Bulgarian Journal of Agricultural Science, 13 (2007), 651-656
National Centre for Agrarian Sciences

Optimization of Nutritive Media Composition for Xylanase Production by *Aspergillus Awamori*

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Abstract

VELKOVA, Z. I., V. K. GOCHEV, G. KOSTOV and A. ATEV, 2007. Optimization of nutritive media composition for xylanase production by *Aspergillus awamori. Bulg. J. Agric. Sci.,* 13: 651-656

Optimization of nutritive media composition for xylanase production by Aspergillus awamori RUS-8 was carried out. Full adequate regression model was obtained by Central Composition Plan. Maximum xylanase activity was achieved in optimized nutritive media on a following composition (g/L): wheat bran-20.02; corn cobs-30.85; corn steep liquor-20.38; $NaNO_3$-2.20; KH_2PO_4-1; KCl-0.5; $MgSO_4$-0.5 and $FeSO_4$-0.01.

*Key words***:** xylanase, *Aspergillus awamori*, optimization

Introduction

D-xylans are the most abundant noncellulosic polysaccharides in hard wood and annual plants, where they account for 20÷35 % of the total dry weight. Due to the structural heterogeneity of the xylans, xylan-degradating enzyme systems include several hydrolytic enzymes. The best known of these are endo-β-1,4-xylanases which hydrolyze xyloologo-saccharides to D-xylose, but several accsssory enzyme activities are necessary for debranching the substituted xylans (Poutanen et al., 1991). Xylanases can be used for bioconverstion of lignocellulosic materials (Mandels et al., 1985) and for more specific hydrolytic purposes, such as the production of oligosaccharides from isolated xylans (Sasaki et al., 1993). Other

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potential applications of crude xylanase preparations can be found in the food industry (Wong, 1993). From industrial point of view filamentous fungi are particularly interesting producers of xylanases, but different factors such as nutritive media composition, substrate pretreatment and fermentation conditions affect the xylanase production (Haltrich et al., 1996). The choice of an appropriate substrate and nutritive media composition is of great importance for the successful production of xylanases. The substrate not only serves as carbon and energy source, but also provides the necessary inducing compounds for the enzyme production. Since the cost of the substrate and media composition play a crucial role in the economics of a xylanase production process many researches will be focused on the selection of appropriate substrates that result in a high xylanase production. The importance of type and concentration of the carbon source has been pointed out in several optimization studies (Gomes et al., 1992; Haltrich et al., 1993; Purkarthofer et al., 1993 and Smith & Wood, 1991). Mathematical-statistic methods for modeling allow to determinate the optimal nutritive media composition that yielded in a higher xylanase activity with a few experiments.

The aim of present study was to determinate the optimal nutritive media composition for xylanase production by *Aspergillus awamori*.

Materials and Methods

Strains. For the purposes of present study *Aspergillus awamori* RUS-8 was used as a xylanase producer strain. The strain was obtained from microbial culture collection of Dep."Biotechnology" at Sofia University "Kliment Ohridski" and was stored on Potato Dextrose Agar (PDA, Difco).

Media. For obtaining of vegetative inoculums modified Mandel's media of a following composition (g/L): Glucose-20; wheat bran-10; corn steep liquor-10; NH_4Cl-1; KH_2PO_4-2; $CaCl_2$-0.4; $MgSO_4$-0.3 and urea- 0.3 were used.

For production of xylanase basal media of a following composition (g/L): Wheat bran-20; corn cobs-40; corn steep liquor-20; $NaNO_3$-2; KH_2PO_4-1; KCl-0.5; $MgSO_4$-0.5 and $FeSO_4$-0.01 was used.

Cultivation conditions. The obtaining of vegetative inoculum was carried out in 500 mL Erlenmeyer flasks containing 50 mL of modified Mandel's media inoculated with spore suspension at concentration 1.10^5 spore/mL. The flasks were cultivated at 28°C, 220 min^{-1} for 24 h. Xylanase biosynthesis was carried out in 500 mL Erlenmeyer flasks containing 50 mL basal media inoculated with 10 % (v/v) vegetative inoculum. The cultivation was carried out at 28°C, 220 min^{-1} for 96 h.

Xylanase assay. Xylanase activity (XA) was determined by the Bailey's method (1992) using 1 % oat spelt xylan (Sigma) as substrate. One U of XA was defined as the amount of enzyme releasing 1 ìmol xylose in one minute reaction.

Mathematical-statistic procedure. for modeling of nutritive media composition for xylanase production by *A. awamori* RUS-8 central composition plan (CCP, Box-Wilson method) was used (Ott, 1987 and Vuchkov, 1986). XA was treated as target function (Y). The selected managing parameters that influenced the target function were coded by the following equation:

$$X_i=(Z_i-Z_i^o)/\Delta Z_i \qquad (1)$$

Where X_i was the coded value of the

variable Z_i; Z_i^o was the natural value of the variable in the center of the plan; ΔZ_i was the interval of variation of variable i.

Measurement of pH and biomass. The final pH value of the cultural broth was determined by potentiometrical means, whereas the bimass was determined gravimetrically after drying at 105°C.

Results and Discussion

On the basis of literature (Gomes et al., 1992; Haltrich et al., 1993; Purkarthofer et al., 1993 and Smith & Wood, 1991) and our previous researches (data not published) wheat bran (X_1), corn cobs (X_2), corn steep liquor (X_3) and $NaNO_3$ (X_4) were studied as factors affecting XA (Y) and the center of the experimental phase area was determined. The rest of nutritive media ingredients were fixed on a constant level. The levels of variation of selected factors were determined by equations (2) and were presented in Tabl.1.

$$x_{high} = x_o + \Delta i \quad (2)$$
$$x_{low} = x_o - \Delta i$$

x_o was the center of the experiment

The area where optimum of the regression model is located is almost stationary and it is significantly nonlinear. For this reason for adequate description of optimum area, mathematical models from a higher stage must be used (Labrev, 1994).

$$Y = b_o + \Sigma b_i X_i + \Sigma b_{ij} X_i X_j + \Sigma b_{ii} X_i^2 \quad (3)$$

Such models can be obtained by CCP (Box-Wilson method). CCP was preferred because it allows estimate the influence of each affecting factor independently and in the aggregate on the basis of comparatively few experiments. Experimental design and results were presented in Table 2. Applying the CCP regression model (4) was obtained which has been fitted to experimental data.

$$Y = 61.7958 + 0.330377.\ X_1 - 0.595313.\ X_2 + 2.41088.\ X_3 + 1.20358.\ X_4 - 6.83256.\ X_1^2 + 1.65625.\ X_1.\ X_2 + 0.01875.\ X_1.\ X_3 + 0.34375.\ X_1.\ X_4 - 8.53601\ X_2^2 - 0.90625.\ X_2.\ X_3 + 0.89375.\ X_2.\ X_4 - 3.27081\ X_3^2 + 0.23125.\ X_3.\ X_4 - 3.13531\ X_4^2 \quad (4)$$

Analysis of the regression equation obtained by CCP showed that the obtained model adequate described the experimental data. Correlation coefficient between the model and experimental results was R^2=78.3951. The free member of the equation (4) possessed the highest positive affect on the target function. This fact proved that the optimal value was located in the area around the center of the experimental plan. Lower values of coefficients in front of other factors in equation (4) demonstrated their weak affect on the target function. Coefficients in front of square members in equation (4) demonstrated

Table 1

Levels of variation of affecting factors

Level	X_1, g/L	X_2, G/l	X_3, g/L	X_4, g/L
x_{high} (1)	10	10	10	1
x_o (0)	20	40	20	2
x_{low} (-1)	30	70	30	3

Table 2

Plan of the experiments

№		Factor X_1	Factor X_2	Factor X_3	Factor X_4	XA, U/mL	Observed value
1	1	0	0	0	0	60.1	61.7958
2	1	0	0	0	0	62.1	61.7958
3	1	0	0	0	0	60.3	61.7958
4	1	0	0	0	0	61.7	61.7958
5	1	-1	-1	-1	-1	32.7	38.9091
6	1	1	-1	-1	-1	33.7	35.5323
7	1	-1	1	-1	-1	37.6	34.431
8	1	1	1	-1	-1	40.1	37.6792
9	1	-1	-1	1	-1	45.8	45.0434
10	1	1	-1	1	-1	42.1	41.7416
11	1	-1	1	1	-1	35.2	36.9402
12	1	1	1	1	-1	39.2	40.2635
13	1	-1	-1	-1	1	41.3	38.3788
14	1	1	-1	-1	1	34.9	36.377
15	1	-1	1	-1	1	33.9	37.4756
16	1	1	1	-1	1	43.2	42.0989
17	1	-1	-1	1	1	39.8	45.438
18	1	1	-1	1	1	42.2	43.5113
19	1	-1	1	1	1	44.6	40.9099
20	1	1	1	1	1	48.6	45.6082
21	1	-1.60717	0	0	0	47.1	43.6164
22	1	1.60717	0	0	0	43.3	44.6783
23	1	0	-1.60717	0	0	47.8	40.7041
24	1	0	1.60717	0	0	33.8	38.7906
25	1	0	0	-1.60717	0	51	49.4726
26	1	0	0	1.60717	0	57.8	57.222
27	1	0	0	0	-1.60717	53.7	51.763
28	1	0	0	0	1.60717	55.8	55.6317

their strong negative affect on target function. An increase in the substrate concentration from 10 to 30 g/L proved to be beneficial for *A. awamori* (Smith & Wood, 1991) but probably the significantly increased concentration of substrates in nutritive medium caused substrate inhibition. The obtained results were proved by

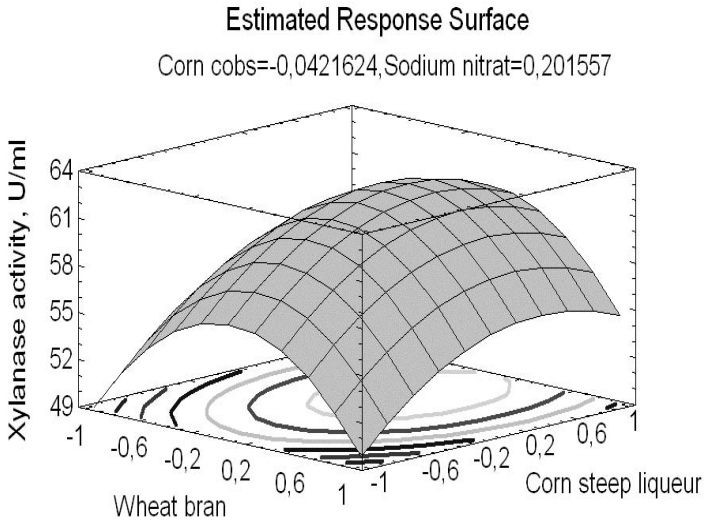

Fig. 1. Response surface (Factors were presented with coded values)

literature (Gomes et al., 1994; Haltrich et al., 1996 and Smith & Wood, 1991). The highest negative affect on the Y possessed the increased concentration of X_2 in the medium. Probably the decrease of enzyme activity caused by the high concentration of corn cobs dues to the impeded oxygen and nutritive compounds mass diffusion and to overcome this problem more intensive aeration and stirring is a must. Optimal levels of the studied factors at which the target function XA has a maximum (62.3936 U/mL) were as follows: wheat bran (X_1)-20.0248 g/L; corn cobs (X_2)-30.853 g/L; corn steep liquor (X_3)-20.381 g/L and $NaNO_3$ (X_4)-2.200 g/L. The response surface was presented in Figure 1. The main difference between basal and optimized nutritive media composition was concern with the lower ratio between concentration of wheat bran and corn cobs. The obtained results were proved by experimental fermentation carried out in optimized nutritive media.

Conclusion

Adequate regression model describing the optimal nutritive media composition for xylanase production by *A. awamori* RUS-8 was obtained by Central Composition Plan. Maximum xylanase activity was achieved on optimized nutritive media on a following composition (g/L): wheat bran-20.0248; corn cobs-30.8530; corn steep liquor-20.3810; $NaNO_3$-2.20; KH_2PO_4-1; KCl-0.5; $MgSO_4$-0.5 and $FeSO_4$-0.01.

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Received January, 12, 2007; accepted August, 3, 2007.