XYLANASE PRODUCTION UNDER SOLID STATE FERMENTATION BY *ASPERGILLUS NIGER*

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(Received: March 16, 2010 – Accepted in Revised Form: September 15, 2011)

doi: *10.5829/idosi.ije.2011.24.03b.01*

Abstract Central composite orthogonal design was applied to quantify relations of xylanase production, loss of dry matter and change of pH with four critical variables during solid state fermentation of a mixture of wheat bran and wheat straw on which *Aspergillus niger* CCUG 33991 was cultivated. The studied variables included the percentage of wheat straw, wheat bran, temperature, moisture content, and fermentation time. The second-order quadratic model predicted the xylanase activity at a suitable set of conditions namely 29°C, 55% moisture content, and 50 hours of fermentation to be 1465 U/g of fermented dry matter which differed less than 6% from measured value at this set of conditions. Furthermore, the obtained models predicted dry matter loss and pH with less than 17% error for the same conditions. The results were used for xylanase production in an intermittently mixed forcedly aerated pilot. Applicability of models for prediction of pilot scale SSF performance was investigated. The results showed considerable deviations of measured trends from model predicted ones. Presence of mixing, temperature and moisture shocks and better oxygen supply are of possible reasons of deviations.

Keywords Xylanase, Solid State Fermentation, Pilot, *Aspergillus niger*, Response Surface

چکیده با بهره گیری از روش سطح پاسخ و طرح آماری مرکب مرکزی متعامد رابطه بین فعالیت زایلاناز تولیدی در کشت جامد یک سویه آسپرژیلوس نیجر CCUG 33991 بر مخلوط سبوس گندم و کاه گندم با چهار متغیر مهم تخمیر حالت جامد بدست آمد. متغیر های بررسی شده شامل درصد کاه، دما، رطوبت و مدت زمان تخمیر بودند. مدل درجه دوم حاکی از فعالیت زایلاناز در شرایط مناسب °C ۲۹، میزان رطوبت ۵۰ درصد، زمان تخمیر ۵۰ ساعت و فعالیت آنزیم U/g ۱٤٦٥ مواد جامد پیشگوئی نمود که با کاهش وزن ۶ درصد حاصل شد. در ضمن کاهش وزن مواد جامد و pH در طی فرایند بررسی شدکه حاکی از ۱۷ درصد انحراف با مدل بود. مطالعات فلاسک به تخمین حالت جامد با مدل نیمه صنعتی توسعه یافته که انحراف از حالت اولیه به دلایل شرایط هم زدگی، شوک رطوبت و انتقال اکسیژن مناسب می باشد.

1. INTRODUCTION

Xylanases are used for improvement of nutritional value of feed, bleaching of the pulp, clarification of fruit juices, improving the quality of bread, extraction of plant oil, coffee and starch and

bioconversion of lignocellulosic materials for production of the fuels and other chemicals [1]. Fungi are the most potent xylanase producers. On an industrial scale, xylanases are produced mainly by *Aspergillus* and *Trichoderma* spp.[2] . Xylanase are usually inducible enzymes secreted in media containing pure xylan or xylan-rich residues. Several reports have shown that agricultural residues such as wheat bran, rice straw, corncob, bagasse and wheat straw induced xylanase synthesis[1]. Wheat bran is nutrient base that contains a blend of complex carbohydrates as cellulose (64%) and hemicelluloses (36%), and also a source of substituted insoluble xylan, in addition to 64% digestible nitrogen. Moreover, it remains loose even under moist conditions during the solid state fermentation (SSF) mode of culturing thereby providing a large surface area and efficient aeration [3].

Optimization of conditions is required for reducing cost of enzyme production. Response surface methodology (RSM), is a powerful tool by which optimization have been successfully employed for enzyme production [4, 5]. Due to their large number, optimization experiments are usually conducted in lab scale within small flasks. However applicability of the results for large scale production is seldom tested. A lab scale intermittently mixed rotating drum type SSF bioreactor having a working volume of 10 liter is used for xylanase production along with other enzymes on 500g dry matter batches of several lignicellulosic substrates [6-10]. Referring to air flow pattern and presence of cooling jacket, conduction seems to be the main metabolic heat removal mechanism and so the bed moisture content remained constant through the fermentation. The temperature also held constant due to small diameter of the bed holding cylinder and relatively slow growth of fungi over lignoclluloses. However the conditions may change if the bioreactor is scaled up. Increasing of diameter will elongate heat transfer path and hence temperature and water vapor pressure gradients will develop within the bed which the latter leads to moisture gradients.

The problem of metabolic heat removal and its impact on xylanase production is visible in another SSF attempt in which *A.sulphureus* was cultiva ted on wheat bran in 30cm×45cm×5cm trays [11]. Wheat bran is a reach medium and supports large metabolic heat production rates of fast growing fungi like *Aspergillus.* This fact restricts bed thickness to a few centimeters for trays and bioreactors in which conduction is the main heat transfer mechanism. Bed temperature has been raised to 45°C and moisture has been fallen to 20% even in mentioned 5cm thick trays. Restriction of the bed thickness, which constraints scale up to two dimensions, can be efficiently reduced by through aeration instead of surface aeration. Xylanase production in through aerated SSF pilots is not sufficiently investigated yet.

The aim of this work was to test applicability of flask culture optimizations for prediction of through aerated SSF pilot performance. Response surface method was used to maximize flask culture xylanase activity by determination of suitable values for three major variables affecting solid state fermentation of *Aspergillus niger* CCUG 33991 cultivated on wheat bran, i.e. temperature, moisture content and time of fermentation. Since the wheat straw is reported to induce xylanase production in some cases due to its high xylan content [12], it was also included in the experiments as the fourth factor. Considering the fact that direct determination of cell mass is nearly impossible in solid state fermentation, the dry matter loss could be used as an indirect criterion for following up the growth. It also is important from the economical viewpoint of the process. Variation of the fermenting medium pH is another indicator of the state of culture. Hence, dry matter loss and fermented substrate pH were also modeled along with xylanase activity. Proficiency of the resulted correlations for prediction of xylanase activity, along dry matter loss and pH of the medium, was tested for pilot scale SSF process.

2. MATERIALS AND METHODS

2.1. Microorganism *Aspergillus niger* CCUG 33991 was grown on PDA slants at 30°C and stored at 4°C until use. Spore suspensions were prepared by adding sterile distilled water containing five drops of Tween 80 and scraping the mycelium with a sterile pipette for use as inoculums. The spores were counted in a Neubauer chamber.

2.2. Growth Media and conditions Solid substrate was wheat bran mixed in different proportions with wheat straw. Wheat bran had a particle size distribution of: >1.00 mm, 35.7%; between 0.595 mm and 1.00 mm, 39.4%; between

0.5 mm and 0.595 mm, 9.7%; between 0.5 mm and 0.420 mm, 8.3%; between 0.297 mm and 0.420 mm, 4.3%; <0.297 mm, 2.6%. Wheat straw was ground in order to its particle size become about wheat bran particle size. Distilled water was used to adjust the moisture content of the substrate to desired levels. In calculation of desired initial moisture content, accompanying water of wheat bran and wheat straw as well as inoculums suspension were taken into account. The ratio of wheat straw in the solid substrate and the moisture content of culture medium were varied according to the experimental design.

2.3. SSF Pilot The pilot consisted of saturated air supplying system, bioreactor, and control system. Air, supplied by a multichannel blower, was saturated by upward flow within a column at top of which a circulated stream of temperature adjusted water was sprayed. A cyclone demisted the air flow which was then flowed in a pipeline on which five electrical heaters were mounted to warm the air to attain desired relative humidity. The air flow rate was adjusted using a rotameter and a globe valve and it was finally entered into bioreactor after being sterilized by passing through a multilayer filter.

The bioreactor was of intermittently mixed forcedly aerated through circulation type. Substrate was loaded to a 40cm×75cm×10cm (W×L×H) perforated bottom tray which was horizontally mounted on a second tray in which air stream was uniformly distributed for passing through the bed. The bed was periodically mixed by a blade type rotating mixer which traveled forward and backward along the bed over a drive shaft. The mixing was controlled via controlling rpm and operating time of the shaft electromotor. A perforated pipe was installed over the mixer for spraying of makeup water. It was fed by a peristaltic pump. The trays and mixing apparatus were installed in an insulated cabinet. A boiler was used to supply steam for pasteurization of the substrate bed and bioreactor. An electrical heater was provided for warming of air stream which was used to dry fermented solids.

Operation of the pilot was automatically controlled by a computer plus data acquisition and controlling elements. Using the Labview software, a program was developed to monitor record and control the SSF based on a previously reported mathematical model [13]. In this model online measured difference of air stream temperature between inlet and outlet of the bioreactor is used to assess metabolic heat production rate. This rate is in turn used to calculate rates of metabolic water production, dry matter reduction, and evaporation. By this way both weight and moisture of the bed are continuously estimated. When the moistures falls to a lower limit, the controller program calculates amount of makeup water required for returning it to an upper limit and send a command to peristaltic pump to supply the water. The program at the same time turns the mixer on to mix the bed for a preselected length of time. Mixing is independent from water supply and could be performed at selected time intervals.

2.4. Flask Culture Five grams portions of culture medium were placed in 250 ml Erlenmeyer flasks which plugged with cotton wool before autoclaving at 121°C for 30 min. The flasks were allowed to come to ambient temperature and then inoculated with 1 ml of spore suspension containing 5×10^7 spores. The flasks were incubated under static conditions at various temperatures and for various periods of time according to experimental design. To prevent fermenting substrates from drying, incubator atmosphere was kept near saturation by placing a tray of water in it. After fermentation, the remained weight of fermented medium in each flask along with its moisture content was measured. To determine the latter, contents of the flask was thoroughly mixed and one gram was removed and dried at 100°C for 20 hours.

2.5. Pilot Culture 4000 g of wheat bran was loaded into the bioreactor tray and uniformly moistened to desired value using water supplying and mixing system. It was then steamed for one hour and cooled by sterile air stream. 20 g of fully sporulated bran produced by flask culture was distributed over the bed and the mixer was turned on to mix spores and substrate evenly. The saturated air stream was established and the control program was turned on after setting its inputs to desired values. The inputs were mixer rpm, mixing interval, mixing time length, lower and upper limits of bed moisture, and relative humidity of air stream at bioreactor entrance. At the end of fermentation, saturated air stream was replaced by dry air stream which was warmed to 60°C by air heater. Finally

the dry fermented solids were unloaded from the bioreactor and weighed. At given times samples from different locations of the bed were taken and mixed and used for assay of enzyme activity, moisture, and pH. Operating conditions of bioreactor is presented in Table 1.

2.6. Enzyme Extraction For flask cultures, 40 ml distilled water was added to residual content of each flask and mixed on a shaker (160 rev/min) at room temperature for 45 min. The mixture was then filtered and clarified filtrate was used for measuring pH and enzyme assay. For the samples taken from bioreactor, 4 g of the sample was held in a flask and extracted by the same method.

2.7. Enzyme Assay Xylanase (3.2.1.8) activity was assayed by measuring the rate of release of reducing sugar from birch wood xylan (Sigma, USA) which was dissolved in 0.1 M acetate buffer (pH 5.0). The reaction mixture containing 1 ml of 1.5% (w/v) xylan solution and 0.1 ml suitably diluted enzyme (diluted by the same buffer) was incubated at 40°C for 20 min. The reaction was stopped by adding 0.5 ml of dinitrosalcylic acid (DNS) reagent and amount of the reducing sugar liberated was measured by DNS method using xylose solution as standard reference [14]. One unit of xylanase activity (U) was defined as the amount of enzyme that released 1 μmol xylose per min under the assay conditions. Xylanase activity was expressed as U per gram of fermented dry matter (U/g-fdm). Each test was performed in duplicate and the average value was used.

2.8. Experimental Design and Statistical Analysis Response surfaces method and central composite orthogonal design for four factors were used in this investigation to determine the effects of following variables on xylanase production: the ratio of wheat straw in solid substrate, fermentation temperature, moisture content and time of fermentation. The variables were coded according to Eq. (1):

$$x_i=(X_i - X_0)/\Delta X_i \quad (1)$$

where, X_i is real value and x_i is coded value of the independent variable, X_0 is its value at center point and ΔX_i is its step change value. The levels of the variables investigated in this study are given in Table 2. Totally 53 treatments were performed. The second order polynomial models were used to quantify correlation between xylanase activity, pH and remained dry matter with the mentioned four variables:

$$y=b_0+\Sigma b_i x_i + \Sigma b_{ii} x_i^2 + \Sigma b_{ij} x_i x_j \quad (2)$$

where, y is the predicted response (xylanase activity, pH, or dry matter), x's are the levels of the

TABLE 1. Operating conditions of bioreactor

Experiment No.	Mixing interval (h)	Moisture limits (g/g)	Air flow rate (l/min)	Inlet air temperature (^{0}C)	Maximum bed mean temperature# (^{0}C)
Run 1	2	0.45-0.6	400	34.5	36
Run 2	5	0.5-0.6	400	34.5	36
Run 3	2	0.5-0.55	250	33.5	36
Run 4	2	0.5-0.6	400	32	33.5
Run 5	5	0.5-0.6	400	28	29.5

#- calculated as average of inlet and outlet air temperatures at time of maximum metabolic heat production rate

TABLE 2. Range of variables at different levels for the central composite experimental design

Independent variables	Symbols	Levels					Step change
		-1.414	-1	0	1	1.414	ΔX
Weight ratio of wheat straw in culture medium (%)	S	8.8	15	30	45	51.2	15
Temperature (^{0}C)	T	25.8	27	30	33	34.2	3
Moisture content (%)	M	40.8	45	55	65	69.1	10
Time of fermentation (h)	I	31.7	40	60	80	88.3	20

independent variables, and *b*'s are various coefficients. Values of coefficients were calculated based on least error regression. Their significances were determined by ANOVA. Optimum conditions were determined using the developed model for xylanase activity (Table 3). Design of experiments and analysis of results were performed using SAS 9 software (Statistical Analysis of System, USA).

3. RESULTS AND DISCUSSION

The xylanase activity varied in the range of 125-1378 U/g-fdm implying considerable effect of selected variables on the xylanase production. The resultant model based on uncoded value of variables is as follows:

$$\text{Xylanase activity (U/g-fdm)} = -14159.3 - 38.0S + 600.7T + 95.8\times M + 144.0I + 0.56S^2 - 10.6T^2 - 1.4MI - 0.60I^2 \quad (3)$$

The model F-value and P-value were 26.58 and 0.0001, respectively showed that the predictive model is statistically significant at 95% ($P<0.05$) confidence level. The coefficient of determination (R^2) for predictive model was 0.827, indicating that 82.7% of the variability in the response could be explained by the model.

3.1. Effect of Studied Variables on Xylanase Production The effect of wheat straw: Since wheat straw contains 50% (w/w) hemicelluloses [12], it would be expected that favorably affect xylanase production. However, an increase of wheat straw in medium from 9% to 30% reduced the xylanase activity. So it seems that wheat straw acted merely as a neutral filling agent. On the other hand at the ratios o/ver than 40%, the effect of wheat straw on xylanase production was positive possibly due to unavailability of wheat bran, i.e. some germinated spores were only in contact with wheat straw and used it as the sole available substrate. Wheat straw has been known for being suitable for xylanase production as sole substrate or in mixture with wheat bran [5, 15-19]. However, fermentation times were longer than one we used in our experiments, for example as long as 7 days [7]. It seems that under studied conditions the presence of wheat straw in wheat bran substrate doesn't enhance xylanase production.

3.2. Effect of Temperature An increase in temperature up to 29°C increased the xylanase activity, however further increase in temperature exhibited adverse effect. These results showed that the optimum temperature for xylanase production by *A.niger* CCUG 33991 in the conditions of this study was 29°C. A similar trend has been reported in some other literatures [7, 11, 20]. The optimum temperature for xylanase production by a strain of *A.niger* cultivated on a mixture of corncob, wheat bran and wheat straw [21] and*A.foetidus* grown on corncob [22] has been reported 28°C and 30°C, respectively.

TABLE 3. Results of ANOVA for the xylanase activity response

Source	DF	SS	MS	F	Pr>F
S	1	157643	157634.3	5.91024	0.01988
T	1	456754	456754.5	17.1252	0.00018
M	1	428848	428847.6	16.0789	0.00027
I	1	634523	634523.5	23.7904	0.0001
S*S	1	277495	277494.8	10.4042	0.00258
S*T	1	25200	25200.13	0.94483	0.33718
S*M	1	18240	18240.5	0.68389	0.41341
S*I	1	84666	84666.13	3.17442	0.08279
T*T	1	160109	160108.7	6.00301	0.01899
T*M	1	26335	26335.13	0.98739	0.32666
T*I	1	5832	5832	0.21866	0.64273
M*M	1	963.182	963.1842	0.03611	0.85029
M*I	1	2595781	2595781	97.3246	0.0001
I*I	1	1004222	10042222	37.6517	0.0001
Model	8	5677357	709669.6	26.5805	0.0001
Error	44	1174749	26698.84		
(Lack of fit)	16	1019262	63703.86		0.0001
(Pure Error)	28	15548.2	5553.114		
Total	52	6852106			
Coefficient of determination (R^2=0.827)					

3.3. Effect of Moisture Content Initial moisture content is one of the key factors influencing xylanase production. The highest xylanase activity was obtained when the moisture content had its highest value .i.e. 69%. Many researchers have reported the similar effect [4, 7, 8, 11, 19, 20, 22, 23] (Table 4).

3.4. The Effect of Time of Fermentation The xylanase activity reached to a maximum value within a period of 45-60 hours and then decreased probably due to inactivation or hydrolysis by secreted proteases of fungi. This trend is observed in some other reports [2, 22, 24].

3.5. pH and Loss of Dry Matter Models Fitting Eq. (2) to experimental data of pH and percentage of dry matter loss resulted following equations:

$$pH = -8.79 + 0.015S + 0.193T + 0.062M + 0.199I - 0.0004SI - 0.002TI - 0.0007I^2; \quad R^2 = 93.2\% \qquad (4)$$

TABLE 4. Comparison of xylanase production from different strains grown on lignocellulosic materials

Microorganism	Substrate	Moisture content (%)	Fermentation time (h)	Temperature (°C)	Xylanase activity (U/g)	Activity assay conditions (pH, °C)	Ref
A.niger F3, F4	Bran+ cottonseed	54.5	30-36	30	1761	4.6. 40	[23]
A. fischeri Fxn 1	Wheat bran	60	72	30	1024	6, 50	[25]
A.niger N218	Corncob+ wheat Bran+ straw	60	72	28	2989	5, 55	[20]
A.niger P 602 mutant	Corncob+ wheat Bran+straw	60	64	28-32	6320	5, 55	[20]
A.niger CBS 11042	Wheat bran + straw	70	72	30	2500		[26]
A.niger KK2 mutant	Pretreated Rice straw	65	120	28	5071	4.8, 50	[6]
A. foetidus	Corncob	84	96	30	3065	5.3, 50	[21]
Trichoderma longibrachiatum	Wheat bran + wheat steaw	55	96	25	593	4.8, 54	[24]
Thermoascuc aurantiacus	Bagasse	81	240	45	2700	5, 50	[8]
Thermoascuc aurantiacus	Wheat straw	80	168	49	5465	5, 50	[17]
Sporotrichum thermophile	Wheat straw	80	163	49	320	5, 50	[12]
Fusarium oxysporum	Corn stover	80	144	27	1840	6, 50	[22]
Melanocarpus albomyces	Wheat straw	86	96	45	7760	6, 70	[9]
Paecilomyces thermophila J18	Wheat straw	83	192	50	18580	6.5, 50	[13]
A.niger CCUG 33991	Wheat bran	55	50	29	1546	5, 40	This work

$$DM_LOSS (\%) = -76.016 - 0.159S + 0.586T + 2.193M + 0.252I - 0.018M^2 \quad R^2 = 87.2 \tag{5}$$

The coefficient of determination (R^2) of these models indicates acceptable precision of the responses.

3.6. Experimental Verification of The Optimal Conditions Respective points of maximum xylanase activity could be found using Eq. (3). The optimum levels of variables, in the range studied, were 8.8% (w/w) wheat straw in dry culture medium, 28.8°C, 69% moisture content and 32 hours of fermentation. Under these optimized conditions, on the base of predictive model, xylanase activity of 1663 (U/g-fdm) was obtained. However the moisture content of 69% isn't suitable for enzyme production in large-scale bioreactors because it leads to conglomeration of the substrate or sticking of the particles to the wall of the reactor that affect adversely on aeration and growth of hyphae. Moreover the substrate becomes more susceptible to bacterial contamination. For this reason, the suitable water content of 55% was selected. Figure 1 shows the isoresponse contour plot for moisture content of 55% and 10% wheat straw. It can be seen that in these conditions maximum xylanase activity of 1114 (U/g-fdm) predicted by the model at 29°C after 50 hours fermentation. This set of conditions was selected as optimum operating point and an experiment with five replications was performed to confirm the accuracy of the model at this point. The average of replicates resulted in the xylanase activity of 1368 (U/g-dfm) which is 18.6% higher than model prediction confirming suitable validity of the model. The average measured values of pH and dry matter loss for the selected optimum operating point were 4.83 and 32.2%, respectively. Eqs. 4 & 5 predictions were 5.5 and 26.8%, respectively. Xylanase activity per unit weight of fermented substrate was seen to be increased with decreasing the ratio of wheat straw in the medium. It suggests that higher xylanase activities could be achieved by further decrease in the ratio of wheat straw in the medium. This fact was investigated by performing a test with pure wheat bran as the medium. Other conditions were set to optimum·operating point. The mean xylanase activity of five replicates was1546 (U/g-fdm) which is 13% higher than one obtained with 10% wheat straw. This result confirmed that wheat straw acted only as the ineffective filler. Extrapolation of Eq. 3 predicts this value to be 1465. Measured and predicted pH and dry matter loss were 4.9 and 21.6% v.s. 5.5 and 18.7% respectively.

3.7 Pilot Result Five experiments were performed in the bioreactor to check for large scale applicability of flask culture optimization results and to investigate effect of some important variables on achievable xylanase activity (Table 3).

Moisture content of the bed varies due to evaporation, metabolic water production and dry matter consumption. It could not be held strictly constant at optimum value since it needs continuous mixing and continuous distribution of infinitesimal amounts of water uniformly over the bed particles. Therefore the bed moisture should be controlled within an interval. Wider intervals are easier to control while narrower ones provide more uniform growth conditions. Three moisture intervals were investigated as is given in Table 1. Temperature also could not be held uniform within the bed and a temperature gradient exists in the direction of air flow. The gradient could be reduced by increasing air flow rate which is in turn restricted by economic considerations. Inlet air temperature mid mean temperature of the air through the bioreactor can demonstrate temperature situation of the bed. Referring to Table 1, three mean temperatures were investigated in the experiments.

A further operational parameter exists in investigated bioreactor type which is not present in flask culture. It is mixing which, destroying hyphae, has adverse effect on some filamentous fungi. However it is needed for uniform water replenishment and also for preventing the bed from being shrunk which channelize air flow [25]. The largest safe time interval between two successive mixing was observed to be five hours. Longer intervals led to appearance of lumps which were not completely destroyed by further mixing. So, effect of mixing interval on target enzyme was investigated by including two mixing intervals of 5h and 2h in experiment conditions.

Figure 2 illustrates trend of xylanase production for different experiments. It is seen that temperature has most pronounced effect on xylanase activity. Reducing mean temperature of the bed

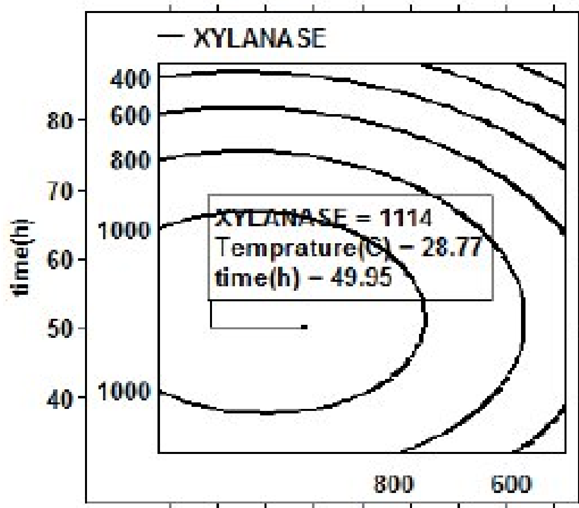


Figure 1. Contour plot of the calculated response surface at fixed levels of wheat straw (10%) and

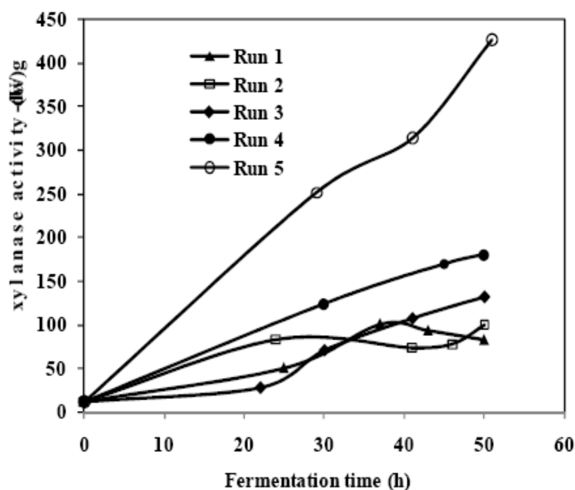


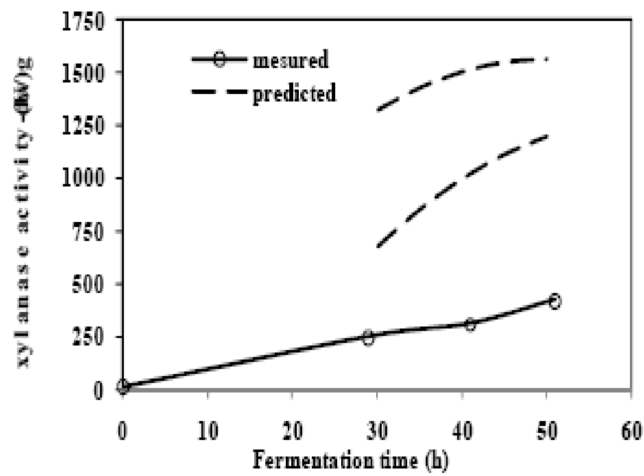
Figure 2. Trends of change in xylanase activity of fermenting wheat bran in bioreactor. Experiment conditions for different runs are given in Table 2

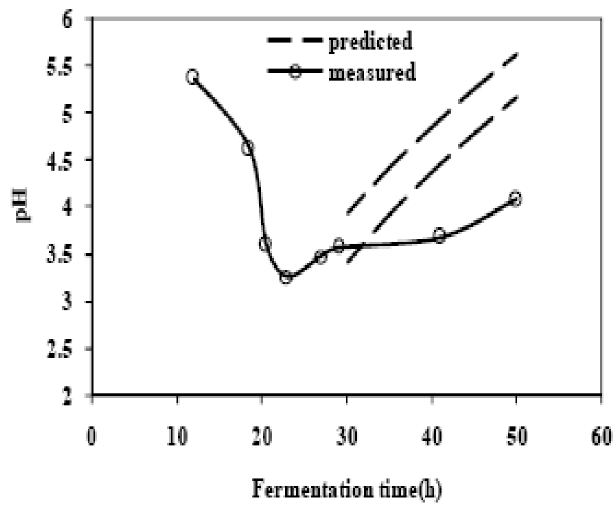
from 36°C to 33°C and 29.5°C increased enzyme activity from 100 to 180 and 450 U/g respectively. This is while changes of mixing interval, moisture limits and air flow rate in runs 1, 2 and 3 caused minor differences in mentioned activity.

Predictions of empirical correlations (Eqs 3-5) for run 5 are compared with actual results in Figure 3. It should be noted that correlations are not valid for fermentation times shorter than 30h. The input parameters of correlations namely moisture and temperature are not constant during the time nor uniform within the bed. So the correlation outputs are calculated for various sets of input values and their upper and lower limits are depicted th in the Figure 3. For example for the 40 hour of fermentation, correlation 3 gives lowest xylanase activity value at moisture of 50% and 32 °C to be 998 U/g and highest one at 60% and 28°C to be 1538 U/g. Referring to Figure 3, attained xylanase activity is significantly lower than predicted ones. Measured pH changes also deviate from predictions, pH reduction could be related to fungal growth and metabolic reaction and increase in pH is related to xylanase production. The same is true for dry matter loss. Dry matter loss could not be measured directly during the fermentation, however its measurement at the end of fermentation differed only 4% from controller estimation (Table 5). Dry matter loss is directly proportional to released metabolic heat.

Therefore the Figure 3 implies that overall metabolic activity was higher in bioreactor than in flask at the same conditions. This may be a resulted from better oxygen supply in through aeration mode. Other metabolic activities such as release of xylanase or metabolism of organic acids are influenced by bioreactor conditions and are not predictable from flask results. One possible deviation reason is mixing which is unavoidable in bioreactor.

One experiment showed that hand shaking of flask culture at 5 h intervals will cause a 22% decrease in xylanase activity compared with stagnant flask culture (data not shown). Another deviation reason seems to be stresses caused by sudden temperature changes in fungus microclimate due to translocations resulted from intermittent mixing or sudden moisture changes due to intermittent water replenishments.





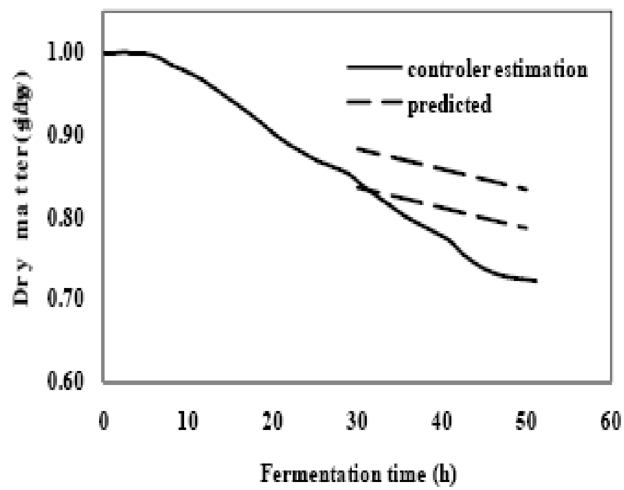


Figure 3. Comparison of experimental results of run 5 with predictions of correlations obtained from response surface analysis of flask culture experiments

TABLE 5. Derivation of model predictions from experimental measurements

Factors				Xylanase activity (U/g)		
S	T	M	I	Prediction of model	Experimental measurement	Error of predictive model
15	27	45	40	432.8	374.5	-15.6
15	27	45	80	750.5	744	-0.9
15	27	65	40	1210	1376.5	12.1
15	27	65	80	388	179	-116.8*
15	33	45	40	219.1	225.5	2.8
15	33	45	80	536.8	401.5	-33.7
15	33	65	40	995.8	1200	17.0
15	33	65	80	174.3	130.5	-33.6
45	27	45	40	307.5	273.5	-29.3
45	27	45	80	625	627.5	0.4
45	27	65	40	1084	956	-13.4
45	27	65	80	262.4	192	-36.7
45	33	45	40	93.5	301	68.9*
45	33	45	80	411.2	334	-23.1
45	33	65	40	870.2	834	-4.3
45	33	65	80	49	276.5	82.4*
8.79	30	55	60	1077	1128	4.5
51.21	30	55	60	899	857.5	-4.9
30	25.76	55	60	694	955.5	27.4
30	34.24	55	60	392	140	-179.9*
30	30	40.86	60	588	694	15.3
30	30	69.14	60	880	815.5	-7.9
30	30	55	31.72	432	223	-93.8*
30	30	55	88.28	76	294.5	74.2*
30	30	55	60	734	713	-2.9

*Data represent are the mean of value of replicates

5. CONCLUSION

A second-order polynomial model obtained using response surface methodology for the xylanse activity excrerted by a strain of *A. niger* cultivated on wheat bran. It could predict xylanase activity under favorable operational conditions adequately. Appropriate models for prediction of the pH and dry matter loss during fermentation process were also developed. Presence of wheat straw in the fermentation medium had no positive effect on xylanse production. As wheat straw is a source of polymeric compound, higher degradation needed pretreatment, so including wheat straw would have given different results. Further studies for production of xylanase in pilot-scale bioreactor showed considerable deviations from predictions of correlative model which may com partly from mixing and oxygen supplying mode and a main source is possibly sudden change in temperature and moisture which results from intermittent mixing and water replenishment.

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