Optimization of glucoamylase production by *Mucor indicus*, *Mucor hiemalis*, and *Rhizopus oryzae* through solid state fermentation

*Mucor indicus*, *Mucor hiemalis*, ve *Rhizopus oryzae* tarafından üretilen glukoamilazın katı hal fermentasyonu ile optimizasyonu

Abstract: Objective: Glucoamylase is a hydrolyzing enzyme with several industrial applications. Glucoamylase was produced via a solid state fermentation by three naturally occurring zygomycetes fungi of *Mucor indicus*, *Mucor hiemalis*, and *Rhizopus oryzae* on wheat bran.

Methods: The effects of cultivation temperature, medium moisture content, and cultivation time on the enzyme production were investigated. Experiments were designed with an orthogonal central composite design on the three variables using response surface methodology (RSM).

Results: For glucoamylase production, the optimum temperature and medium moisture content for the three fungi were 26.6°C and 71.8%, respectively. The optimum cultivation time for *M. hiemalis* and *R. oryzae* was 33.1 h, while it was 66.8 h for *M. indicus*. At optimum conditions, glucoamylase production by *M. indicus*, *M. hiemalis*, and *R. oryzae* was respectively 255.3, 272.3, and 1545.3 U per g dry substrate.

Conclusion: *R. oryzae* is a suitable candidate for industrial production of glucoamylase.

Keywords: Glucoamylase, *Mucor hiemalis*, *Mucor indicus*, Optimization, *Rhizopus oryzae*, Solid state fermentation

*Corresponding author: Sanaz Behnam:* Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran, e-mail: behnam-sanaz@yahoo.com

Keikhosro Karimi: Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran, e-mail: karimi@cc.iut.ac.ir

Morteza Khanahmadi: Isfahan Agriculture and Natural Resources Research Centre, Isfahan 81785-199, Iran, e-mail: khanahmadi@abrii.ac.ir

Zahra Salimian: Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran, e-mail: zahra_salimian@yahoo.com
1 Introduction

Glucoamylase is a hydrolyzing industrial enzyme which can degrade both amylose and amylopectin and produce glucose [1,2]. Glucoamylase is commercially used in the production of glucose and fructose juice, baking for improving bread quality, beverage pharmaceuticals, and detergents industries [3–5].

Solid state fermentation (SSF) and submerged fermentation are common ways of enzymes production [6]. In solid-state fermentation, the microorganisms (mainly fungi) grow on moist solid materials in the absence of free-flowing water. The water, which is needed for microbial activities, is available in the solid matrix of the substrate [7–9]. Solid state fermentation is more advantageous than submerged fermentation, since its capital and operating costs are lower; less space is needed, simpler equipment and media are used, and less wastewater is produced [6,10–12]. Among the microorganisms used in SSF, filamentous fungi can grow on complex solid substrates and produce a wide variety of extracellular enzyme [13]. The amount of enzyme production depends on the fermentation conditions; therefore, optimization of the conditions is essential to decrease the cost of enzyme production [14].

This work was aimed to investigate the ability of three fungal strains of Mucor indicus, Mucor hiemalis, and Rhizopus oryzae to produce glucoamylase on wheat bran via the solid state fermentation. Wheat bran is an ideal substrate for solid state fermentation whose bed has an appropriate porosity for fungal growth and air stream. These fungal strains have several industrial applications. They are separated from edible sources with high abilities for ethanol production from cellulosic sources. They can convert substrate containing inhibitors. Their biomass has a high nutritional value and contains considerable amounts of chitosan and glucosamine as valuable components [15–17]. To our knowledge, there is no investigation on optimization of glucoamylase production by these strains on wheat bran. Furthermore, solid state fermentation as an advantageous method for enzyme production than submerged fermentation was applied. Response Surface Methodology (RSM) was used to design the experiments at various medium temperatures, moisture contents, and cultivation times. A quadratic model as a function of the three mentioned parameters was evaluated for glucoamylase production by the fungi. Finally, the parameters effects on the enzyme production were studied and their optimum values to obtain the highest enzyme amount were reported.

2 Materials and Methods

2.1 Microorganisms and fermentation conditions

Three different species of fungi: Mucor indicus (CCUG 22424), Mucor hiemalis (CCUG 16148), and Rhizopus oryzae (CCUG 28958) were obtained from the Culture Collection, University of Göteborg, Sweden. The strains were maintained on plates containing 40 g l\(^{-1}\) glucose, 10 g l\(^{-1}\) peptone, and 15 g l\(^{-1}\) agar and kept at 32°C for 5 days to grow and then stored at 4°C until use. Sterile distilled water containing 0.1% (v/v) Tween 80 (Polyoxyethylene Sorbitan Monooleate) was used to separate spores. An amount of 10 g moistened wheat bran was added to different Erlenmeyer flasks and autoclaved at 121°C for 20 min. After cooling, the flasks were inoculated with the spore suspension and kept at 30°C for 7 days to grow the spores on the bran surface. Afterwards, 50 ml sterile distilled water containing 0.1% (v/v) Tween 80 and sterile glycerol were added to the flasks to separate the spores from the bran to be kept in micro tubes at -20°C.

Fermentation was carried out in 250-ml flasks containing 10 g dry wheat bran. They were autoclaved, cooled, and inoculated with spore suspensions (1000 spores per g dry bran). Medium was moistened by addition of water. The amount of added water was adjusted such that the bed moisture after addition of spore suspension was equal to that required for each experiment. The flasks were put in an incubator at experiment’s temperature. A dish filled with water was put in the incubator to moisten the air in the incubator and reduce water evaporation from the substrate. Under such conditions the substrate moisture remains nearly constant during the fermentation course.

2.2 Enzyme extraction and assay

One unit of the enzyme activity (U) was defined as the amount of enzyme required to liberate 1 μmol of the reducing sugar (glucose) per min under the assay conditions. The yields were expressed as U per gram dry substrate (gds).

At the desired fermentation time, the enzyme produced in each flask was extracted by adding 90 ml distilled water and mixing in a rotary shaker (100 rpm) at room temperature for 30 min. Then the samples were filtered and the filtrates were analyzed for glucoamylase activities. The filtrate was diluted by citrate buffer. Dilution should be such that at the end of following stages, glucose concentration will be around 1 g/l. In these experiments, dilution was performed
two times (one ml of the filtrate was mixed with one ml citrate buffer.) Afterwards, one ml of distilled water and 0.1 ml of the diluted sample were mixed in a 10-ml tube. Then one ml of the substrate solution (1 w/w % of soluble starch in 50 mM citrate buffer) was added, mixed, and placed in a water bath at 60°C for 15 min. Afterwards, the reaction tube was immediately placed in boiling water for 5 min to inactivate the enzyme and terminate the reactions. The concentration of the released glucose was measured using 3,5-dinitrosalicylic acid (DNS) method of Miller [18].

### 2.3 Experimental design and statistical analysis

In order to study the effect of temperature, medium moisture content, and cultivation time on the enzyme production and to find the optimum values to get the highest amount of glucoamylase, Response Surface Methodology (RSM) was used. An orthogonal central composite design on the three factors with five levels (–1.682, -1, 0, +1, and +1.682) was applied. The ranges in which these variables were studied are listed in Table 1. The suggested experiments, obtained by SAS (Statistical Analysis System) software, are presented in Table 2. All experiments were performed in duplicate and the average values for the enzyme activities were reported. A quadratic model expressing glucoamylase activity as a function of temperature, medium moisture content, and cultivation time was also fitted to the experimental data. SPSS (PASW Statistics 18) and Matlab 7 softwares were used to perform all statistical analyses and the process optimization, respectively.

### 3 Results and Discussion

#### 3.1 Empirical models for glucoamylase production by the fungi

The experiments suggested by the software with the three variables of temperature, medium moisture content, and cultivation time were performed and the enzyme activities produced by the fungi were measured and presented in Table 2. A quadratic equation was fitted to the experimental enzyme activity as follows:

\[ \text{Enzymes activity (U/gds)} = a_0 + a_1 T + a_2 W + a_3 I + a_4 T^2 + a_5 W^2 + a_6 I^2 + a_7 TW + a_8 TI + a_9 WI + a_{10} FI \]

where \( a_0 \) is the intercept, \( a_1, a_2, \) and \( a_3 \) are linear coefficients, \( a_4, a_5, \) and \( a_6 \) are squared coefficient, and \( a_7, a_8, \) and \( a_9 \) are interaction coefficients.

#### Table 1: The experimental domain (\( \alpha=1.682 \)).

| Independent variables | Symbol (unit) | Range and level |
|-----------------------|--------------|-----------------|
|                       |              | \(-\alpha-1 \) | 0 | +1 | +\( \alpha \) |
| Temperature (oC) T    | C            | 26.59 | 30 | 35 | 40 | 43.4 |
| Moisture content (W/W) W (%) | % | 38.18 | 45 | 55 | 65 | 71.81 |
| Time I (h)            |              | 33.18 | 40 | 50 | 60 | 66.81 |

#### Table 2: Experimental design and results for glucoamylase production (U/gds) by the fungi.

| NO. | Uncoded level | Fungus | M. indicus | M. hiemalis | R. oryzae |
|-----|---------------|--------|------------|-------------|-----------|
|     | T (oC) | W (%) | I (h) |    |    |    |    |    |    |
| 1   | 30.00 | 45.00 | 40.00 | 96.00 | 77.93 | 910 |
| 2   | 30.00 | 45.00 | 60.00 | 121.0 | 13.09 | 885 |
| 3   | 30.00 | 65.00 | 40.00 | 49.50 | 143.3 | 1158 |
| 4   | 30.00 | 65.00 | 60.00 | 161.5 | 66.60 | 645 |
| 5   | 40.00 | 45.00 | 40.00 | 64.50 | 28.84 | 825 |
| 6   | 40.00 | 45.00 | 60.00 | 35.00 | 22.65 | 890 |
| 7   | 40.00 | 65.00 | 40.00 | 88.50 | 76.12 | 880 |
| 8   | 40.00 | 65.00 | 60.00 | 64.00 | 130.9 | 770 |
| 9   | 26.59 | 55.00 | 50.00 | 126.0 | 76.12 | 900 |
| 10  | 43.41 | 38.18 | 50.00 | 56.50 | 37.68 | 814 |
| 11  | 35.00 | 13.18 | 50.00 | 73.00 | 9.32 | 870 |
| 12  | 35.00 | 71.82 | 50.00 | 88.00 | 104.3 | 843 |
| 13  | 35.00 | 55.00 | 33.18 | 66.00 | 89.27 | 978 |
| 14  | 35.00 | 55.00 | 66.82 | 110.5 | 24.53 | 736 |
| 15  | 35.00 | 55.00 | 50.00 | 58.00 | 26.90 | 857 |
| 16  | 35.00 | 55.00 | 50.00 | 74.00 | 17.15 | 805 |
| 17  | 35.00 | 55.00 | 50.00 | 70.00 | 23.93 | 819 |
| 18  | 35.00 | 55.00 | 50.00 | 80.00 | 17.59 | 829 |
| 19  | 35.00 | 55.00 | 50.00 | 77.00 | 23.86 | 803 |
| 20  | 35.00 | 55.00 | 50.00 | 58.00 | 15.28 | 736 |
| 21  | 35.00 | 55.00 | 50.00 | 64.00 | 23.80 | 829 |
| 22  | 35.00 | 55.00 | 50.00 | 86.00 | 29.93 | 775 |
| 23  | 35.00 | 55.00 | 50.00 | 67.00 | 26.90 | 857 |

In order to evaluate the significance of the regression model, analysis of variance (ANOVA) was used and the results are presented in Table 3. The low values of the probability (p-value), which are obtained by the Fisher F-test, as well as high values of \( R^2 \) indicated that the model has a high significance and its adequacy is confirmed [19]. Generally, \( R^2>0.75 \) shows the aptness of the model. According to Table 3, the adequacy of quadratic models for glucoamylase production by the three fungi was confirmed, since all \( R^2 \) values are higher than 0.9. Therefore, the model explained more than 90% of the variability in the response. The coefficients of Equation 1 for the glucoamylase production by the fungi were obtained and the significance of each coefficient was determined (Table 4). Lower p-values show that the corresponding coefficients are more significant [20].
For glucoamylase production by *M. indicus*, the interaction term of temperature and time was highly significant (p<<0.05). The linear term of moisture content, the interaction term of moisture content and time, and the second order term of temperature were equally significant and the remaining terms were insignificant.

For the enzyme production by *M. hiemalis*, the interaction terms of moisture content with temperature and time were insignificant, while other terms were highly significant.

For glucoamylase production by *R. oryzae*, the interaction term of moisture content and time, and the interaction term of temperature and time were highly significant, the linear term of temperature was significant, and the remaining terms were insignificant.

When a term of Equation 1 has a high significance, it would be a limiting factor. This means that a small change in its value would considerably change the enzyme production [21]. The magnitude and the sign of coefficients indicated the effects of the parameters on the enzyme production.

In order to investigate the effects of cultivation conditions on glucoamylase production by the fungi, the equations obtained for enzyme production were used and the simultaneous effects of two variables on the enzyme production were studied by fixing the third one at the central value (Figs. 1–4).

### 3.2 Effect of temperature on the enzyme production by the fungi

Temperature is reported to be the most important factor influencing the solid state fermentation [22]. The effect of temperature on glucoamylase production by the three fungi was investigated. For this purpose, the moisture content was chosen as 55% (the central point of the studied domain) and the variation of enzyme activity with time for different values of temperatures were studied.

In solid state fermentation for glucoamylase production by *M. indicus*, higher temperatures favored the enzyme production at initial stages of cultivation, while low temperatures were preferred at long incubation times (Fig. 1). At 50 h, which was the central point of the time domain, the best temperature for obtaining the highest glucoamylase activity was 26.6°C.

### Table 3: Regression analysis (ANOVA) for glucoamylase production by the fungi.

| Fungus    | SSM   | SSR   | DFM | DFR | MSM | MSR | F-value | p-value | R²   |
|-----------|-------|-------|-----|-----|-----|-----|---------|---------|------|
| *M. indicus* | 15928.1 | 1654.7 | 9   | 13  | 1769.7 | 127.2 | 13.9    | 0.000   | 0.906 |
| *M. hiemalis* | 32277.1 | 1696.7 | 9   | 13  | 3586.3 | 130.5 | 27.5    | 0.000   | 0.950 |
| *R. oryzae*  | 181716.8 | 19883.7 | 9   | 13  | 20190.7 | 1529.5 | 13.2    | 0.000   | 0.901 |

SS: Sum of square; DF: Degree of freedom; MS: Mean square. Subscripts: M: Model; R: Residual.

### Table 4: Regression coefficients of equation 1 for glucoamylase production by the fungi.

| Fungus     | *M. indicus* | *M. hiemalis* | *R. oryzae* |
|------------|--------------|---------------|-------------|
| Term       | Coefficient  | p-value       | Coefficient | p-value   | Coefficient | p-value |
| a₀         | 466.37       | 0.138         | 2647.51     | 0.000     | 2657.6      | 0.022   |
| a₁         | -7.584       | 0.458         | -70.523     | 0.000     | -103.3      | 0.010   |
| a₂         | -13.69       | 0.011         | -18.834     | 0.001     | 29.092      | 0.094   |
| a₃         | 5.798        | 0.225         | -35.837     | 0.000     | -21.33      | 0.199   |
| a₄         | 0.272        | 0.032         | 0.573       | 0.000     | 0.661       | 0.116   |
| a₅         | 0.148        | 0.087         | 0.092       | 0.277     | -0.183      | 0.521   |
| a₆         | -0.478       | 0.000         | 0.476       | 0.000     | 1.233       | 0.001   |
| a₇         | 0.030        | 0.308         | 0.143       | 0.000     | 0.163       | 0.120   |
| a₈         | 0.115        | 0.013         | 0.061       | 0.152     | -0.829      | 0.000   |
| a₉         | 0.057        | 0.063         | 0.143       | 0.000     | 0.165       | 0.116   |

### Table 5: Optimum conditions and enzyme activity for production of glucoamylase by the fungi.

| Parameter's values | *M. indicus* | *M. hiemalis* | *R. oryzae* |
|--------------------|--------------|---------------|-------------|
| T (°C)             | 26.6         | 26.6          | 26.6        |
| W (%)              | 71.8         | 71.8          | 71.8        |
| I (h)              | 66.8         | 33.1          | 33.1        |
| Model’s activity (U/gds) | 255.3      | 272.3         | 1545.3      |
| Experimental activity (U/gds) | 240.1       | 260.5         | 1496.3      |
According to Fig. 2 and 3, lower temperatures were preferred at short cultivation times for glucoamylase production by *M. hiemalis* and *R. oryzae*, as the corresponding enzyme activities were higher. A different trend was observed at longer times. However, after 50 h, 26.6°C was still the optimum temperature for glucoamylase production.

Since the fungal growth was affected by temperature, the enzyme production is dependent on temperature. The physiological changes due to high temperatures in enzyme production are not well known. However, it is reported that high temperatures may limit the synthesis of essential proteins for fungal growth and other physiological processes [23]. Similar result was obtained for glucoamylase production by *Aspergillus* species on wheat bran in which the optimum temperature was 30°C [24].

### 3.3 Effect of medium moisture content

For an enzyme production, medium moisture content has been reported to be an important factor in solid state fermentation [11]. Low and high medium moisture contents influence the growth of the fungus, enzyme biosynthesis [25,26], and the physical properties of the solid substrate [27]. Therefore, it is necessary to find the optimum moisture content, which is dependent on the type of the substrate, end product, and nutrient requirements of the fungus [28]. In this paper, the effect of moisture content on glucoamy-
Glucamylase activity was achieved at initial stages of culture. For all moisture contents and cultivation times, maximum activity was obtained at a minimum value and then increased with elapsing time. For longer times, the glucamylase activity showed a direct relation with moisture content. For the central point of the time region (50 h), the highest enzyme activity was obtained at the highest moisture content.

For M. hiemalis, glucamylase production showed a parabolic behavior with a minimum point as a function of moisture content. With increasing the time, descending branch disappeared gradually and increasing trend was observed. For the cultivation time of 50 h, the highest enzyme activity was obtained at the highest moisture content.

For R. oryzae, glucamylase production increased with increasing the moisture content at short times (I<37 h). For fermentation times between 37 h and 55 h, the enzyme activity decreased to a minimum value and then increased with increasing the moisture content. For longer times, the glucamylase activity showed a direct relation with moisture content. For the central point of the time region (50 h), the highest enzyme activity was obtained at the highest moisture content. For low medium moisture content, glucamylase production increased with increasing the moisture content at short times (I<42 h). For 42 h<I<55 h, the enzyme activity showed a parabolic behavior with a minimum point with increasing moisture content. For longer times, the ascending branch of the parabola vanished and a decreasing trend was observed.

Generally, moisture content is an important factor affecting enzymes production. For instance, low moisture contents reduce mass transfer and solubility of nutrients and increase water tension, which decreases metabolic and enzymatic activity [21,29–31]. There are several reports on the favorable effect of high moisture contents on enzyme production. It may be resulted from the fact that at high moisture contents, the fungal growth is faster and the enzyme production initiates earlier [24,30]. Ellaiah et al. [24] indicated that the initial moisture content of 80% was the optimum amount for glucamylase production by an Aspergillus species.

### 3.4 Effect of cultivation time

For low medium moisture content, glucamylase production by M. indicus showed a parabolic behavior (with a minimum point) with time, and higher enzyme activity was observed at initial stages of fermentation. With increasing the medium moisture content, the descending branch of the glucamylase- time curve gradually disappeared and the enzyme production increased (Fig. 4a).

For M. hiemalis, glucamylase activity decreased to a minimum value and then increased with elapsing time. For all moisture contents and cultivation times, maximum glucamylase activity was achieved at initial stages of cultivation (Fig. 4b).

Glucamylase production by R. oryzae increased with time for low moisture contents. For intermediate moisture contents, the linear trend changed to a parabolic behavior with a minimum point. For large moisture contents, the ascending branch disappeared and the trend of glucamylase activity as a function of time was descending (Fig. 4c).

According to the results, cultivation time affects glucamylase production by the fungi. Short cultivation times provide conditions for the economical enzyme production. For glucamylase production by M. hiemalis and R. oryzae, lower cultivation times favor the enzyme production. Catabolite repression by glucose released from the substrate hydrolysis may reduce the enzyme activity at longer times. Furthermore, other byproducts may inhibit the fungal growth and affect the enzyme formation [32].

In contrast, the maximum glucamylase production by M. indicus was observed at the longest time within the fungus autolysis stage.

### 3.5 Optimum conditions for glucamylase production

According to Table 2, glucamylase production by the fungi varies considerably with cultivation time, temperature, and moisture content. Thus, it is necessary to find the conditions at which the highest enzyme activity could be obtained.

The optimum conditions for glucamylase production by the fungal strains are presented in Table 5. Accordingly, for glucamylase production, R. oryzae showed the highest enzyme activity (1545 U/gds) with a considerable difference with M. indicus and M. hiemalis enzyme activity. The optimum temperature and moisture content for the three fungi were 26.6°C and 71.8%, respectively. Maximum glucamylase activity was achieved at the shortest time (33.1 h) for M. hiemalis and R. oryzae, whereas it was obtained at the longest time (66.8 h) for M. indicus. Furthermore, the optimum conditions for these strains were verified and some experiments were performed at optimum conditions. As it is observed in Table 5, glucamylase activities at the optimum conditions are appropriately close to the values predicted by mathematical models confirming that the models appropriately describe glucamylase production by these strains.

### 4 Conclusions

Appreciable amounts of glucamylase can be produced by solid state fermentation of R. oryzae, M. indicus, and M. hiemalis.
malis on wheat bran. The optimum temperatures and moisture content for efficient glucoamylase production by the fungi were the same (26.6°C and 71.8%), while their optimum cultivation times (33.1 h for *M. hiemalis* and *R. oryzae* and 66.8 h for *M. indicus*) were different. The maximum glucoamylase production was obtained by *R. oryzae* (1545.3 U/gds) with a considerable difference with that of *M. indicus* (255.3 U/gds) and *M. hiemalis* (272.3 U/gds).

Conflict of interest: None declared.

5 References

[1] Elegado F, Fujio Y. Selection of raw-starch digestive glucoamylase producing Rhizopus strain. J Gen Appl Microbiol 1993; 39:541–6.

[2] Ono S, Hiromi K, Zinbo M. Kinetic studies of glucoamylase, I. The influence of chain length of linear substrates on the rate parameter. J Biochem 1964; 55:315–20.

[3] Nigam P, Singh D. Enzyme and microbial system involved in starch processing. Enz Microb Technol 1995; 17:770–8.

[4] Nguyen QD, Rezissy-Szabo JM, Claeyssens M, Stals I, Hoschke A. Purification and characterization of amylolytic enzymes from thermophilic fungus Thermomyces lanuginosus strain ATCC 34626. Enz Microb Technol 2002; 31:345–52.

[5] Chen J, Li DC, Zhang YQ, Zhou QX. Purification and characterization of a thermostable glucoamylase from Chaetomium thermophilum. J Gen Appl Microbiol 2005; 51(3):175–81.

[6] Pandey A, Soccol CR, Nigam P, Soccol VT. Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. Bioreossur Technol 2000; 74(1):69–80.

[7] Cannell E, Moo-Young M. Solid state fermentation systems. Process Biochem 1980; 15(5):2–7.

[8] Durand PA. La fermentation en milieu solide. Biofutur 1998; 181(181):41–3.

[9] Raghavaran SKS, Ranganathan TV, Karanth NG. Some engineering aspects of solid-state fermentation. Biochem Eng J 2003; 13(2):127–35.

[10] Ferreira G, Boer CG, Peralta RM. Production of xylanolytic enzymes by *Aspergillus* tamarii in solid state fermentation. FEMS Microbiol Lett 1999; 173(2):335–9.

[11] Weiland P. Principles of solid state fermentation. In (Ed. Zadražil F, Reinner P) Treatment of Lignocellulosics with White Rot Fungi. Elsevier Applied Science, London 1988. p. 64–76.

[12] Kalogeris E, Iniotaki F, Topakas E, Christakopoulos P, Pekos D, Macris BJ. Performance of an intermittent agitation rotating drum type bioreactor for solid-state fermentation of wheat straw. Bioressour Technol 2003; 86(3):207–13.

[13] Badhan AK, Chadha BS, Kaur J, Saini HS, Bhat MK. Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora* sp. IMI 387099. Bioressour Technol 2007; 98(3):504–10.

[14] Maciel GM, Vandenberghe LPS, Haminik CWI, Fendrich RC, Bianca BED, et al. Xylanase production by *Aspergillus* niger LPB 326 in solid-state fermentation using statistical experimental designs. Food Technol Biotechnol 2008; 46(2):183–9.

[15] Karimi K, Brandberg T, Edebo L, Taherzadeh MJ. Fed-batch cultivation of *Mucor* indicus in dilute-acid lignocellulosic hydrolyzate for ethanol production. Biotechnol Lett 2005; 27(18):1395–400.

[16] Karimi K, Zamani A. *Mucor* indicus: biology and industrial application perspectives: a review. Biotechnol Adv 2013; 31(4):466–81.

[17] Millati R, Edebo L, Taherzadeh MJ. Performance of Rhizopus, Rhizomucor and *Mucor* in ethanol production from glucose, xylose and wood hydrolysates. Enz Microb Technol 2005; 36(2–3):294–300.

[18] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959; 31 426–8.

[19] Francis F, Sabu A, Nampoothiri KM, Ramachandram S, Ghosh S, et al. Use of response surface methodology for optimizing process parameters for the production of alpha-amylose by *Aspergillus* oryzae. Biochem Eng J 2003; 15(2):107–15.

[20] Myers RH, Montgomery DC, Anderson-Cook CM. Response surface methodology: product and process optimization using designed experiments. Wiley, New York 2002.

[21] Adinarayana K, Eliaiah P, Srinivasulu B, Devi RB, Adinarayana G. Response surface methodological approach to optimize the nutritional parameters for neomycin production by *Streptomyces marisnensis* under solid state fermentation. Process Biochem 2003; 38(11):1565–72.

[22] Krishna C. Solid-state fermentation systems-an overview. Crit Rev Biotechnol 2005; 25(1-2):1–30.

[23] Gawande PV, Kamat MY. Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. J Appl Microbiol 1999; 87(4):511–9.

[24] Eliaiah P, Adinarayana K, Bhavani Y, Padmaja P, Srinivasulu B. Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species. Process Biochem 2002; 38(4):615–20.

[25] Nishio N, Tai K, Nagai S. Hydrolysis production by *Aspergillus* niger in solid state cultivation. European J Appl Microbiol Biotechnol 1979; 8(4):263–70.

[26] Ramesh MV, Lonsane BK. Critical importance of moisture content of the medium in alpha amylase production by *Bacillus* licheniformis *M* 27 in a solid state fermentation system. Appl Microbiol Biotechnol 1990; 33(5):501–5.

[27] Bakri Y, Jacques P, Thonart P. Xylanase production by *Penicillium* canescens 10-10c in solid-state fermentation. Appl Biochem Biotechnol 2003; 105-106:737–48.

[28] Lonsane BK, Ghildyal NP, Budiaetman S, Ramakrishna SV. Engineering aspects of solid state fermentation. Enz Microb Technol 1985; 6(6):258–65.

[29] Archana A, Satyanarayana T. Xylanase production by thermophilic *Bacillus licheniformis* A99 in solid-state fermentation. Enzym Microb Technol 1997; 21(1):12–7.

[30] Kalogeris E, Christakopoulos P, Pekos D, Macris BJ. Studies on the solid-state production of thermostable endoxylanases from *Thermoascus aurantiacus*: Characterization of two isozymes. J Biotechnol 1998; 60(3):155–63.

[31] Wang XJ, Bai JG, Liang YX. Optimization of multienzyme production by two mixed strains in solid-state fermentation. Appl Microbiol Biotechnol 2006; 73(3):533–40.

[32] Feroza B, Begum S, Hossain M. Production of glucoamylase by *Aspergillus* niger in Liquid culture and Determination of its Cultural Condition. Bangladesh J Sci Indian Res 1998; 33(2):309–11.