Carbohydrate Polymers 86 (2011) 249–254

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Optimization of solid-state medium for the production of inulinase by *Aspergillus ficuum* JNSP5-06 using response surface methodology

Han-Qing Chen [a], Xiao-Ming Chen [b], Tian-Xiang Chen [b], Xue-Ming Xu [b], Zheng-Yu Jin [b,*]

[a] School of Biotechnology and Food Engineering, Hefei University of Technology, 193 Tunxi Road, Hefei, Anhui 230009, PR China
[b] State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu 214122, PR China

ARTICLE INFO

Article history:
Received 3 March 2011
Received in revised form 2 April 2011
Accepted 18 April 2011
Available online 28 April 2011

Keywords:
Response surface methodology
Optimization
Aspergillus ficuum JNSP5-06
Inulinase production
Solid-state fermentation

ABSTRACT

In the present study, response surface methodology (RSM) was employed to optimize the composition of medium based on Plackett–Burman design and Box–Behnken design for the production of inulinase by *Aspergillus ficuum* JNSP5-06 using solid-state fermentation (SSF). Inulin, $NH_4H_2PO_4$ and corn steep liquor were found to have significant effects on inulinase production by the Plackett–Burman design. The concentrations of the three compositions above were further optimized using a Box–Behnken design. The results showed that the final concentration of medium optimized with RSM was 11.47% inulin, 0.76% $NH_4H_2PO_4$ and 5.71% corn steep liquor by employing wheat bran as the solid substrate. Under optimized medium, the average inulinase activity reached 205.63 U/g initial dry substrate.

1. Introduction

Inulin occurs as a carbohydrate reserve mainly in the roots and tubers of Jerusalem artichoke, chicory, dandelion, burdock, and dahlia. It consists of linear chains of β-2,1-linked D-fructofuranose molecules terminated by a glucose residue through a sucrose-type linkage at the reducing end (Vandamme & Derycke, 1983). Recently, inulin has received attention as a relatively inexpensive and abundant material for the production of fructose and fructo-oligosaccharides, which are extensively used in pharmaceutical industry and food industry (Vandamme & Derycke, 1983).

Inulinases are potentially useful enzymes for the production of high fructose syrups from inulin (Ettalibi & Baratti, 1987). Fructose production by inulin hydrolysis is more advantageous than conventional process based on starch, which includes the action of α-amylase, amyloglucosidase, and glucose isomerase, yielding only 45% of fructose in the final product due to the thermodynamical equilibrium of the reaction. However, hydrolysis of inulin by inulinase can yield products with 95% of fructose (Kim & Rhee, 1989).

Traditionally, inulinase is produced by submerged fermentation (Gill, Sharma, Harchand, & Singh, 2003; Kalil, Suzan, Maugeri, & Rodrigues, 2001; Selvakumar & Pandey, 1999a). In recent years, inulinase can also be produced by solid-state fermentation (SSF) (Bender, Mazutti, Oliveira, Luccio, & Treichel, 2006; Chen, Wang, & Li, 2007; Mazutti, Bender, Treichel, & Luccio, 2006; Selvakumar & Pandey, 1999b).

Compared with submerged fermentation, SSF has many advantages for the production of enzymes such as high productivity, simple technique, low production cost, low energy requirement, less wastewater production, and better product recovery (Couto & Sanromán, 2006; Singhania, Patel, Soccol, & Pandey, 2009). Moreover, the crude fermented products from SSF can be used directly as the enzyme sources for biosynthesis and biotransformation.

In recent years, inulinase production by SSF has attracted more attention. Under the optimized conditions, inulinase activity of 391.9 U/g of dry fermented bagasse from *Kluyveromyces marxianus* NRRL Y-7571 was obtained by SSF (Mazutti et al., 2006). After the optimization of solid state medium for the production of inulinase by *Kluyveromyces* sp. S120 using response surface methodology, the average inulinase activity of 409.8 U/g initial dry substrate was achieved (Chen et al., 2007). Sheng et al. (2009) also reported that inulinase activity of 420.9 U/g of dry substrate was reached in the solid-state fermentation by marine yeast *Cryptococcus aureus* G7a.

In our previous study, a filamentous fungus, *Aspergillus ficuum* JNSP5-06 isolated from soil sample can produce inulinase (Chen, Chen, et al., 2009). However, the effect of solid-state fermentation on the production of inulinase by *A. ficuum* JNSP5-06 is still unknown.

Appropriate fermentation medium is very important for SSF, because medium composition can significantly affect product yield.

* Corresponding author. Tel.: +86 510 85913299; fax: +86 510 85913299.
E-mail addresses: hanqchen@yahoo.com.cn (H.-Q. Chen), jinlab2008@yahoo.com (Z.-Y. Jin).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.04.044

Table 1
The Plackett–Burman design for nutrient screening in inulinase production.

Variable	Nutrient	Levels		*t*-Value	$P>\|t\|$	Ranking
Nutrient code		Low (−1)	High (+1)			
A	$NH_4H_2PO_4$ (%)	0.4	0.8	5.7372	0.0105	2*
B	NaCl (%)	0.5	1.0	−0.98636	0.3967	6
C	KH_2PO_4 (%)	0.1	0.2	1.4452	0.2442	5
D	Inulin (%)	5.0	10.0	6.6252	0.0070	1**
E	$MgSO_4$ (%)	0.05	0.1	0.78861	0.4879	7
F	$ZnSO_4$ (%)	0.01	0.02	−1.6266	0.2023	4
G	Corn steep liquor (%)	3.0	6.0	4.4336	0.0213	3*
H	Tween-80 (%)	0.5	1.0	−0.75309	0.5061	8

* Statistically significant at 95% of probability level.
** Statistically significant at 99% of probability level.

The conventional change-one-factor-at-a-time approach, has been used for medium optimization, but it was laborious and time-consuming. Response surface methodology (RSM) can overcome the drawbacks of conventional method and has been extensively used in the optimization of fermentation media (Dutta, Dutta, & Banerjee, 2004; Gao et al., 2009; Li et al., 2008; Paseephol, Small, & Sherkat, 2007; Rao, Chul-Ho, & Rhee Sang-Ki, 2000; Reddy, Ramesh, Mrudula, Reddy, & Seenayya, 2003; Wei, Zheng, Liu, & Zhu, 1998; Xiong, Liu, Song, & Ji, 2004). RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions (Kalil, Maugeri, & Rodrigues, 2000). It is a statistically designed experimental protocol in which several variables are simultaneously varied. In RSM, the experimental responses of design of experiments are fitted to quadratic function.

In this study, RSM was used to optimize medium composition and culture condition for inulinase production by *A. ficuum* JNSP5-06 in solid-state fermentation.

2. Materials and methods

2.1. Microorganism and medium

A. ficuum JNSP5-06 was isolated from a soil sample (Chen, Chen, et al., 2009). The strain was maintained on agar slant medium at 4 °C. The medium contained the following components (g/L): glucose 15, yeast extract 10, peptone 10, agar 20.

2.2. Inoculum preparation

Inocula were prepared in a medium containing (g/L) yeast extract 10, peptone 20 and inulin 10. A loop of cells from the slant were transferred into a 250 mL conical flask containing 30 mL culture medium and incubated on a rotary shaker operating at 200 rpm and 30 °C for 24 h.

2.3. Solid-state fermentation

Wheat bran purchased from the local market was used as the solid substrate for inulinase production. Ten grams of wheat bran was supplemented with 12 mL of distilled water containing 10% inulin, 0.4% $NH_4H_2PO_4$, 0.5% NaCl, 0.05% $MgSO_4$, 0.01% $ZnSO_4$ on the basis of dry solid substrate. The substrates were set at initial pH 6.0 in 250 mL conical flasks, sealed with hydrophobic cotton and autoclaved at 121 °C for 30 min. The cooled substrates were inoculated with a 4% inoculum level, mixed carefully under strictly aseptic conditions with sterile glass rods, and then incubated in a chamber with relative humidity above 80% at 30 °C for 72 h in a static mode.

2.4. Extraction of inulinase

When fermentation was completed, a weighed quantity of the fermented matter was transferred to 250 mL conical flasks with the addition of 20 volumes of distilled water (w/v, based on initial dry weight of the substrate) and the mixture was mixed thoroughly on a rotary shaker (150 rpm) at room temperature (20 ± 2 °C) for 60 min. The mixtures were filtered through muslin cloth. After centrifugation of the filtrate at 1503 × *g* and at 4 °C for 10 min, the supernatant was collected as the crude enzyme solution and the total volume was recorded (Chen, Chen, Chen, Xu, & Jin, 2011).

2.5. Inulinase activity assay

The inulinase activity was determined by measuring the reducing sugars released from the hydrolysis of sucrose. Briefly, 0.2 mL of suitably diluted enzyme extract was mixed with 2 mL of sucrose solution (2%, w/w) in 0.2 mol/L acetate buffer (pH 4.5). The reaction was carried out at 50 °C for 30 min and then terminated by boiling for 5 min. The reducing sugar concentration of the reaction mixture was analyzed by DNS method (Kalil et al., 2001). One unit of inulinase activity was defined as the amount of enzyme that produces 1 μmol of reducing sugar per minute under the assay conditions used in this study.

2.6. Screening of the supplemental nutrients using a Plackett–Burman design

Plackett–Burman design, an efficient technique for medium component optimization (Naveena, Altaf, Bhadriah, & Reddy, 2005), was employed for screening fermentation parameters that significantly influenced inulinase production. Each independent variable was tested at two levels, high and low, which were denoted by (+) and (−), respectively. In the present study, the supplemental nutrients were screened by a Plackett–Burman design for eight variables at two levels.

2.7. Optimization of the supplemental nutrients using a Box–Behnken design

Once the critical variables were screened, a Box–Behnken design for three independent variables, each at three levels with three replicates at the center point (Francis et al., 2003), was employed to fit the polynomial models:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

where *Y* was the yield of inulinase, β_0 was the intercept term, β_1, β_2 and β_3 were linear coefficients, β_{12}, β_{13} and β_{23} were interaction

coefficients, β_{11}, β_{22} and β_{33} were squared coefficients, and X_1, X_2 and X_3 were coded independent variables.

2.8. Statistical analysis

SAS package (Version 8.0, SAS Institute Inc., Cary, NC, USA) was used for the experiment design and regression analysis. The statistical significance of regression coefficients was 95%. The optimum levels of variables were obtained by graphical analysis.

3. Results and discussion

3.1. Screening of significant nutrients using a Plackett–Burman design

Plackett–Burman design is a powerful technique for screening important variables. In the present study, it was used to analyze the effect of eight variables on inulinase production (Table 1). In the experiment design, each row represents an experiment and each column represents an independent variable. The sign + and − represent the two different levels (high and low) of the independent variable under investigation (Table 2). The yield of inulinase, determined for each experiment design, was shown in Table 2. The analysis of variance (ANOVA) for the experiment design was calculated, and the significant levels of each medium variable were determined by *t*-test (Table 1). It indicated that inulin, corn steep liquor and $NH_4H_2PO_4$ had significant influence on inulinase production (Table 1). All the other insignificant variables were neglected and optimum combination of these three variables was further analyzed by a Box–Behnken design.

3.2. Further optimization of the nutrients using a Box–Behnken design

Based on the results of Plackett–Burman design, three variables including inulin, corn steep liquor and $NH_4H_2PO_4$, which significantly influenced inulinase production, were further investigated for their optimum combination using a Box–Behnken design. The design and results of the experiments carried out by the Box–Behnken design are shown in Table 3. The results were analyzed by ANOVA on SAS package and the regression model was obtained as

$$Y_1 = 204.47 + 12.6625X_1 - 5.1875X_2 - 4.8525X_3 - 21.58375X_1^2 + 0.3825X_1X_2 - 0.7225X_1X_3 - 19.77375X_2^2 - 8.1125X_2X_3 - 21.45375X_3^2 \quad (2)$$

where Y_1 is the inulinase yield (%), X_1 is the inulin concentration (%), X_2 is the $NH_4H_2PO_4$ concentration and X_3 is the corn steep liquor concentration. The ANOVA of the quadratic regression model demonstrated that Eq. (2) was a highly significant model, as was evident from the Fisher's *F*-test with a very low probability value [($P_{model} > F$) = 0.0004082] (Table 4). The goodness of fit of the model was checked by determination coefficient (R^2). In this case, the R^2 value of 0.9861 indicated that 98.61% of the total variability in the response could be explained by this model. A regression model with $R^2 > 0.9$ was considered as having a very high correlation (Chen, Bai, et al., 2009). Therefore, the present R^2-value reflected a very good fit between the observed and predicted responses, and implied that the model is reliable for predicting inulinase production. The value of the adjusted determination coefficient (AdjR^2 = 0.9611) confirmed the significance of the model as well. Among model terms, X_1, X_1^2, X_2^2 and X_3^2 were very significant with a probability of 99%, while X_2, X_3 and X_2X_3 with a probability of 95% (Table 4).

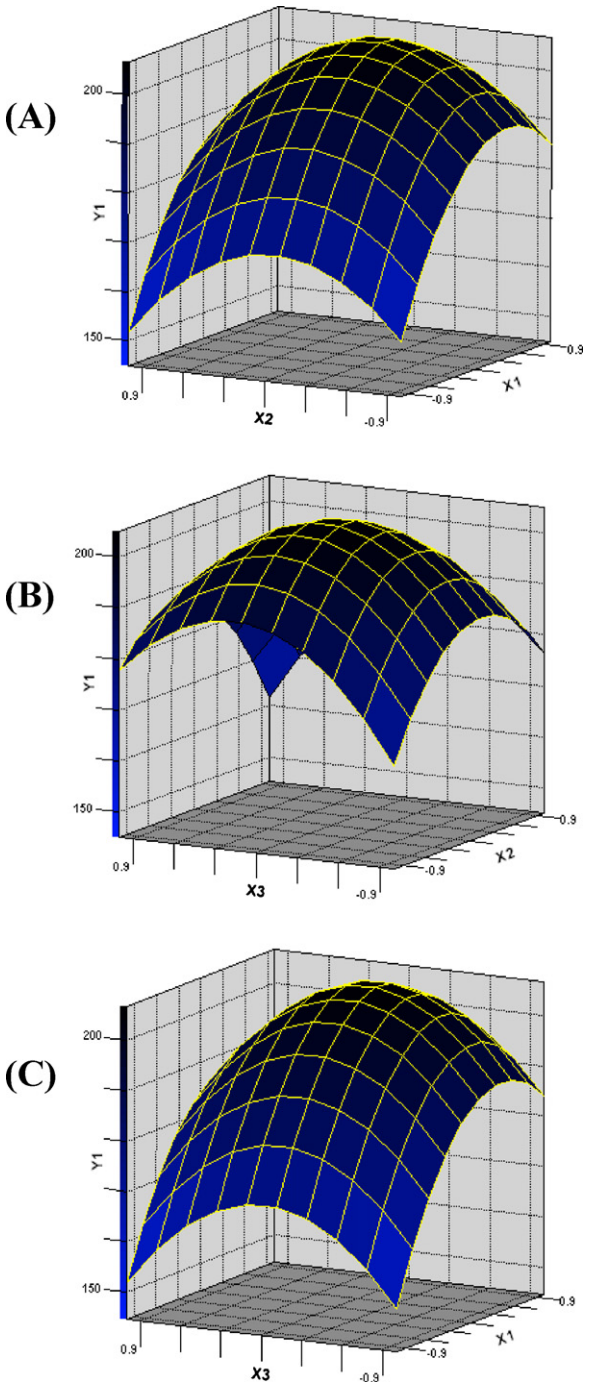

Fig. 1. Response surface plots of inulinase production. (A) Effect of inulin (X_1) and $NH_4H_2PO_4$ (X_2) on inulinase production (Y_1) (supplemented with 5.71% corn steep liquor). (B) Effect of $NH_4H_2PO_4$ (X_2) and corn steep liquor (X_3) on inulinase production (Y_1) (supplemented with 11.47% inulin). (C) Effect of inulin (X_1) and corn steep liquor (X_3) on inulinase production (Y_1) (supplemented with 0.76% $NH_4H_2PO_4$).

Table 4 also indicates that the interaction between X_2 and X_3 had significant influence on inulinase yield.

The response of inulinase production to the concentrations of inulin, $NH_4H_2PO_4$ and corn steep liquor for the above regression model is plotted in Fig. 1. Three-dimensional graphs were generated for the pair-wise combination of the three factors, while keeping the other one at their optimum levels. Graphs were given here to highlight the roles played by various factors and also to emphasize the roles played by the physical constraints *vis-à-vis* the biosynthetic aspects in the final yield of the inulinase.

Table 2
The Plackett–Burman design variables (in coded levels) with inulinase activity as response.

Run	Variable levels								Inulinase activity (U/gds[b])
	A[a]	B	C	D	E	F	G	H	
1	+1	+1	−1	+1	+1	−1	+1	−1	199.21
2	+1	−1	−1	−1	+1	+1	+1	−1	170.30
3	−1	+1	+1	+1	−1	+1	+1	−1	176.85
4	−1	+1	−1	−1	−1	+1	+1	+1	140.67
5	+1	−1	+1	+1	−1	+1	−1	−1	179.26
6	+1	−1	+1	−1	−1	−1	+1	+1	182.45
7	−1	+1	+1	−1	+1	−1	−1	−1	144.45
8	−1	−1	−1	−1	−1	−1	−1	−1	139.35
9	+1	+1	+1	−1	+1	+1	−1	+1	155.34
10	−1	−1	+1	+1	+1	−1	+1	+1	178.28
11	+1	+1	−1	+1	−1	−1	−1	+1	174.78
12	−1	−1	−1	+1	+1	+1	−1	+1	162.21

[a] The symbols were the same as those in Table 1.
[b] gds = grams of initial dry substrate.

The optimal concentrations for the maximum inulinase production based on the model were calculated and the value was 11.47%, 0.76% and 5.71% for inulin, $NH_4H_2PO_4$ and corn steep liquor, respectively. By substituting levels of the factors into the regression equation, the maximum predictable response for inulinase production was calculated and was experimentally verified. The maximum inulinase production obtained experimentally using the optimized medium was 205.63 U/gds, which was in agreement with the predicted value of 206.85 U/gds by the RSM regression study.

Chen et al. (2007) reported that the average inulinase activity (409.8 U/g of initial dry substrate) produced by *Kluyveromyces* sp. S120 was obtained after optimization of solid-state medium using RSM. Mazutti et al. (2006) also reported that under the optimized conditions obtained using RSM, the extra-cellular inulinase concentration in solid-state culture of *K. marxianus* NRRL Y-7571 reached

Table 3
The Box–Behnken design for optimizing nutrient concentration.

Run	Nutrient						Inulinase activity (U/gds)
	Coded levels			Actual levels			
	X_1	X_2	X_3	X_1	X_2	X_3	
1	−1	−1	0	5.0	0.4	6.0	158.65
2	−1	+1	0	5.0	1.2	6.0	148.23
3	+1	−1	0	15.0	0.4	6.0	177.23
4	+1	+1	0	15.0	1.2	6.0	168.34
5	0	−1	−1	10.0	0.4	3.0	164.23
6	0	−1	+1	10.0	0.4	9.0	173.35
7	0	+1	−1	10.0	1.2	3.0	169.36
8	0	+1	+1	10.0	1.2	9.0	146.03
9	−1	0	−1	5.0	0.8	3.0	151.21
10	+1	0	−1	15.0	0.8	3.0	183.96
11	−1	0	+1	5.0	0.8	9.0	140.35
12	+1	0	+1	15.0	0.8	9.0	170.21
13	0	0	0	10.0	0.8	6.0	205.14
14	0	0	0	10.0	0.8	6.0	203.95
15	0	0	0	10.0	0.8	6.0	204.32

X_1 = inulin (%), X_2 = $NH_4H_2PO_4$ (%), X_3 = corn steep liquor (%).

Table 4
Analysis of variance for the experimental results of the Box–Behnken design.

Factor[a]	Standard error	Sum of square	Degree of freedom	*F* value	*P*-value	Significance
X_1	1.4733	1282.711	1	73.8724	0.0003516	**
X_2	1.4733	215.2813	1	12.3982	0.0168991	*
X_3	1.4733	188.3741	1	10.8486	0.0216245	*
X_1^2	2.1686	1296.996	1	74.6951	0.0003425	**
X_2^2	2.1686	1219.994	1	70.2604	0.0003958	**
X_3^2	2.1686	1699.434	1	97.8718	0.0001800	**
$X_1 \times X_2$	2.0835	0.5852	1	0.0337	0.8615515	
$X_1 \times X_3$	2.0835	2.0880	1	0.1203	0.7428799	
$X_2 \times X_3$	2.0835	263.2606	1	15.1608	0.0114821	*
Model		6168.714	9	39.4735	0.0004082	**
Error		86.8194	5			
Total SS[b]		6255.5334	14			

[a] X_1 = inulin, X_2 = $NH_4H_2PO_4$, X_3 = corn steep liquor.
[b] Sum of square.
* Statistically significant at 95% of probability level.
** Statistically significant at 99% of probability level.

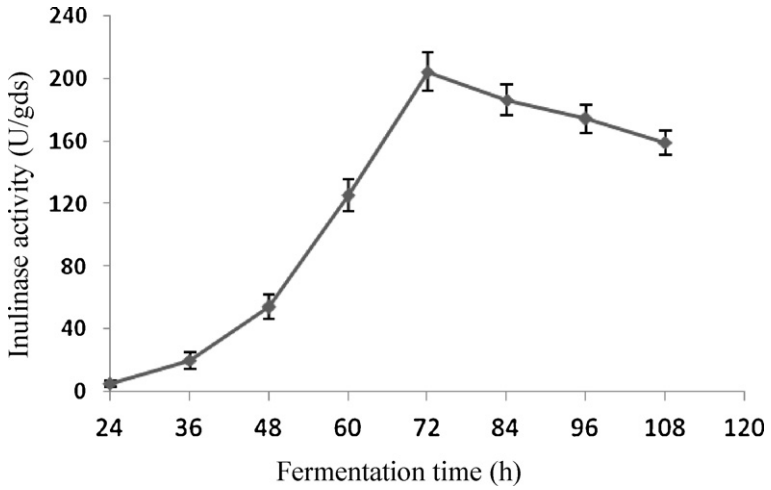

Fig. 2. Time course of the inulinase production by *Aspergillus ficuum* JNSP5-06 during the solid-state fermentation. Data are given as means ± SD, $n = 3$.

391.9 U/g of dry fermented bagasse after 96 h of fermentation. Moreover, Sheng et al. (2009) have shown that the highest inulinase production (420.9 U/g of dry substrate) by the marine yeast strain *C. aureus* G7a was reached within 120 h of the solid-state fermentation under the optimal conditions. However, in this study, we found that the maximum inulinase production of 205.63 U/g dry substrate was obtained in the solid-state fermentation culture of *A. ficuum* JNSP5-06 under the optimized medium, which was lower than that of previous studies, indicating that the difference of inulinase production may be related to the strains and compositions of solid state fermentation.

Carbon compounds are the sources of carbon skeleton and energy of microorganism cells, while nitrogen sources provide N element with protoplasm and other structure of cells. Inulin is not only a significant carbon source, but also an important inducer for inulinase production by *A. ficuum* JNSP5-06. However, higher inulin concentration can lead to catabolic repression, consequently lowering the enzyme yield, which is in agreement with Fig. 1A and C. Inulin is a relatively inexpensive and abundant substrate. It can also be replaced by the extraction of Jerusalem artichoke, chicory or dahlia in industry. Corn steep liquor, which provides nitrogen element, vitamins and other nutrients for medium, is an important nitrogen source for inulinase production by *A. ficuum* JNSP5-06. Corn steep liquor has been reported to be the best nitrogen source for inulinase production by *Actinomycete* strain (Gill et al., 2003) and *Kluyveromyces* sp. Y-85 (Wei et al., 1998) in submerged fermentation. On the other hand, higher concentration of corn steep liquor can also reduce the inulinase production as shown in Fig. 1B and C. This could be due to the complex nature of corn steep liquor and some of its constituents at higher concentration might have a toxic effect on inulinase production. NH_4^+ was observed to enhance the inulinase production (Table 1). However, higher concentration of ammonium was also found to inhibit inulinase synthesis, which is also in agreement with Fig. 1A and B, presumably because of the more release of ammonium ions. Therefore, with the increase in the amount of inulin, corn steep liquor or $NH_4H_2PO_4$, higher concentration components in medium would repress inulinase activity, although the cell growth usually slowly continued. On the other hand, with the decrease of the media components, both the cell growth and the inulinase production would also quickly decrease.

3.3. Time course of fermentation

The time courses of the inulinase production by *A. ficuum* JNSP5-06 during SSF under the optimal conditions obtained from RSM were monitored for 108 h. As shown in Fig. 2, after 36 h of fermentation, the production of inulinase dramatically increased. The inulinase production reached a maximum (204.2 U/gds) after 72 h of solid-state fermentation. It also suggested that inulinase produced by *A. ficuum* JNSP5-06 might be one example of a growth-associated product.

4. Conclusions

After the optimization by using RSM, the optimal medium composition for the inulinase production by *A. ficuum* JNSP5-06 during the solid-state fermentation were found to be 11.47% inulin, 0.76% $NH_4H_2PO_4$ and 5.71% corn steep liquor by employing wheat bran as the solid substrate. Under the optimized conditions, the maximum inulinase activity of 205.63 U/g of dry substrate was reached in the solid-state fermentation culture of *A. ficuum* JNSP5-06.

Acknowledgements

This study was supported by the National High Technology R & D Program of China (Grant no. 2006AA10Z333), Natural Science Foundation of Jiangsu Province (Grant no. BK2008003) and Research Program of State Key Laboratory of Food Science and Technology, Jiangnan University (Project No. SKLF-MB-200804).

References

Bender, J. P., Mazutti, M. A., Oliveira, D. D., Luccio, M. D., & Treichel, H. (2006). Inulinase production by *Kluyveromyces marxianus* NRRL Y-7571 using solid state fermentation. *Applied Biochemistry and Biotechnology*, *129–132*, 951–958.

Chen, H.-Q., Chen, X.-M., Chen, T.-X., Xu, X.-M., & Jin, Z.-Y. (2011). Extraction optimization of inulinase obtained by solid state fermentation of *Aspergillus ficuum* JNSP5-06. *Carbohydrate Polymers*, *85*, 446–451.

Chen, H. Q., Chen, X. M., Li, Y., Wang, J., Jin, Z. Y., Xu, X. M., et al. (2009). Purification and characterization of exo- and endo-inulinase from *Aspergillus ficuum* JNSP5-06. *Food Chemistry*, *115*, 1206–1212.

Chen, X., Wang, J., & Li, D. (2007). Optimization of solid-state medium for the production of inulinase by *Kluyveromyces* S120 using response surface methodology. *Biochemical Engineering Journal*, *34*, 179–184.

Chen, X. C., Bai, J. X., Cao, J. M., Li, Z. J., Xiong, J., Zhang, L., et al. (2009). Medium optimization for the production of cyclic adenosine 3′,5′-monophosphate by *Microbacterium* sp. no. 205 using response surface methodology. *Bioresource Technology*, *100*, 919–924.

Couto, S. R., & Sanromán, M. Á. (2006). Application of solid-state fermentation to food industry—A review. *Journal of Food Engineering*, *76*, 291–302.

Dutta, J. R., Dutta, P. K., & Banerjee, R. (2004). Optimization of culture parameters for extracellular protease production from a newly isolated *Pseudomonas* sp. using response surface and artificial neutral network models. *Process Biochemistry*, *39*, 2193–2198.

Ettalibi, M., & Baratti, J. C. (1987). Purification, properties and comparison of invertase, exoinulinase and endoinulinase of *Aspergillus ficuum*. *Applied Microbiology and Biotechnology*, *26*, 13–20.

Francis, F., Sabu, A., Nampoothiri, K. M., Ramachandran, S., Ghosh, S., Szakacs, G., et al. (2003). Use of response surface methodology for optimizing process parameters for the production of α-amylase by *Aspergillus oryzae*. *Biochemical Engineering Journal*, *15*, 107–115.

Gao, H., Liu, M., Liu, J., Dai, H., Zhou, X., Liu, X., et al. (2009). Medium optimization for the production of avermectin B1a by *Streptomyces avermitilis* 14-12A using response surface methodology. *Bioresource Technology*, *100*, 4012–4016.

Gill, P. K., Sharma, A. D., Harchand, R. K., & Singh, P. (2003). Effect of media supplements and culture conditions on inulinase production by an *Actinomycete* strain. *Bioresource Technology*, *87*, 359–362.

Kalil, S. J., Maugeri, F., & Rodrigues, M. I. (2000). Response surface analysis and simulation as a tool for bioprocess design and optimization. *Process Biochemistry*, *35*, 539–550.

Kalil, S. J., Suzan, R., Maugeri, F., & Rodrigues, M. I. (2001). Optimisation of inulinase production by *Kluyveromyces marxianus* using factorial design. *Applied Biochemistry and Biotechnology*, *94*, 257–264.

Kim, C. H., & Rhee, S. K. (1989). Fructose production from Jerusalem artichoke by inulinase immobilized on chitin. *Biotechnology Letters*, *11*, 201–206.

Li, X., Xu, T., Ma, X., Guo, K., Kai, L., Zhao, Y., et al. (2008). Optimization of culture conditions for production of *cis*-epoxysuccinic acid hydrolase using response surface methodology. *Bioresource Technology*, *99*, 5391–5396.

Mazutti, M., Bender, J. P., Treichel, H., & Luccio, M. D. (2006). Optimization of inulinase production by solid-state fermentation using sugarcane bagasse as substrate. *Enzyme and Microbial Technology*, *39*, 56–59.

Naveena, B. J., Altaf, M., Bhadriah, K., & Reddy, G. (2005). Selection of medium components by Plackett–Burman design for production of L(+) lactic acid by *Lactobacillus amylophilus* GV6 in SSF using wheat bran. *Bioresource Technology*, *96*, 485–490.

Paseephol, T., Small, D., & Sherkat, F. (2007). Process optimisation for fractionating Jerusalem artichoke fructans with ethanol using response surface methodology. *Food Chemistry*, *104*, 73–80.

Rao, J. K., Chul-Ho, K., & Rhee Sang-Ki, K. (2000). Statistical optimization of medium for the production of recombinant hirudin from *Saccharomyces cerevisiae* using response surface methodology. *Process Biochemistry*, *35*, 639–647.

Reddy, R. M. P., Ramesh, B., Mrudula, S., Reddy, G., & Seenayya, G. (2003). Production of thermostable β-amylase by *Clostridium thermosulfurogenes* SV2 in solid-state fermentation: Optimization of nutrient levels using response surface methodology. *Process Biochemistry*, *39*, 267–277.

Selvakumar, P., & Pandey, A. (1999a). Comparative studies on inulinase synthesis by *Staphylococcus* sp. and *Kluyveromyces marxianus* in submerged culture. *Bioresource Technology*, *94*, 123–127.

Selvakumar, P., & Pandey, A. (1999b). Solid state fermentation for the synthesis of inulinase from *Staphylococcus* sp. and *Kluyveromyces marxianus*. *Process Biochemistry*, *34*, 851–855.

Sheng, J., Chi, Z., Yan, K., Wang, X., Gong, F., & Li, J. (2009). Use of response surface methodology for optimizing process parameters for high inulinase production by the marine yeast *Cryptococcus aureus* G7a in solid-state fermentation and hydrolysis of inulin. *Bioprocess and Biosystems Engineering*, *32*, 333–339.

Singhania, R. R., Patel, A. K., Soccol, C. R., & Pandey, A. (2009). Recent advances in solid-state fermentation. *Biochemical Engineering Journal*, *44*, 13–18.

Vandamme, E. J., & Derycke, D. G. (1983). Microbial inulinases: Fermentation process, properties and applications. *Advances in Applied Microbiology*, *29*, 139–176.

Wei, W. L., Zheng, Z. H., Liu, Y. Y., & Zhu, X. S. (1998). Optimizing the culture conditions for higher inulinase production by *Kluyveromyces* sp. Y-85 and scaling-up fermentation. *Journal of Fermentation and Bioengineering*, *86*, 395–399.

Xiong, Y. H., Liu, J. Z., Song, H. Y., & Ji, L. N. (2004). Enhanced production of extracellular ribonuclease from *Aspergillus niger* by optimization of culture conditions using response surface methodology. *Biochemical Engineering Journal*, *21*, 27–32.