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Full Length Research Paper

Optimization of the alpha amylase production from *Bacillus amyloliquefaciens* IIB-14 via parameter significance analysis and response surface methodology

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In this article, we report the optimization of bacterial extracellular alpha amylase production from *Bacillus amyloliquefaciens* IIB-14, a locally isolated bacterial culture, using response surface methodology (RSM). Different agricultural by-products were evaluated for enzyme activity under solid state fermentation (SSF). Of those, wheat bran (10 g) was found to be optimal when moistened with 0.02 M phosphate buffer at a level of 1:1.25. A marked improvement in enzyme productivity (52.06 U/mg/min) was observed at 72 h after the cultivation at 40°C (pH 7.2). The pre-grown bacterial inoculum (24 h old) was used at a level of 20%, v/v. The combined effect of cultural and nutritional variables on bacterial amylase was further investigated using RSM. The procedure limited the number of actual experiments performed while allowing for possible interactions between the optimal components including temperature, pH and inoculum. No previous work has used statistical analysis in determining the interactions among these parameters for alpha amylase production. The *p*-value of the coefficient for quadratic effect was 0.002, suggesting that this was the principal experimental variable having maximal effect on enzyme productivity. The results are economically significant ($p \leq 0.05$) and marked the commercial utility of the isolated bacterial culture.

Key words: Agricultural by-products, alpha amylase, *Bacillus* spp., fermentation, response surface methodology, solid substrate fermentation.

INTRODUCTION

Alpha amylase (endo-1,4-α-D-glucan glucanohydrolase) is an extra-cellular endo-enzyme that randomly cleaves α-1,4 linkages between adjacent glucose units in the linear amylose and branched amylopectin chain of the starch molecule and ultimately generates glucose, maltose and maltotriose units (Dey et al., 2001; Liu and Xun, 2008). The enzyme can be derived from several sources such as plants, animals and microorganisms. Among the microbes, the enzyme has been reported from fungi, yeasts, bacteria and actinomycetes. The most abundantly used bacterial amylases have been derived from *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus stearothermophilus* (Aiyer, 2005; Kiran and Chandra, 2008; Mrudula, 2010). Alpha amylase constitutes approximately 25% of the world enzyme market covering many industrial processes such as sugar, textile, paper, brewing, baking, distilling industries, preparation of digestive aids, fruit juices, starch syrups, detergents and pharmaceuticals (Teodoro and Martins, 2000; Hmidet et al., 2009). These applications have stressed to search a better fermentation process for enzyme productivity. Both submerged (SmF) and solid state fermentations (SSF) have been exploited; the later has been found to be more

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advantageous compared to the former being a simple and cost effective technique with better product recovery from the fermented mash culture (Mulimani et al., 2000; Abdullah et al., 2006).

The excretion of products of metabolism as a part of survival strategy of microbes in certain environments and over productivity of enzymes by media manipulation is considered to be a better strategy (Tanyildizi et al., 2007; Mahdavi et al., 2010). The operational parameters interact and influence each other's effect on the response during a batch process. The limitation of a single factor optimization can be eliminated by different techniques such as response surface methodology (RSM) which is used to explain the combined effects of all the variables involved. It is summarized as a collection of statistical tools and techniques for constructing and exploring an approximate functional relationship between a response variable and a set of design variables (Kiran and Chandra, 2008). The most extensive application of RSM can be found in situations where several input variables influence the performance measure (called the response) in a way that is difficult to describe. It might be possible to derive an expression for the performance measure based on the response values obtained from experiments at some particular combination of the input variables (Soni et al., 2003). However, no previous work has used RSM to optimize alpha amylase productivity when temperature, initial pH and inoculum size were the interacting response parameters.

The medium composition and fermentation design greatly affects both the growth and productivity of extracellular enzymes from microorganisms. Cost and availability of the substrate are important considerations, and therefore the selection of an appropriate substrate plays a vital role in the development of an efficient SSF process (Mulimani et al., 2000; Haq et al., 2003; Akpan and Adelaja, 2004). The present study was designed to evaluate different agricultural by-products like rice bran, maize bran, rice husk, wheat bran, barley bran, mustered cakes and wheat straw and also to optimize the cultural conditions such as incubation period, temperature, pH, moisture content, size and age of inoculum for alpha amylase productivity from *Bacillus amyloliquefaciens* IIB-14 under SSF.

MATERIALS AND METHODS

Organism and inoculum preparation

The strain *Bacillus amyloliquefaciens* IIB-14, a locally isolated bacterial culture was obtained from the available stock culture of the Institute of Industrial Biotechnology (IIB), Government College University Lahore Pakistan. The culture was maintained on the nutrient broth (NB) agar (pH 7) slants and stored at 4°C in a cold-cabinet. Fifty ml of NB medium (containing nutrient broth 0.8%) was used and a loopful of bacterial culture was aseptically transferred and agitated at 200 rpm in a rotary shaking incubator for 24 h. The temperature was maintained at 37°C.

Fermentation procedure and conditions

Various agricultural by-products including rice bran, maize bran, rice husk, wheat bran, barley bran, mustered cakes and wheat straw were evaluated using solid SSF. Ten grams of wheat bran as substrate (found optimal) was moistened with 0.02 M phosphate buffer (pH 7) at a ratio of 1:1. One ml of the inoculum (2.35×10^7 CFU) was aseptically transferred to each flask. The flasks were placed in an incubator at 37°C for 72 h. The contents of flasks were shaken twice a day to ensure maximal air (or oxygen) supply and a better bacterial growth. All the experiments were run parallel in a set of triplicates.

Enzyme extraction

After the optimal incubation period, 100 ml of 0.02 M phosphate buffer (pH 7) was transferred to each flask. The flasks were rotated in a shaking incubator at 200 rpm for 1 h. The fermented mash was centrifuged at 6500 × g for 15 min. The supernatant was used as the enzyme source.

Enzyme assay

Alpha amylase was estimated according to the method of Rick and Stegbauer (1974). The enzyme extract (0.5 ml) was added to a test tube containing 0.5 ml of 1.0% soluble starch solution. A blank containing 0.5 ml of distilled water with 0.5 ml of starch (1.0%) was run in parallel. The tubes were incubated at 60°C for 10 min. Then 1.0 ml of DNS (3, 5-Dinitrosalicylic acid) was added to each test tube. The tubes were placed in boiling water for 5 min and cooled at room temperature. The contents of tubes were diluted up to 10 ml with distilled water. The absorbance of reaction mixture was determined at 546 nm using a UV/VIS spectrophotometer and was converted to milligram (mg) of maltose from the standard curve established against maltose.

One enzyme unit is defined as the amount of enzyme that hydrolyses 1.0 mg of starch (0.1%) in 10 min at 40°C and pH 7.2.

Determination of protein content

Total protein content was estimated by taking 0.1 ml of the enzyme extract and 5 ml of Bradford reagent in a test tube. A blank containing 0.1 ml of distilled water with 5 ml of the Bradford reagent was run in parallel. The optical density was measured at 595 nm using a spectrophotometer. The slope of the curve was used for protein estimation from bovine serum albumin (BSA)-standard (Bradford, 1976).

Optimization of batch culture conditions

The medium and cultural conditions optimized for alpha amylase productivity included incubation period (12, 24, 36, 48, 60, 72, 84, 96 h), temperature (34, 37, 40, 43, 46°C), diluents (distilled water, phosphate buffer, citrate buffer and acetate buffer), substrate to diluent ratio, pH (6.6, 6.8, 7.0, 7.2, 7.4 and 7.6), age and size (5.0, 10, 15, 20, 25 and 30%) of inoculum.

Application of RSM

RSM for alpha amylase productivity was studied (Dey et al., 2001). The experimental design was a 2^2 full factorial central composite experimental plan. The data of the factors were chosen after a series of preliminary experiments. Twenty experiments were conducted using a face central composite statistical design ($\alpha = 1$)

Table 1. Experimental design using RSM.

Run	Temp. (°C)	pH	Inoculum (%, v/v)	Alpha amylase production (U/mg/min)
1	34	6.6	5	18.34 ± 1.6
2	34	6.6	10	18.95 ± 1.2
3	37	6.6	10	21.06 ± 2.1
4	37	6.8	10	22.52 ± 1.7
5	37	6.8	15	22.98 ± 1.6
6	37	7	15	27.24 ± 2.5
7	40	6.8	15	32.15 ± 1.9
8	40	6.6	15	32.46 ± 2.4
9	40	6.6	20	33.08 ± 2.4
10	37	7	25	36.45 ± 3.5
11	37	7	15	37.12 ± 3.6
12	40	7.2	20	51.96 ± 3.7
13	43	6.8	15	45.62 ± 3.8
14	43	7	20	44.15 ± 4.5
15	43	6.6	25	44.01 ± 4.1
16	34	7	15	36.98 ± 3.4
17	37	7.4	15	35.42 ± 3.2
18	37	6.8	15	31.28 ± 3.1
19	46	6.6	20	29.16 ± 3.4
20	46	7	30	27.94 ± 3.3

The optimal parameters include temperature, pH and inoculum size for RSM analysis. ± indicate the standard deviation (sd) among three parallel replicates in each set of the separate run. The values representing enzyme production in the last column differ significantly from each other at p≤ 0.05.

for the study of three critical factors each at different levels. The levels of factors used in the experimental design are listed in Table 1. The second order polynomial coefficients were calculated and analyzed (Spss-10, Version-4.0, USA). The basic strategy consisted of four steps: procedures to move into optimal region, behavior of the response in the optimal region, estimation of the optimal conditions and verification. The variable levels of V_i were coded as v_i according to the following indexed equation such that V_0 corresponds to the central value, where v_i is the dimensionless value of an independent variable; V_i is the real value of the variable; V_0 is the real value of the variable at the center point and ΔV_i is the step change.

$$v_i = \frac{V_i - V_0}{\Delta V_i} \qquad i = 1,2,3, \ldots\ldots, k$$

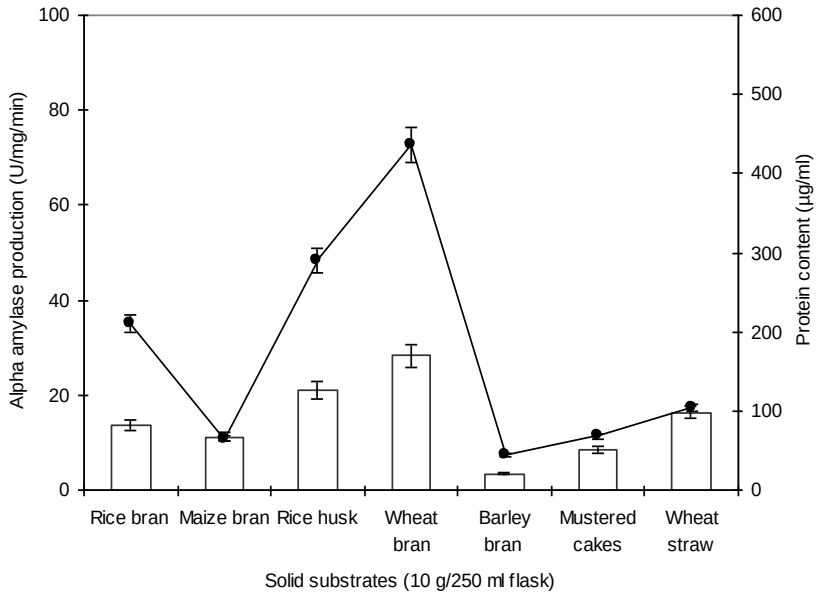
Statistical depiction

The treatment effects were compared by the protected least significant difference method given by Snedecor and Cochran (1980). Each experiment was performed in a set of three parallel triplicates and the standard deviation of each experiment ranged from 1.2 to 4.5. The significant difference among the replicates has been presented as Duncan's multiple ranges in the form of probability (p) value.

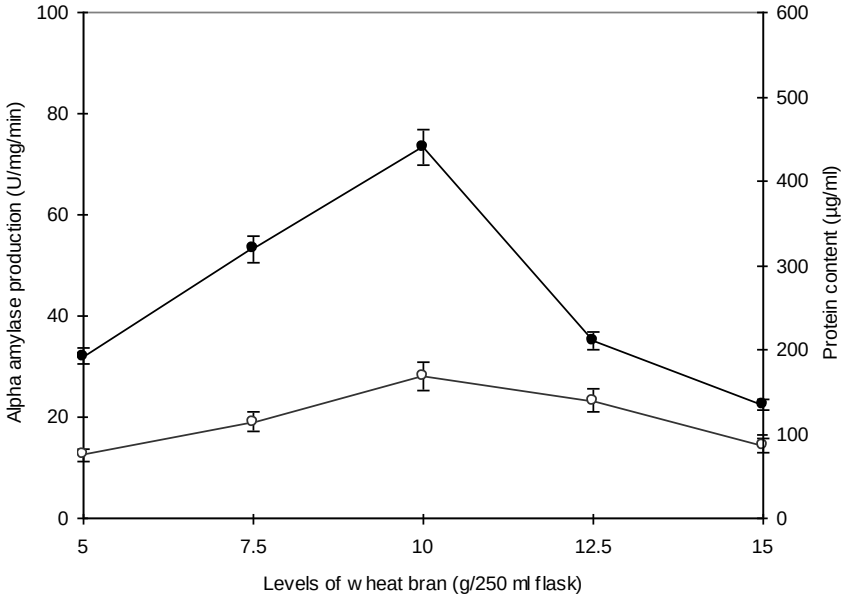
RESULTS AND DISCUSSION

The selection of a suitable substrate for a fermentation process is a significant factor for SSF. In the present study, different agricultural by-products such as rice bran, maize bran, rice husk, wheat bran, barley bran, mustered cakes and wheat straw as basal carbon source were evaluated (Figure 1). Rice bran gave 13.54 U/mg/min enzyme activities. An extremely lower enzyme productivity (3.44 U/mg/min) was observed with barley bran. It might be due to the fact that barley bran contained very low contents of nutrients, which were essential for alpha amylase productivity (Dhanya et al., 2009). However, wheat bran supported maximal enzyme productivity (28.26 U/mg/min) and protein content (436 µg/ml), probably due to the fact that wheat bran contained high concentration of nutrients and provided large surface for the growth of microorganism substantiating the findings reported earlier (Anto et al., 2006). However, Mulimani et al. (2000) obtained maximum enzyme productivity with rice bran as a solid medium. The other substrates such as maize bran, rice husk, mustered cakes and wheat straw gave 11.16, 21.05, 8.39 and 16.32 U/mg/min enzyme, respectively.

The level of wheat bran was varied from 5 to 15 g for alpha amylase productivity (Figure 1b). Wheat bran (5.0 g) gave 12.5 U/mg/min enzyme productivity, which was increased with the increase in wheat bran concentration from 5 to 10 g for each batch culture. Maximal enzyme activity (27.98 U/mg/min) and protein content (440 µg/ml) was achieved when 10 g of wheat bran was used (Figure 1a). In contrast to present finding, previously 20 g of



(a)



(b)

Figure 1. Evaluation of (a) solid substrates and (b) substrate concentration for alpha amylase productivity from *B. amyloliquefaciens* IIB-14. Incubation period 72 h, pH 7.0, temperature 37°C. Y-error bars indicate standard deviation (±sd) among the three parallel replicates. Enzyme activity (U/mg/min) -○-, Protein content -●-.

wheat bran was however found to be the best for maximizing the enzyme productivity (Tanyildizi et al., 2007). Increasing wheat bran concentration (12.5 g) other than the optimal, the enzyme productivity was noticeably declined to 23.26 U/mg/min. It was further reduced to 14.42 U/mg/min when 15 g of wheat bran was used.

The optimization of incubation period is critical for the optimal growth of the bacterial cells with significant alpha amylase productivity (Lulko et al., 2007). Batch culture fermentations were carried out from 12 to 96 h after incubation (Figure 2). Enzyme productivity was found extremely low (2.32 U/mg/min) at 12 h of incubation,

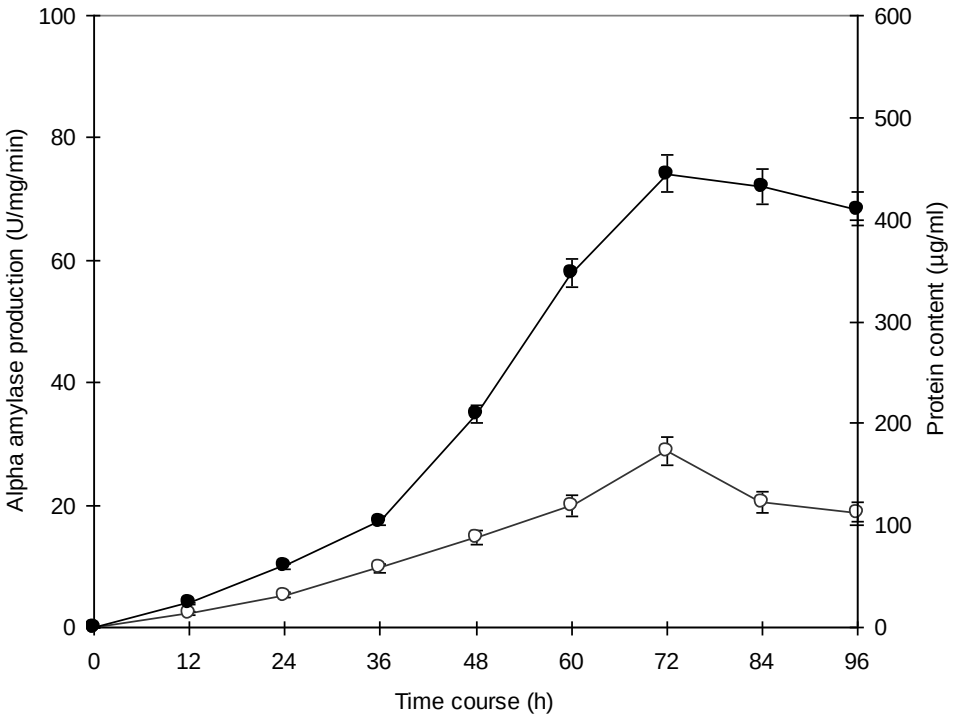


Figure 2. Alpha amylase productivity from *B. amyloliquefaciens* IIB-14. Temperature 37°C, pH 7.0. Y-error bars indicate standard deviation (±sd) among the three parallel replicates. Enzyme activity (U/mg/min) -○-, Protein content -●-.

probably due to the fact that bacteria entered the lag phase of growth, where they acclimatized with the environment. It was gradually increased with increase in incubation period from 12 to 60 h after inoculation. However, a sharp increase in enzyme productivity was obtained from 60 to 72 h and incubation for 72 h was found to be optimal for both enzyme productivity (28.92 U/mg/min) and protein content (445 µg/ml). Hmidet et al. (2009) found that the enzyme productivity was maximal when bacteria entered in the late exponential phase of growth. The activity was markedly declined by further increasing the incubation period from 72 to 84 h. At 96 h, it was reduced to 18.84 U/mg/min possibly due to the depletion of nutrients, accumulation of by-products such as proteases, toxins, inhibitors and proteolytic activity (Mulimani et al., 2000; Teodoro and Martins, 2000; Aiyer, 2005).

Temperature is a highly sensitive parameter for alpha amylase productivity. Microbial fermentations were carried out from 34 to 46°C (Figure 3). At 34°C, the enzyme productivity was found to be very low (21.08 U/mg/min). At low temperature, bacterial growth was not encouraging (Declerck et al., 2003). However, maximum enzyme activity (33.65 U/mg/min) and protein content (446 µg/ml) was obtained at 40°C. It might be due to the fact that the growth of bacteria was maximal at optimum temperature. By further increasing the temperature (43°C), there was a sharp decline in the enzyme productivity (27.14 U/mg/min). However, at 46°C, the enzyme activity was markedly reduced to 23.82 U/mg/min, probably due to the reduction in moisture content of the fermented mash that resulted in enzyme denaturation.

The moisture content in the solid substrate is one of the most critical parameters in SSF that directly influences growth of the organism and subsequent alpha amylase productivity (Mrudula 2010). The effect of different diluents such as distilled water, tap water, citrate buffer, phosphate buffer and acetate buffer was investigated (Figure 4). Extremely low level of enzyme activity (2.4 U/mg/min) was obtained with distilled water. It might be due to the fact that distilled water has no micro- or macro-nutrients, keeping the pH variable as an unpredictable variable which did not support microbial growth. However, enzyme activity (34.12 U/mg/min) and protein content (462 µg/ml) was maximum when wheat bran was moistened with phosphate buffer. Saxena et al. (2007) found that phosphate ions may not only support the growth of organism but also act as stimulators of alpha amylase. Maximum enzyme productivity was obtained when wheat bran was moistened with tap water containing 1.0% Tween-40 and 1.0 mM $MgSO_4$ (Mulimani et al., 2000). Other diluents such as tap water, citrate buffer and acetate buffer gave 11.08, 20.52 and 13.32 U/mg/min enzyme, respectively.

In the present study, substrate to diluent (phosphate

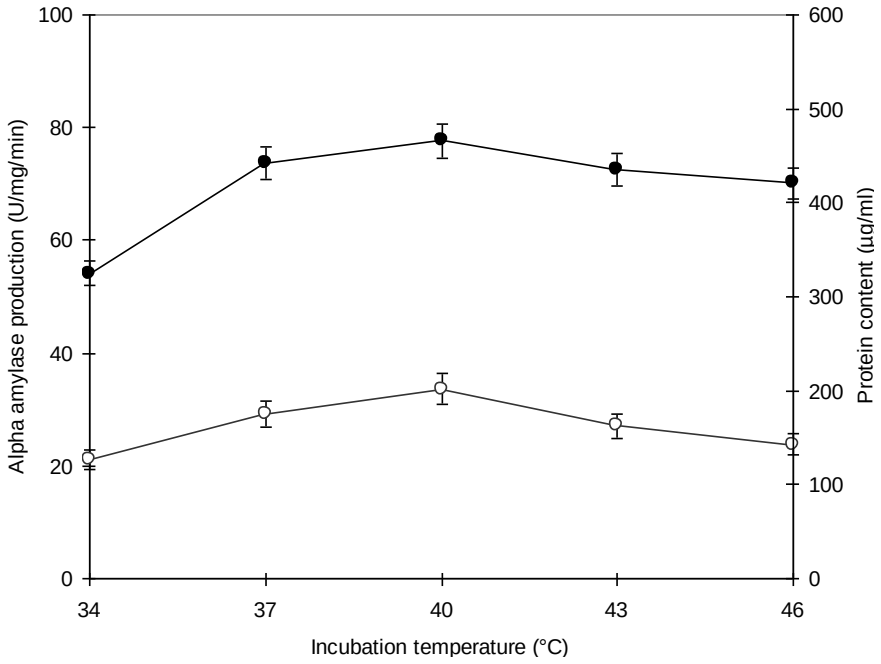


Figure 3. Effect of temperature on alpha amylase productivity from *B. amyloliquefaciens* IIB-14. Incubation period 72 h, pH 7.0, substrate to diluent ratio 1:1. Y-error bars indicate standard deviation (±sd) among the three parallel replicates. Enzyme activity (U/mg/min) -○-, Protein content -●-.

buffer) ratio varied from 1:0.5 to 1:2 (Figure 4a). The lowest enzyme activity (20.68 U/mg/min) was obtained when the ratio was adjusted to 1.0:0.5. This is as a result of the fact that the lower moisture content caused a reduction in the solubility of nutrients of the solid substrate and its degree of swelling, but with higher water tension (Declerck et al., 2003). However, enzyme productivity was sharply improved with the increase in ratio of substrate to phosphate buffer from 1:0.5 to 1.0:1.25. The enzyme productivity (39.52 U/mg/min) and protein content (476 µg/ml) was optimal when phosphate buffer was added to wheat bran at a level of 1.0:1.25. It might be due to the fact that optimal level of moisture content was very essential for air (or oxygen) supply for an aerobic fermentation. Further increase in the concentration of phosphate buffer decreased the enzyme activity. At a ratio of 1.0:2.0, it was markedly reduced to 25.26 U/mg/min, probably due the fact that during SSF, higher moisture level decreases porosity, changes wheat bran particle structure, promotes development of stickiness and lowers the oxygen transfer rate (Declerck et al., 2003).

Medium pH plays a profound role for the growth of organism and subsequent enzyme synthesis (Sodhi et al. 2005). The pH was varied from 6.6 to 7.6 for each batch culture (Figure 5). The enzyme productivity (34.20 U/mg/min) and protein content (320 µg/ml) was found to be very low at pH 6.6, possibly due to the fact that at slightly acidic pH, the bacterial growth was not encouraging. However, it was gradually increased with rise in the pH from 6.6 to 7 and sharply increased from 7.0-7.2. It was found to be maximal when pH of the medium was maintained at 7.2. At pH 7.2, the enzyme productivity was 44.12 U/mg/min and protein content was 496 µg/ml. It might be due to the fact that the organism required slightly alkaline pH for its growth and hence enzyme productivity was increased. In contrast to the present findings, Sajedi et al. (2005) and Mahdavi et al. (2010) obtained optimal enzyme activity at neutral pH and at a pH range of 8-9.5. By further increasing the pH up to 7.4, the enzyme productivity was declined (36.15 U/mg/min). At pH 7.6, it was reduced to 29.84 U/mg/min, probably due to change in pH other than the optimal inhibited the growth of organism.

Alpha amylase productivity is also affected by the age and size of inoculums (Baysal et al., 2008). The effect of different inoculum size (5.0 to 30%) was investigated (Figure 6). Small size of inoculum, like 5.0% (v/m), resulted in extremely lower enzyme activity (36.44 U/mg/min) and protein content (438 µg/ml) probably due to small number of bacterial cells available in the production medium. However, it was gradually increased with increase in the inoculum size from 5-15% while sharply increased at 15-20%. Optimal enzyme productivity was 51.28 U/mg/min and protein content was 522 µg/ml, respectively, when 20% (v/m) inoculum was used. It was due to the fact that bacterial cells were sufficient as reported previously (Hagihara et al., 2001;

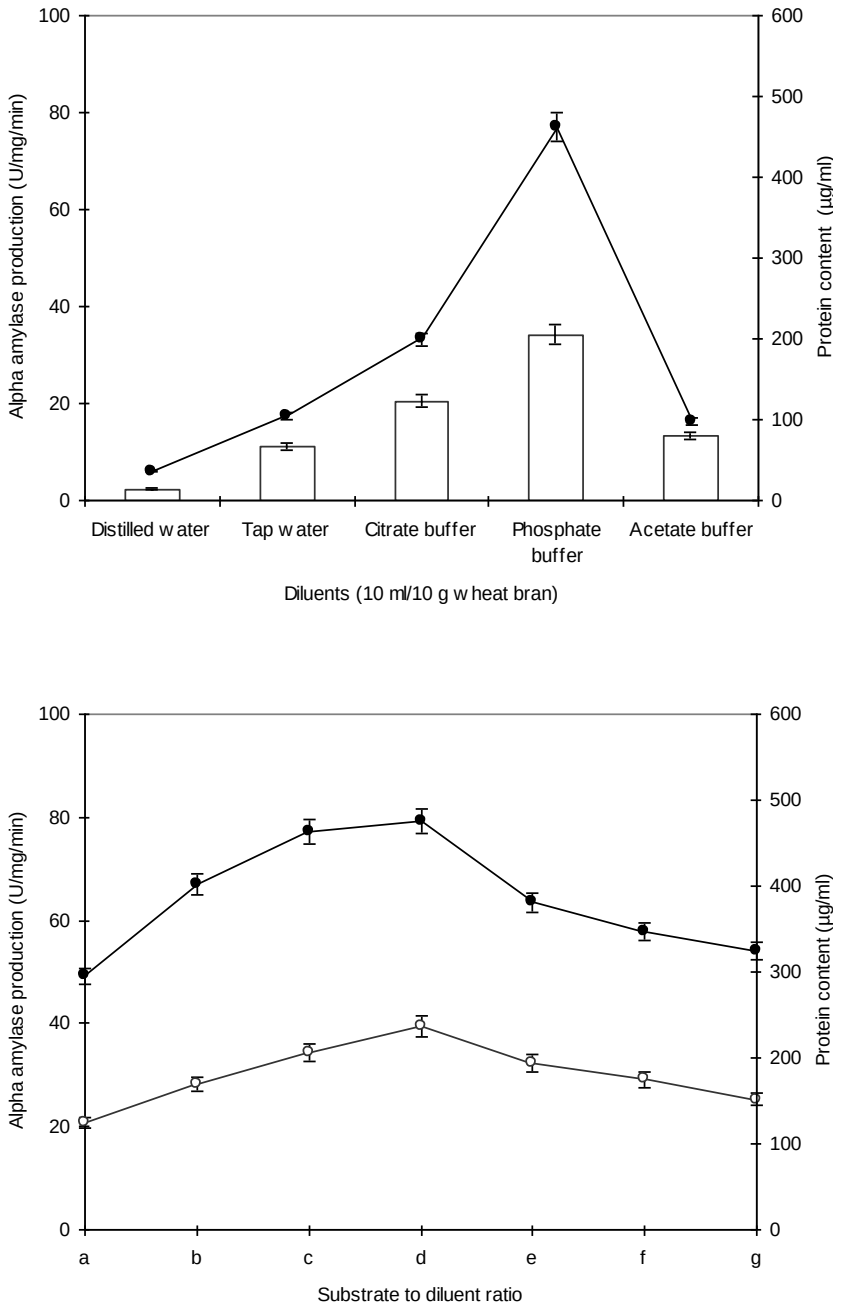


Figure 4. Effect of (a) different diluents and (b) substrate to diluent ratio on alpha amylase productivity from *B. amyloliquefaciens* IIB-14. Temperature 40°C, pH 7.0, incubation period 72 h. Y-error bars indicate standard deviation (±sd) among the three parallel replicates. Enzyme activity (U/mg/min) -○-, Protein content -●- (a. 1:0.5, b) 1:0.75, c. 1:1, d. 1:1.25, e. 1:1.5, f. 1:1.75, g. 1:2).

Aiyer, 2005). By further increasing the size of inoculum to 25%, there was a mark decline (42.35 U/mg/min) in enzyme productivity. It was further reduced to 38.72 U/mg/min, when 30% inoculum was used. This may be due to the limiting nutrients at higher inoculum size other than the optimal.

The age of inoculum was varied from 8 to 40 h. Enzyme productivity was found to be extremely low (32.15 U/mg/min), when 8 h old inoculum was used, probably due to the pre mature growth of bacterial cells. It was gradually increased when the age of inoculum was further increased from 8 to 16 h (Figure 6a). However, optimal enzyme activity (52.06 U/mg/min) and protein content (519 µg/ml) was obtained with 24 h old inoculum.

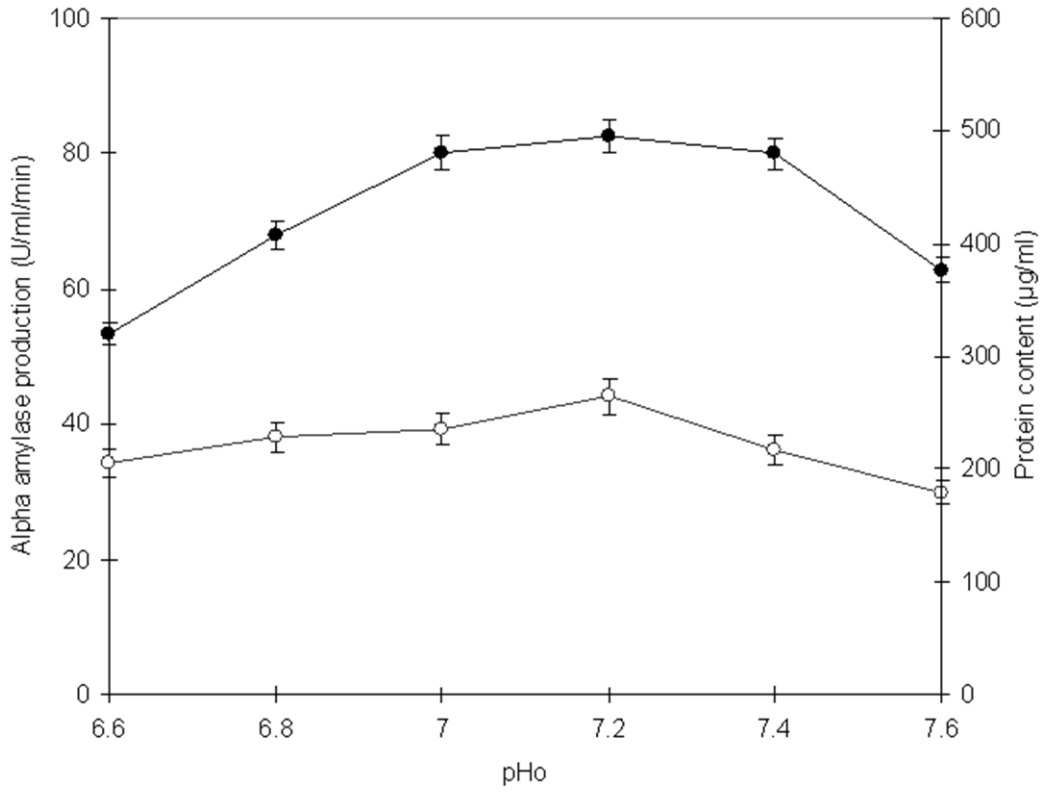


Figure 5. Effect of pH on alpha amylase productivity from *B. amyloliquefaciens* IIB-14. Temperature 40°C, incubation period 72 h, substrate to diluent ratio 1:1.25. Y-error bars indicate the standard deviation (±sd) among the three parallel replicates. Enzyme activity (U/mg/min) -○-, Protein content -●-.

It might be due to the fact that bacteria were in their active state of growth. By further increasing the age of inoculum (32 h old), there was a mark decline in enzyme productivity. Further decline in enzyme activity (33.14 U/mg/min) was recorded at an inoculum of 40 h. It was due to the accumulation of other by products such as secondary and tertiary metabolites or proteolyses (Baysal et al., 2008).

Our results showed that the incubation temperature, initial pH and size of inoculum markedly influenced the production of alpha amylase under solid state fermentation by the selected isolate *B. amyloliquefaciens* IIB-14. Thus, the optimal levels and significance of coefficients were determined by *t*- and *p*-values (Table 2). The larger the magnitude of *t*-value and smaller the *p*-value indicated the high significance of the corresponding coefficient. The variable with largest effect was the squared term of optimal level of diluent to substrate ratio (Dey et al., 2001). Furthermore, the linear effect of diluent and quadratic effect of pre-grown inocula were more significant than other factors. The great importance of agricultural by-products such as solid wheat bran for alpha amylase productivity is very much emphasized in literature (Teodoro and Martins, 2000; Abdullah et al., 2006). This effect of basal substrate might result from conversion of raw starch into reducing sugars (as maltose) by entering to the pathway for formation of metabolic energy. The coefficient for linear effect of starch and relatively linear effect of incubation period, temperature and the interactive effect of age of inoculum and substrate to diluent ratio may be noteworthy to some extent (Sodhi et al., 2005; Tanyildizi et al., 2007). The probability value of the coefficient of quadratic effect of optimal level of substrate was fairly high (0.0812), indicating that only 6.98% of the model affected by this variable. Since linear and quadratic effect of size and age of inoculum were significant, suggesting that they can act as limiting nutrients and a little variation in their concentration may alter the rate of product formation (Aiyer, 2005).

The analysis of variance (ANOVA) for amylolytic biosynthesis is presented in Table 3. The ANOVA of quadratic regression model demonstrates that the model is highly significant, as is evident from the Fisher's *F*-test with a very low probability value ($P_{model}>F$ = 0.002). Relatively lower value of coefficient of variation (CV = 28.96%) indicates a better precision and reliability of batch culture experiments carried out. The model presented a high determination coefficient (R^2 = 0.1572) suggesting ~96.5% of the variability in the response (Lulko et al., 2007). An adequate precision of 9.42 indicated an optimal gesture for the signal-noise ratio.

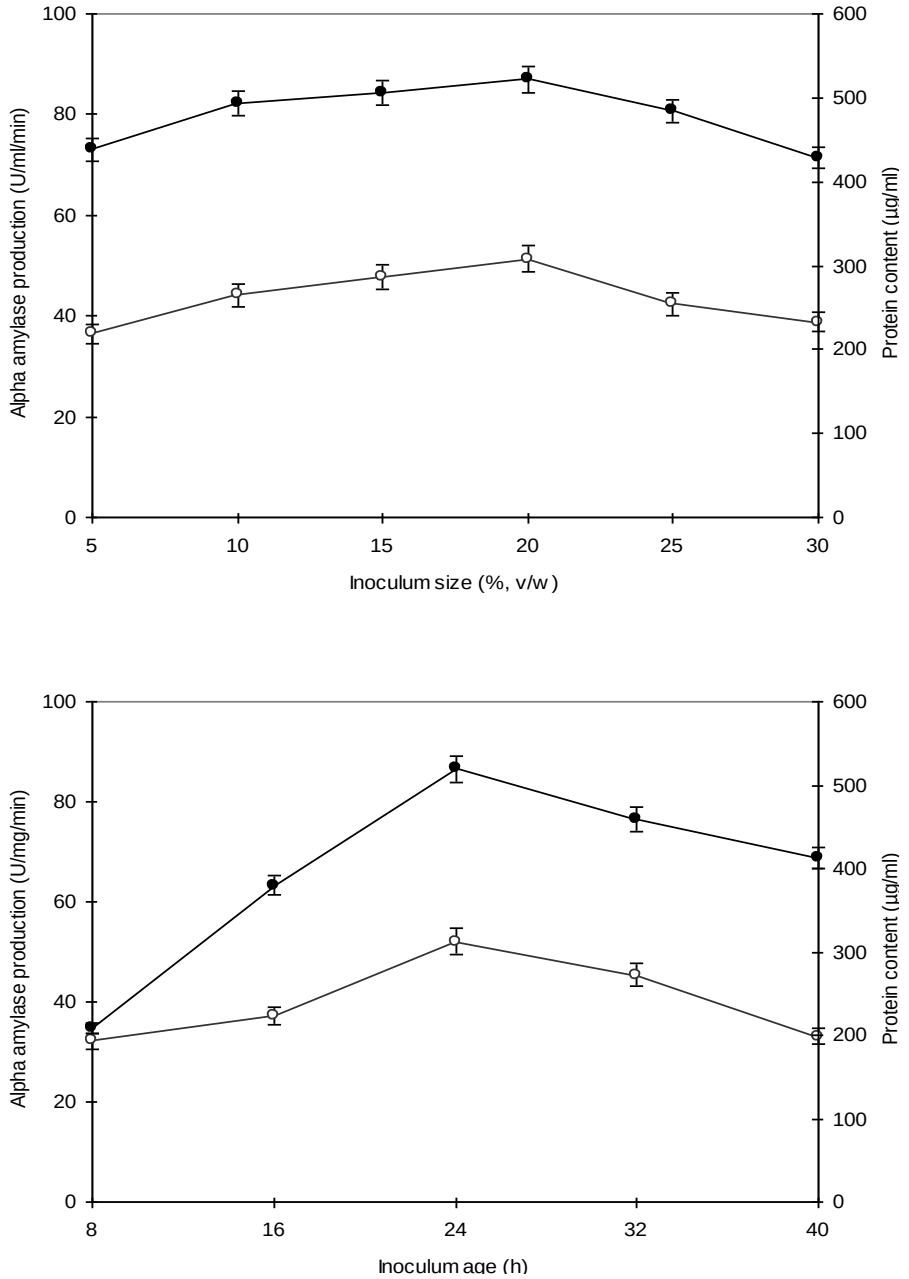


Figure 6. Effect of inoculum (a) size and (b) age on alpha amylase productivity from *B. amyloliquefaciens* IIB-14. Temperature 40°C, pH 7.2, incubation period 72 h. Y-error bars indicate standard deviation (±sd) among the three parallel replicates. Enzyme activity (U/mg/min) -○-, Protein content -●-.

The analysis gives the value of the model and determines the requirement of a more complex model with a better fit. However, if the *F*-test for lack of fit is significant, then a more complicated model is needed. The value of the adjusted determination coefficient was also found to be very high indicating a high significance where Y is the response that is alpha amylase activity (Hagihara et al., 2001; Soni et al., 2003; Baysal et al., 2008). V_1, V_2, V_3 and V_4 were coded values of the test variables incubation period, pH, carbon source and pre-grown bacterial inoculum, respectively. These results show that the model chosen can satisfactorily explain the optimal fermentation process parameters in batch culture.

Conclusion

In the present studies, wheat bran supported maximal

Table 2. Significance of regression coefficient for the least-squares fit parameter.

Model term	Parameter estimate	Standard error of mean	*t*-value	*p*-value
Intercept	36.15	5.48	25.06	0
V_1	3.14	7.36	9.38	0.002
V_2	−0.81	7.36	9.84	0.0244
V_3	4.01	7.36	−2.28	0.0812
V_4	2.08	7.36	2.22	0.002
$V_{1,2}$	7.92	11.86	−5.94	0.1572
$V_{1,3}$	0.91	11.86	−1.68	0.0431
$V_{1,4}$	4.63	11.86	7.13	0.1598
$V_{2,3}$	−2.69	11.86	4.14	0.1275
$V_{2,4}$	6.12	11.86	−0.28	0.0265
$V_{3,4}$	−3.76	11.86	−1.68	0.0884

Table 3. ANOVA for quadratic model*.

Source(s)	Sum of squares	Degree of freedom	Mean square	*F*-value	< *p* >
Model	695.35	6	85.35	28.96	0.002
Interaction	52.48	3	35.82	16.04	0.042
Linear	35.66	5	28.78	21.32	0.05
Square	146.51	9	112.56	42.15	0.003
Residual error	80.84	13	5.32		
Lack of fit	29.42	9	9.42	7.28	0.0682
Pure error	7.86	2	4.18		
Total	792.84	22			

*R^2 = 0.9171, CV = 24.56%.

alpha amylase productivity from *B. amyloliquefaciens* IIB-14 using solid state fermentation. A noticeable 10-fold enhancement in the enzyme activity was achieved by optimizing cultural conditions and nutritional requirements. RSM being an efficient and relatively simple technique can save time and material during batch culture studies. The use of full factorial design where the main point was to reveal the influence of concentrations of macronutrients on enzyme productivity allowed a rapid screening of large experimental domains in search of optimal fermentation conditions. This article provides a detailed study that used statistical analysis to determine the optimal levels and interaction among the incubation temperature, pH, and inoculum size for enzyme production. The results of the statistical analysis showed that the fit of the model was good in all cases. In addition, the economic significance ($p \leq 0.05$) of the experimental trials marked the commercial utility of the isolated bacterial culture. Structural characterization and enzyme stability at elevated temperature is the focus of further studies.

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