Optimization of β-Mannanase Production from *Bacillus licheniformis* TJ-101 Using Response Surface Methodology

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Plackett-Burman design, central composite design and response surface analysis were carried out with the aim of optimizing culture conditions for β-mannanase production from *Bacillus licheniformis* TJ-101. Screening experiments of Plackett-Burman design were firstly employed to evaluate the effects of 16 variables on β-mannanase production. The four identified significant variables, i.e. the concentration of Na_2HPO_4, the concentration of KH_2PO_4, initial pH and medium volume, were further optimized by central composite design and response surface analysis. The optimum values of four critical variables were determined as Na_2HPO_4 6.4 g L^{-1}, KH_2PO_4 0.36 g L^{-1}, initial pH 7.7 and medium volume V = 30.1 mL. Under these conditions, the β-mannanase activity can experimentally reach $a = 523.1 \pm 5.9$ U mL^{-1} (127.7 % increase compared with the enzyme activity before optimization) at the flask level. Moreover, oxygen limitation and initial pH remarkably affected β-mannanase production from *Bacillus licheniformis* TJ-101 as revealed by response surface analysis. Maximum β-mannanase activity increased by 73.7 % in a 6.6 L fermenter using the optimized medium and dissolved oxygen at 20 % saturation.

Key words:

β-Mannanase, *Bacillus licheniformis*, Plackett-Burman design, central composite design, response surface methodology

Introduction

β-Mannanase (β-1,4-D-mannan mannohydrolase; EC 3.2.1.78) is an important hemicellulase, because it plays a key role in randomly cleaving internal β-1,4-D-mannopyranosyl linkages of galactomannan, glucomannan, galactoglucomannan and mannan to produce oligosaccharides such as functional mannan-oligosaccharide with a degree of polymerization of 6–7.[1] Mannanases can be extracted from germinating seeds of terrestrial plants, blue mussel and animals. They have also been effectively prepared from various microorganisms (bacteria, yeast, fungi and so on) by fermentation.[2,5–6] Since several β-mannanases were successfully isolated and characterized from *Aspergillus* sp., *Bacillus* sp., *Bacillus licheniformis*, *fungi* and *Trichoderma harzianum,*[3–4,7–10] the versatile β-mannanases have been widely used in the food, feed, oil and gas, as well as pulp and paper industries.[11–13]

In order to prepare industrial enzymes, the traditional 'one-factor-at-a-time' method was frequently applied to optimize medium composition and production conditions. Because the combined effects of all involved factors are not taken into consideration,[14] unfortunately, this technique is a time-consuming process and sometimes leads to unreliable optimum values of the experiments. These limitations can be overcome by response surface methodology (RSM) to optimize all interaction effects with a minimum number of experiments.[15] Statistical experimental design of RSM has also been used successfully in optimizing fermentation media[16] and the phase system composition of the two-phase system.[17] However, RSM is only reliable for a small number of variables. When the number of variables is more than 5 in statistical optimization of fermentation conditions, the Plackett-Burman design is recommended to find out the significant variables to guarantee the reliabilities of RSM.[18]

In our previous work, the production and purification of neutral β-mannanase from *Bacillus licheniformis* as well as the kinetics of fermentation process were studied for industrial use.[4,8,19,20] It is indispensable to improve strain and optimize fermentation conditions, for reducing cost and increasing productivity are the keys to commercializing most bio-products. A good mutant from UV and heat mutagenesis, *Bacillus licheniformis* TJ-101,

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has been isolated recently. In the present work, the Plackett-Burman design, central composite design and response surface analysis were applied to the optimization of submerged culture conditions to enhance β-mannanase production for the first time. The results of RSM optimization were effectively validated in a flask level and were employed to guide β-mannanase production in a V = 6.6 L fermenter.

Materials and methods

Microorganism, medium and fermentation

A strain of *Bacillus licheniformis* TJ-101 was obtained from *Bacillus licheniformis* with combined UV-heat mutagenesis method,[21] and was maintained at 4 °C on Luria-Bertani (LB) agar slants containing γ = 20 g L^{-1} agar.

Initial culture medium[20] (pH 7.5) with a composition (in g L^{-1}) of konjac powder, 30; meat peptone, 30; corn steep liquor, 5.0; $(NH_4)_2SO_4$, 5.0; Na_2HPO_4, 4.0; KH_2PO_4, 0.30; $MgCl_2$, 0.60; $CaCl_2$, 3.0; $FeSO_4$, 0.01 and Na_2CO_3, 3.0 was sterilized by autoclaving at 121 °C for 30 min, and was cooled to room temperature. The medium composition was progressively modified using statistical design method in the results and discussion section. The optimized medium with a composition (in g L^{-1}) of konjac powder, 30; meat peptone, 30; corn steep liquor, 5.0; $(NH_4)_2SO_4$, 5.0; Na_2HPO_4, 6.4; KH_2PO_4, 0.36; $MgCl_2$, 0.60; $CaCl_2$, 3.0; $FeSO_4$, 0.01 and Na_2CO_3, 3.0 at initial pH of 7.7 was used in the validation experiments.

The inoculum was prepared using the above initial medium according to the procedure described by Feng *et al.*[19] The 500 mL flask fermentation experiments were performed in terms of scheduled medium and cultivation conditions by statistical experimental designs. In the verifying experiments, 30.1 mL of optimized medium with pH of 7.7 was inoculated and cultured at 30 °C and 180 rpm for 48 h. In this work, 5 L modified medium in a 6.6 L fermenter was inoculated after autoclave and fermented at 30 °C and 20 % dissolved oxygen with aeration rate of 0.75 vvm (vol. of air per vol. of medium and per min) for 42 h.

Analysis

Cell mass concentration was determined using the method of Feng *et al.*[19] β-Mannanase activity was determined by mixing 0.1 mL of suitably diluted enzyme solution with 0.9 mL of locust bean galactomannan (w = 0.5 %) in phosphate buffer (c = 50 mmol L^{-1}, pH 7.5) at 60 °C for 10 min.[22] The amount of released reducing sugar was measured via the 3,5-dinitrosalicylic acid (DNS) method. One unit of enzyme activity was defined as the amount of β-mannanase that liberated 1 μmol mannose per minute under the above conditions.

Experimental design and optimization

The culture conditions for β-mannanase production from *Bacillus licheniformis* TJ-101 were optimized via two stages: Plackett-Burman design and central composite design. The 'Design Expert' software 7.0 (Stat-Ease Inc., Minneapolis, USA) was used for optimization.

(a) Plackett-Burman Design

In the first stage, a Plackett-Burman design for 19 variables, including media composition, culture conditions and three dummy variables at two levels (–1 and +1) in 20 trials, was used for screening the relatively important variables on β-mannanase production (Table 1). The chosen levels (–1 and +1) were based on our previous fermentation study.[20] In this experimental design, the interaction effects may be neglected, and a linear function is used for screening as follows:[23]

Table 1 – *Values of variables at different levels in Plackett-Burman design for β-mannanase production from Bacillus licheniformis TJ-101*

Variables	Fermentation conditions	High level (+1)	Low level (–1)
X_1	konjac powder, γ/g L^{-1}	35	25
X_2	bone peptone, γ/g L^{-1}	35	25
X_3	corn steep liquid, γ/g L^{-1}	7.0	3.0
X_4	$(NH_4)_2SO_4$, γ/g L^{-1}	7.0	3.0
X_5	Na_2HPO_4, γ/g L^{-1}	6.0	2.0
X_6	KH_2PO_4, γ/g L^{-1}	0.4	0.2
X_7	$MgCl_2 \cdot 6H_2O$, γ/g L^{-1}	0.8	0.4
X_8	$CaCl_2$, γ/g L^{-1}	4.0	2.0
X_9	$FeSO_4$, γ/g L^{-1}	0.02	0.00
X_{10}	Na_2CO_3, γ/g L^{-1}	4.0	2.0
X_{11}	pH before sterilization	8.0	6.0
X_{12}	temperature, θ/°C	35	25
X_{13}	cultivating time, t/h	52	44
X_{14}	medium volume, V/mL	60	40
X_{15}	shaking speed, rpm	200	160
X_{16}	inoculum fraction, φ/%	4.0	2.0
X_{17}, X_{18}, X_{19}	dummy variables	–	–

$$a = \beta_0 + \sum \beta_i X_i \qquad (i=1\ldots\ldots k) \qquad (1)$$

where a is the β-mannanase activity, X_i are 19 evaluated variables as shown in Table 1, and β_i are the regression coefficients reflecting the main effects of X_i on a. The effect levels of each tested variable $E(X_i)$ may be calculated by the following equation:

$$E(X_i) = \frac{2(\Sigma X_{i,\max} - X_{i,\min})}{N} \qquad (2)$$

where, $X_{i,\max}$ and $X_{i,\min}$ are the β-mannanase productions presented respectively at the maximum and minimum level for each measured variable, and N is the number of experiments.

(b) Central composite design (CCD)

Table 2 – *Experimental range and levels of four key variables used in a 2^4 full factorial central composite design*

Variables	Fermentation conditions	Range and levels				
		–2	–1	0	1	2
X_1	Na_2HPO_4 (g L^{-1})	3	4.5	6.0	7.5	9.0
X_2	KH_2PO_4 (g L^{-1})	0.2	0.3	0.4	0.5	0.6
X_3	initial pH	7	7.5	8	8.5	9
X_4	medium volume (mL)	20	30	40	50	60

CCD which was introduced by Box and Wilson[24] is one of the most popular experiment designs in response surface methodology. In the second stage, a set of 30 experiments from CCD was performed to establish an empirical model of the fermentation process and to estimate the precise optimum of four critical culture conditions, i.e. the concentration of Na_2HPO_4, the concentration of KH_2PO_4, initial pH and medium volume. The effect of each variable on enzyme production was studied at five different levels (Table 2), and four variables were coded according to eq. (3):

$$x_i = \frac{(X_i - X_i^0)}{\Delta X} \qquad i = 1,2,3,4 \qquad (3)$$

where x_i is the coded value of the tested variable X_i, X_i^0 is the value of X_i on the centre point, and ΔX is the step change value. To estimate the optimal point, the experimental results were fitted with a second order polynomial equation as follows:

$$a_i = \alpha_0 + \sum_1^4 \alpha_i X_i + \sum_1^4 \alpha_{ii} X_i^2 + \sum_1^4 i \sum_1^4 j \alpha_{ij} X_i X_j \qquad (4)$$

where a_i is the predicted enzyme activity; α_0 is the offset term; α_i is the ith linear coefficient; α_{ii} is the squared coefficient; and α_{ij} is the ijth interaction coefficient.

As a practical and professional tool, the Design-Expert was applied to analysis and evaluation of the obtained data as well as to sketching iso-response contour plots.

Results and discussion

Screening of important physico-chemical variables by Plackett-Burman design

Plackett-Burman design was used to analyze the effects of 19 variables (including three dummy) on the activity of β-mannanase from *Bacillus licheniformis* TJ-101 (Table 3). As shown in Table 4, four physico-chemical variables, namely the concentration of Na_2HPO_4, the concentration of KH_2PO_4, initial pH, and medium volume influenced the fermentation process significantly due to their high contribution, and were selected for further optimization by RSM in the second stage. The other four variables, composition of konjac flour, composition of corn steep liquor, shaking speed and fermentation time, were not chosen in view of the number (less than 5) of CCD experiments, although they also affected β-mannanase production to a certain extent. Furthermore, contributions of three dummy variables were calculated as 0.66 %, 1.53 % and 0.05 %, respectively in Table 4, which confirmed the validity of the Plackett-Burman design.

Optimization of four key variables by CCD

Na_2HPO_4, KH_2PO_4, initial pH and medium volume were identified as the most significant physico-chemical parameters for the production of β-mannanase from *Bacillus licheniformis* TJ-101. 30 experiments of CCD (Table 5), which included 2^4 trials (16 runs), a star configuration (8 runs) and six centre points (6 runs), were carried out to obtain a quadratic model.

The equation coefficients were calculated using Design Expert, and the result of the second order response surface model was obtained. To explain the production of β-mannanase, the following polynomial equation was established:

$$a = 490.38 + 0.66x_1 - 5.80x_2 - 17.03x_3 - 33.00x_4 - 0.81x_1x_2 - \\ - 4.09x_1x_3 - 11.94x_1x_4 + 5.71x_2x_3 + 1.43x_2x_4 + \\ + 12.67x_3x_4 - 37.70x_1^2 - 11.80x_2^2 - 35.35x_3^2 - 14.29x_4^2 \qquad (5)$$

where a is the response of β-mannanase activity; x_1, x_2, x_3 and x_4 are the coded values of Na_2HPO_4, KH_2PO_4, initial pH and medium volume, respectively.

Table 3 – *Plackett-Burman design method for screening the important effects of the tested variables*

Trail	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}	Enzyme activity a/U mL^{-1}
1	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	177.2
2	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	308.1
3	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	227.4
4	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	196.8
5	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	163.0
6	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	276.3
7	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	379.9
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	51.4
9	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	274.2
10	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	93.7
11	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	357.4
12	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	289.0
13	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	151.4
14	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	156.6
15	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	250.3
16	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	362.2
17	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	245.8
18	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	315.1
19	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	159.4
20	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	292.5

Table 4 – *Significance analysis for the effects of variables on β-mannanase production from Bacillus licheniformis TJ-101 in the Plackett-Burman design*

Variables	Fermentation conditions	$E(X_i)$	Contribution (%)	Significance
X_1	konjac flour (g L^{-1})	41.61	5.54	*
X_2	bone peptone (g L^{-1})	18.61	1.11	*
X_3	corn steep liquid (g L^{-1})	–42.17	5.69	*
X_4	$(NH_4)_2SO_4$ (g L^{-1})	18.15	1.05	*
X_5	Na_2HPO_4 (g L^{-1})	63.09	12.74	significant
X_6	KH_2PO_4 (g L^{-1})	75.95	18.46	significant
X_7	$MgCl_2 \cdot 6H_2O$ (g L^{-1})	37.79	4.57	*
X_8	$CaCl_2$ (g L^{-1})	35.33	3.99	*
X_9	$FeSO_4$ (g L^{-1})	6.17	0.12	*
X_{10}	Na_2CO_3 (g L^{-1})	–11.89	0.45	*
X_{11}	pH before sterilization	50.85	8.27	significant
X_{12}	temperature, θ/°C	31.77	3.23	*
X_{13}	cultivating time, t/h	48.33	7.47	*
X_{14}	medium volume, V/mL	–78.43	19.68	significant
X_{15}	shaking speed, rpm	40.55	5.26	*
X_{16}	inoculum fraction, φ/%	–5.95	0.11	*
X_{17}	dummy variables	14.37	0.66	*
X_{18}	dummy variables	21.87	1.53	*
X_{19}	dummy variables	3.97	0.05	*

*insignificant

Table 5 – *CCD plan for optimizing four critical variables*

Trial	Coded value				Enzyme activity a/U mL^{-1}	
	x_1	x_2	x_3	x_4	actual value	predicted value
1	–1	–1	–1	–1	447.1	449.4
2	–1	–1	–1	1	376.9	379.1
3	–1	–1	1	–1	386.3	386.7
4	–1	–1	1	1	366.2	367.1
5	–1	1	–1	–1	408.5	425.1
6	–1	1	–1	1	367.2	360.5
7	–1	1	1	–1	394.4	385.3
8	–1	1	1	1	382.7	371.4
9	1	–1	–1	–1	470.2	484.4
10	1	–1	–1	1	362.8	366.3
11	1	–1	1	–1	404.3	405.4
12	1	–1	1	1	351.7	338.0
13	1	1	–1	–1	463.4	456.9
14	1	1	–1	1	342.1	344.5
15	1	1	1	–1	400.0	400.7
16	1	1	1	1	346.9	339.0
17	–2	0	0	0	337.3	338.3
18	0	–2	0	0	461.6	454.8
19	0	0	–2	0	398.4	383.0
20	0	0	0	–2	510.5	499.2
21	2	0	0	0	339.2	340.9
22	0	2	0	0	422.1	431.6
23	0	0	2	0	296.9	314.9
24	0	0	0	2	353.3	367.2
25	0	0	0	0	487.2	490.4
26	0	0	0	0	493.4	490.4
27	0	0	0	0	495.5	490.4
28	0	0	0	0	476.3	490.4
29	0	0	0	0	490.1	490.4
30	0	0	0	0	499.8	490.4

The Student's t-value and corresponding P-value of each coefficient are listed in Table 6. The larger t-value and the smaller P-value indicate that the significance of coefficient is higher. In terms of this, the linear and squared effects of initial pH (x_3 and x_3^2) and medium volume (x_4 and x_4^2) in this case are highly significant (P-value < 0.0001),

Table 6 – *The least-squares fit and parameter estimate i.e. the significance of regression coefficient*[*]

Model term	Parameter estimate (α)	Standard error	Computed t-value	P-value
Intercept	490.38	5.17	94.85	–
x_1	0.66	2.59	0.25	0.8014
x_2	–5.80	2.59	–2.24	0.0404
x_3	–17.03	2.59	–6.58	< 0.0001
x_4	–33.00	2.59	–12.74	< 0.0001
x_1^2	–37.70	2.42	–15.58	< 0.0001
x_2^2	–11.80	2.42	–4.88	0.0002
x_3^2	–35.35	2.42	–14.61	< 0.0001
x_4^2	–14.29	2.42	–5.90	< 0.0001
x_1x_2	–0.81	3.17	–0.26	0.8026
x_1x_3	–4.09	3.17	–1.29	0.2159
x_1x_4	–11.94	3.17	–3.77	0.0019
x_2x_3	5.71	3.17	1.80	0.0919
x_2x_4	1.43	3.17	0.45	0.6579
x_3x_4	12.67	3.17	4.00	0.0012

[*] x_1, x_2, x_3 and x_4 are the coded values of Na_2HPO_4, KH_2PO_4, initial pH and medium volume, respectively

as well as the squared effect of Na_2HPO_4 (x_1^2). The high significance of the variables in the quadratic model indicates that these quantities will perform as limiting factors for their small variations can lead to great changes in β-mannanase production.

Analysis of variance (ANOVA) was performed for the established model (eq. (5)). As shown in Table 7, the F-value of β-mannanase production equation is 46.81, and the value of 'Prob (P) > F' is less than 0.0001, which indicates that the model is highly significant; Moreover, determination coefficient (R^2 = 0.9775) is close to 1 for a good statistical simulation, which implies that the model can explain 97.75 % variation in the response. In addition, the high value of the adjusted determination coefficient (0.9565) means high significance of the model, and the desired value of adequate precision (20.57) suggests a sufficient indication for the signal-noise ratio. Usually, the lower the value of variation coefficient (VC), the better the precision and reliability of the experiments. Here, the value of VC is 3.08 %, which demonstrates that the experiments of CCD are very reliable and precise.

The 2D contour plot and the 3D response surface curve were employed to understand how the significant factors influenced the production of

Table 7 – *ANOVA for the established quadratic model*[*]

Source	SS	DF	MS	*F*-value	Prob(*P*) > *F*
model	104 600	14	7471.9	46.51	< 0.0001
residual (error)	2 410	15	160.6		
lack of fit	2 077	10	207.7	3.12	0.1103
pure error	333	5	66.5		
total	107 010	29			

[*]Determination coefficient (R^2) = 0.9775; Adjusted R^2 = 0.9565; Variation coefficient (VC) = 3.08 %; Adequate precision = 20.57; SS, sum of square; DF, degrees of freedom; MS, mean square.

β-mannanase and they were also applied to determine the optimum values of four critical variables at the maximum response, as presented in Fig. 1. These plots describe that little change of initial pH and medium volume may largely alter the enzyme activity of β-mannanase. The significant effect of pH value can be explained in that the growth of *Bacillus licheniformis* TJ-101 largely depends on a suitable pH which has a direct influence on the tricarboxylic acid (TCA) cycle, where critical amino acids for cell growth and β-mannanase synthesis were secreted, in metabolic pathway of *Bacillus licheniformis*.[25] Furthermore, the activity

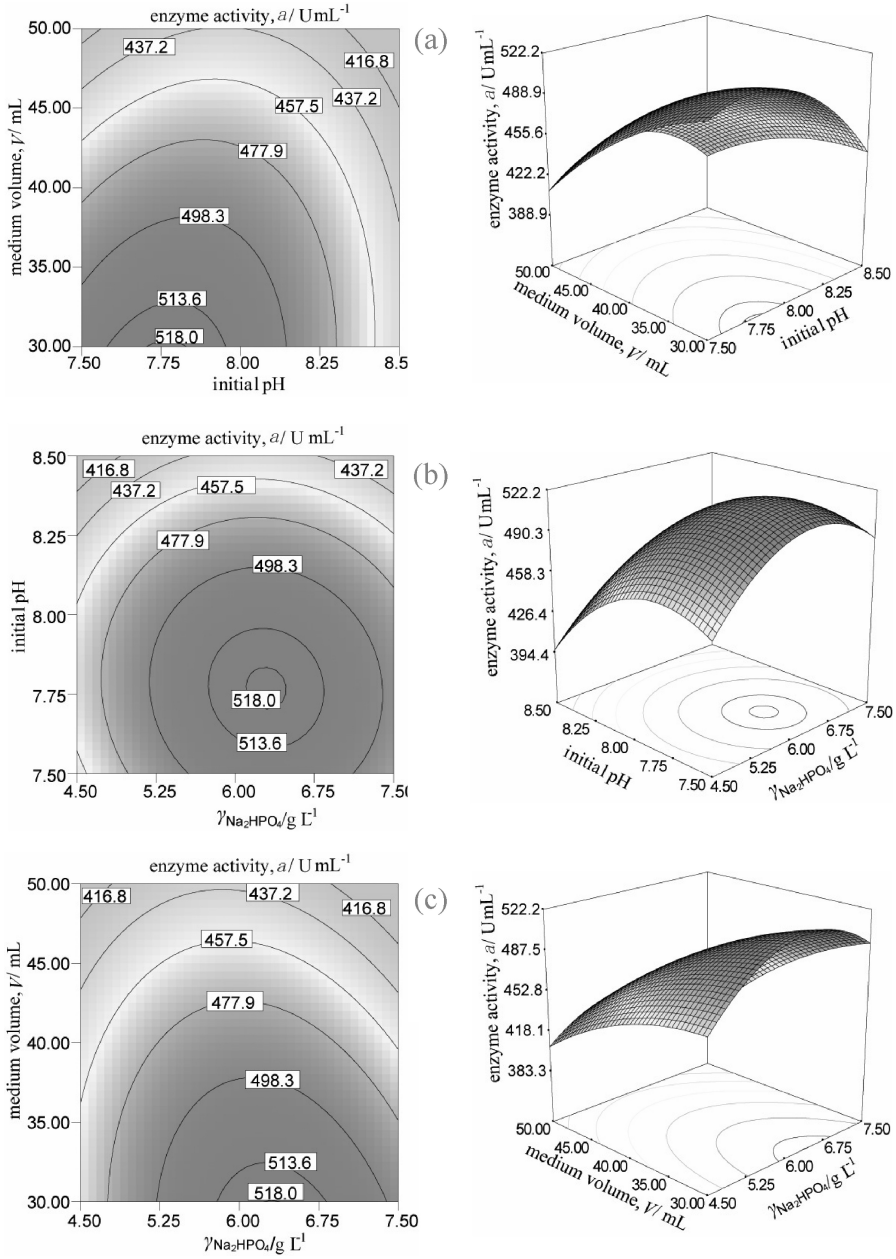

Fig. 1 – *2D contour plots and 3D response surface curves of the quadratic model for β-mannanase production by using CCD: (a) fixed KH_2PO_4 concentration and Na_2HPO_4 concentration at the optimum point of 0.36 g L^{-1} and 6.4 g L^{-1}, respectively; (b) fixed KH_2PO_4 concentration and medium volume at the optimum point of 0.36 g L^{-1} and 30.1 mL, respectively; (c) fixed KH_2PO_4 concentration and initial pH at the optimum point of 0.36 g L^{-1} and 7.7, respectively*

of β-mannanase remarkably increases with decreasing medium volume in that the low medium volume leads to high dissolved oxygen (DO) which may satisfy the demand of cell growth.[19] Generally, oxygen limitation can decrease the metabolic flux of TCA, and consequently reduce biosynthesis of some important amino acids.[26] In engineering, this problem can be solved largely by supplying high oxygen concentration gas or maintaining high dissolved oxygen concentration. According to our previous study,[19] dissolved oxygen fraction at 20 % saturation was the minimum requirement for the high cell mass concentration and enzyme activity. The ultimate solution of oxygen limitation may depend on studying how the amount of dissolved oxygen influences cell growth in the metabolic mechanism, and regulating the corresponding genes of *Bacillus licheniformis* using metabolic engineering to enhance β-mannanase production.

Based on eq. (5) and Fig. 1, the optimum conditions for maximizing the production of β-mannanase were obtained as: Na_2HPO_4 6.4 g L^{-1}, KH_2PO_4 0.36 g L^{-1}, initial pH 7.7 and medium volume of 30.1 mL, respectively, using Design Expert.

Validation of model

Under these optimum conditions, the highest activity of β-mannanase was predicted as 518.3 U mL^{-1} via eq. (5), and the maximum value was determined as 523.1 ± 5.9 U mL^{-1} by performing additional experiments in a flask level. It is obvious that these two values are very close. Compared with enzyme activity before optimization,[20] the maximum value (523.1 ± 5.9 U mL^{-1}) was increased by 127.7 % from 229.7 U mL^{-1}.

Based on the results of response surface analysis, β-mannanase was produced by *Bacillus licheniformis* TJ-101 in a 6.6 L auto-control fermenter at 20 % dissolved oxygen using the optimized medium. Under these conditions, shorter fermentation time, higher cell biomass and β-mannanase activity can be achieved compared with our previous work[19] (see Fig. 2). The maximum enzyme activity was determined as 370.9 ± 3.8 U mL^{-1} at 36 h, which increased by 73.7 % from 213.5 U mL^{-1} [19] in a 6.6 L fermenter level. The gap between the maximum enzyme activity in a flask (523.1 ± 5.9 U mL^{-1}) and in a fermenter (370.9 ± 3.8 U mL^{-1}) will probably be narrowed by statistically optimizing the operation parameters of the 6.6 L fermenter experiments, such as the flow rate of air, mixing speed, system pH and so on.

Conclusion

In this study, the optimal growth conditions for the production of β-mannanase from *Bacillus licheniformis* TJ-101 in a flask level were determined by using Plackett-Burman design and central composite design (CCD) as follows: Na_2HPO_4 6.4 g L^{-1}, KH_2PO_4 0.36 g L^{-1}, initial pH 7.7 and medium volume of 30.1 mL, respectively. Under the optimal conditions, the maximum activity of β-mannanase experimentally reached 523.1 ± 5.9 U mL^{-1}, which is close to the predicted value of RSM and increased by 127.7 % than before optimization (229.7 U mL^{-1}). Statistical analysis of results showed that initial pH and medium volume (dissolved oxygen) remarkably affected β-mannanase production from *Bacillus licheniformis* TJ-101. The 6.6 L fermenter experiment for β-mannanase production was performed at 20 % dissolved oxygen with the optimized medium, and the maximum enzyme activity increased by 73.7 % from 213.5 U mL^{-1} to 370.9 ± 3.8 U mL^{-1}.

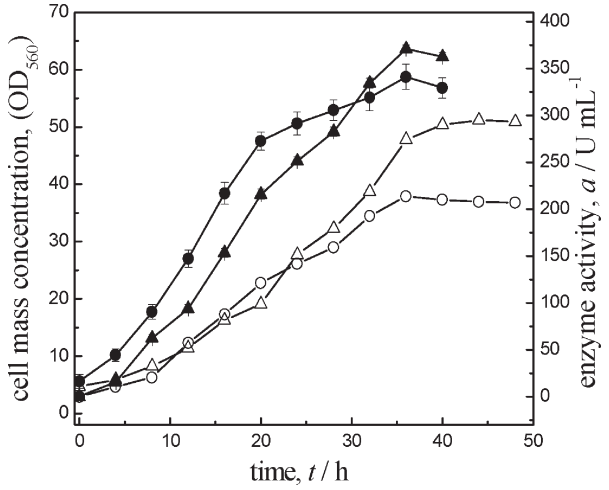

Fig. 2 – *Time course of cell growth and β-mannanase production using optimal medium in a 6.6 L fermenter at 20 % dissolved oxygen: (●) cell mass concentration; (▲) enzyme activity; (○) cell mass concentration in our previous work; (△) enzyme activity in our previous work*

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Figure captions

a – activity, U mL^{-1}
c – concentration, mmol L^{-1}
E – effect levels
F – *F*-value

N – number of experiments
R^2 – determination coefficient
t – time, h
V – volume, mL
w – mass fraction, %
X – variable
x – code value
α – coefficient
β – regression coefficient
γ – mass concentration, g L^{-1}
θ – temperature, °C
φ – volume fraction, %

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