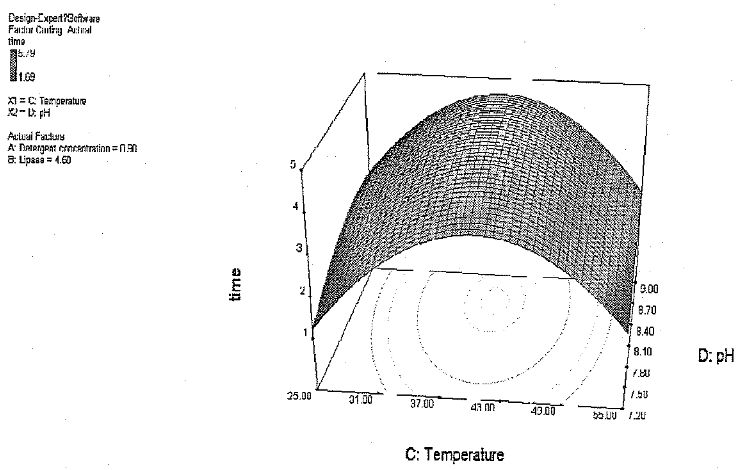

Figure 3 Response surface showing the effect of washing temperature and buffer pH on the wetting time of the washed cotton fabrics

The effect of washing temperature and buffer pH on the wetting time of the washed cotton fabrics at detergent concentration of 0.90g/L and lipase concentration of 4.50g/L was shown in response surface of Figure 3. The wetting time was optimum when the washing temperature was 25-31℃ and the buffer pH was 7.2-7.8. The change of washing temperature had a significant effect on wetting time as shown above, due to the remarkable influence on lipase ability, besides the wetting time was ideal at low washing temperature which was same as the conclusion of Figure 2. Otherwise, the change of buffer pH had a insignificant effect on wetting time, because lipase acted well in the range of weak alkaline environment.

3.4 Confirmation of the washing conditions optimization

On the basis of model fitting, actual washing process was carried out to confirm the washing conditions optimization, the concrete conditions were given by the 'Design Expert' software package (Version 8.05b, 2011) and the results of wetting time were as followed:

Table 5 Confirmation of the washing conditions optimization

Runs	Detergent concentration (g/L)	Lipase concentration (g/L)	Washing temperature (°C)	Buffer pH	Wetting time (s)	Expectations
1	0.60	4.40	25.91	7.20	0.59	1.000
2	0.60	4.94	25.88	7.20	0.58	1.000
3	1.20	4.02	54.99	9.00	0.99	1.000
4	1.20	2.38	54.20	8.92	0.94	1.000
5	1.20	2.57	25.02	8.88	0.93	1.000
6	0.61	3.17	25.96	7.20	0.56	1.000
7	0.60	4.37	25.06	7.29	0.51	1.000
8	1.20	3.27	54.70	8.96	0.96	1.000
9	0.61	6.39	25.03	7.23	0.79	1.000
10	1.19	3.04	54.90	8.94	0.90	1.000
11	0.67	1.01	25.15	7.20	0.72	1.000
12	0.61	4.85	25.68	7.20	0.76	1.000
13	0.60	6.31	25.15	7.24	0.77	1.000

From the table, the results were obvious, the seventh group had the best washing effect, namely the wetting time of the washed cotton fabric was 0.51s, so the optimal washing conditions were as followed: 0.60g/L detergent, 4.37g/L lipase, washing temperature of 25.06℃, buffer pH of 7.29.

4 Conclusions

The Box-Behnken model, a tool of RSM, was suitable to represent the real relationship among the selected factors, viz. detergent concentration, lipase concentration, washing temperature and buffer pH. The mutual effect between two factors was illustrated in response surface plot. The optimal washing conditions were as follows: 0.60g/L detergent, 4.37g/L lipase, washing temperature of 25.06℃, buffer pH of 7.29. The wetting time of the washed cotton fabric was 0.51s under the optimal conditions. The ideal laundry could be conducted under low temperature with the addition of lipase.

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