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Statistical Optimization of Tannase Production by Penicillium sp. EZ-ZH390 in Submerged Fermentation

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Abstract

Tannase has several important applications in food, feed, chemical and pharmaceutical industries. In the present study, production of tannase by mutant strain, Penicillium sp. EZ-ZH390, was optimized in submerged fermentation utilizing two statistical approaches. At first step, a one factor at a time design was employed to screen the preferable nutriments (carbon and nitrogen sources of the medium) to produce tannase. Screening of the carbon source resulted in the production of 10.74 UmL^{-1} of tannase in 72 h in the presence of 14% raspberry leaves powder. A 1.99-fold increase in tannase production was achieved upon further screening of the nitrogen source (in the presence of 1.2% ammonium nitrate). Then the culture condition variables were studied by the response surface methodology using a central composite design. The results showed that temperature of 30°C rotation rate of 85 rpm and fermentation time 24 h led to increased tannase production. At these conditions, tannase activity reached to 21.77 UmL^{-1}, and tannase productivity was at least 3.55 times (0.26 $UmL^{-1}h^{-1}$) in compare to those reported in the literature. The present study showed that, at the optimum conditions, Penicillium sp. EZ-ZH390 is an excellent strain for use in the efficient production of tannase.

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1. Introduction

Tannin acyl hydrolase, also known as tannase, is an enzyme that catalyzes the hydrolysis of ester bonds present in complex tannins, gallotannins and gallic acid esters [1]. Tannase has several important applications in food, pharmaceutical and chemical industries; however, due to high production costs of this enzyme, industrial production is severely restricted [2].

Tannase can be obtained from the various sources (e.g. tannin rich plants), but microbial sources are preferred for its industrial production [3]. The conditions for obtaining the maximal production of tannase depend on two factors: the system utilized and the enzyme source. Different research groups have devoted considerable efforts in finding new sources of tannase [4-8], and designing better production systems [9, 10]. A number of papers have been published recently dealing with the optimization of fermentation systems for the production of tannase [11-14]. Optimization of medium components is the pre-requisite for the better production of microbial enzymes. One factor at a time is a classical, simple method to study the effect of individual medium components on enzyme production.

In most of the studies, optimization was carried out by changing one independent factor at a time while fixing all the others at a certain level. This method requires a large number of experiments to determine the optimum levels, so it is very time consuming. In addition, this approach does not include the interactive effects among the variables and is, therefore, unreliable. Thus, a statistical optimization is preferred [15]. Raaman et al. optimized the extracellular tannase production by *Paecilomyces variotii* in solid-state fermentation (SSF) via changing one factor at a time, and obtained 1.23-fold increase in production of the enzyme [13]. Mohapatra et al. reported the optimization of tannase production by Bacillus licheniformis in submerged fermentation (SmF) using Taguchi's methodology, and obtained 2.18-fold increase in tannase production [16]. Using response surface methodology (RSM), Sharma and coworkers optimized tannase production by *Aspergillus niger*, and obtained 2.01-fold improvement in enzyme production [17]. Naidu et al. optimized tannase production by *Aspergillus foetidus* in SmF utilizing two statistical methods subsequently: at first, a Plackett-Burman design was used to find out the key factors for tannase production, and then these factors were optimized by RSM [18]. With this strategy, a two-fold increase in tannase activity was obtained in comparison to the medium optimized by the conventional one factor at a time method.

With this view, in the present investigation, tannase production by Penicillium sp. EZ-ZH390, a mutant from the recently isolated fungus Penicillium sp-EZ-ZH190 with high productivity was optimized in submerged fermentation utilizing two statistical methods subsequently: first, a one factor at a time design was employed for screening the carbon and nitrogen sources of the medium; then the culture condition factors were optimized by RSM using a central composite design.

2. Materials and Methods

2.1.Microorganism and preparation of inoculums

Penicillium sp. EZ-ZH390, a mutant strain from the fungus Penicillium sp. EZ-ZH190 isolated from the musty tea leaves, was used [3]. The inocula were prepared by addition of 5 mL of 0.01% tween 80 to 10 days old agar slope culture. Then the spore suspension was obtained at the final concentration of 5×105 spores per mL.

2.2. Raw materials

Leaves of raspberry and the green husk of walnut were collected from a local orchard, and green tea leaves were taken from the local farmer's fields. These materials were properly dried at 60°C, and powdered in a grinder.

2.3. Time course of tannase production

The time course of tannase production by Penicillium sp. EZ-ZH390 was carried out in shake flask cultures. Erlenmeyer flasks (250 mL), each containing 50 mL of fermentation medium (basal medium contains: $NaNO_3$, 2.0 gL^{-1}, K_2HPO_4, 1.0 gL^{-1}, KCl, 0.5 gL^{-1}, $MgSO_4$, 0.5 gL^{-1}, $FeSO_4$, 0.01 gL^{-1}, $ZnSO_4$ (trace), $CuSO_4$ (trace) and tannic acid, 10.0 gL^{-1}that were sterilized separately by passing through a cellulose nitrate membrane filter with 25 mm diameter and 0.45 μm pore size) were inoculated with the prepared inocula (2% vv^{-1}) and incubated for 168 h at 30°C and at 100 rpm in a rotary shaker. The culture broth was filtered through filter paper (Whatman No. 1) following the termination of the fermentation at 24 h intervals. The filtrate was centrifuged at 4°C and 12500 g for 15 min, and finally, the tannase activity of the supernatant, biomass (dried weight) and pH was measured.

2.4.Tannase activity assay

Tannase activity was determined spectrophotometrically according to Ibuchi et al. method [19] as described by Zakipour et al [3]. One unit of enzyme activity is defined as the amount of enzyme, which is able to hydrolyze 1 μmol of ester in 1 min.

2.5. Screening of the preferable nutriment for tannase production using one factor at a time method

Different carbon sources including glucose, green tea leaves, raspberry leaves and green husk of walnut powders (10.0 gL^{-1}) were added individually instead of tannic acid in the basal medium to find the highest tannase activity. Then the different contents of the selected carbon source were used in further experiments to find the highest tannase activity. The optimum content of the selected carbon source, which produced the highest tannase enzyme, was used in further experiments for screening the nitrogen sources.

To study the nitrogen sources, two of them (NH_4Cl and NH_4NO_3) were added individually instead of $NaNO_3$ in the basal medium with the optimized content of the selected carbon source to find the highest tannase activity. Then, to study the optimum nitrogen source content, the different contents of the selected nitrogen source, which gave the highest tannase productivity, were used in further experiments to produce the highest tannase enzyme. The optimized contents of the selected carbon and nitrogen sources in the basal medium, which produced the highest tannase enzyme, were used as a culture medium in further experiments to optimize the culture condition factors by RSM. All experiments were performed in triplicate, and the values were expressed as mean ± standard deviation. The SPSS software (ver. 16) was used to analyze the experimental data.

2.6. Optimization of the culture condition factors using RSM

The response surface methodology (RSM) was adapted to determine the optimum levels of the culture condition variables (time and temperature of incubation and rotation rate) for maximum tannase production using a central composite design (CCD). The culture condition variables were assessed at five coded levels (-1.68, -1, 0, +1, +1.68), as shown in Table 1. The experimental design and the results of CCD are listed in Table 2. The tannase activity was taken as the dependent variable or response (Y). A second-order polynomial equation was then fitted to the data using a multiple regression procedure, which resulted in the independent variables. All experiments were performed in duplicate, and Design Expert 7.0 software was used for the regression analysis of the experimental data, to make the RSM design, and also to plot the three-dimensional graphs. The statistical significance of the model equation and its terms was evaluated through the Fisher's test. The fit quality of the second-order polynomial model equation was expressed via the coefficient of determination (R^2) and the adjusted R^2. The fitted polynomial equation was then expressed in the form of three-dimensional plots in order to illustrate the relationship between the responses and the experimental levels of the variables in the study.

3. Results and Discussion

3.1. Time course of tannase production

The time course of tannase production by Penicillium sp. EZ-ZH390 is shown in Figure 1. Tannase production followed the logarithmic growth phase of the fungus with maximum yield occurring at 96 h during the stationary phase of growth and the corresponding pH and biomass of 5.12 and 0.5 gL^{-1}, respectively.

Penicillium sp. EZ-ZH390 produces extracellular tannase when grown in the basal medium. In this study, the use of liquid medium with continuous shaking creates enough substrate-enzyme interfaces for the tannase secretion.

The increase in biomass from day 1 to 7 indicates the utilization of the liberated gallic acid and glucose by Penicillium sp. EZ-ZH390 for cell growth. The growth pattern followed a logarithmic phase from day 2 to 4 and a stationary phase from day 4 to 7 (Fig. 1).

A fall in tannase activity from day 4 to 7 suggests a possible tannase inhibition probably by repression. This seems to be reflected in cell growth because there was no significant increase in biomass from day 5 to 7 (Fig. 1). Gupta et al. reported strong end-product inhibition with gallic acid, which was of the competitive type with *Aspergillus japonicas* tannase production [20].

Bradoo et al. reported maximum production of tannase by *Aspergillus japonicus* after 24 h of incubation at 30°C [21]. Suseela and Nandy observed that both tannic acid and gallic acid were completely decomposed within 3 days by *Penicillium chrysogenum* [22]. However, Bhat et al. harvested *Aspergillus niger* van Teighem tannase after 120 h incubation at 30°C [23].

In this study, there was a fall in pH from the initial pH of 6.4 of the fermentation medium (basal medium) to pH 4.76 on day 3, and by a gradual elevation to 5.12 on day 4 on which highest tannase activity was recorded (Fig. 1). These pH changes indicate that gallic acid molecules were liberated into the medium, thereby lowering the pH of the system. Probably, as the fungus utilized part of the products of hydrolysis (gallic acid and glucose), the pH of the system gradually rose to 5.58 .

Table 1. Experimental variables at different levels used for the RSM approach

Variables	Symbol	Coded levels				
		-1.68	-1	0	+1	+1.68
Temperature (ºC)	A	15	21	30	39	45
Rotation rate (rpm)	B	0	40	100	160	200
Incubation time (h)	C	24	44	72	100	120

Table 2. Central composite of the rotatable design of the variables with Tannase activity as response

Run	Repetition	Variables			Tannase activity (UmL^{-1})
		A	B	C	
1	1	-1	-1	-1	17.63
2	1	+1	-1	-1	17.02
3	1	-1	+1	-1	15.27
4	1	+1	+1	-1	14.68
5	1	-1	-1	+1	19.07
6	1	+1	-1	+1	18.37
7	1	-1	+1	+1	16.63
8	1	+1	+1	+1	15.97
9	1	-1.68	0	0	16.64
10	1	+1.68	0	0	16.12
11	1	0	-1.68	0	17.1
12	1	0	+1.68	0	15.83
13	1	0	0	-1.68	16.26
14	1	0	0	+1.68	19.58
15	1	0	0	0	21.53
16	1	0	0	0	21.58
17	1	0	0	0	21.5
18	1	0	0	0	21.56
19	2	-1	-1	-1	17.66
20	2	+1	-1	-1	17.06
21	2	-1	+1	-1	15.24
22	2	+1	+1	-1	14.64
23	2	-1	-1	+1	19.02
24	2	+1	-1	+1	18.41
25	2	-1	+1	+1	16.6
26	2	+1	+1	+1	16
27	2	-1.68	0	0	16.6
28	2	+1.68	0	0	16.15
29	2	0	-1.68	0	17.06
30	2	0	+1.68	0	15.85
31	2	0	0	-1.68	16.23
32	2	0	0	+1.68	19.62
33	2	0	0	0	21.5
34	2	0	0	0	21.52
35	2	0	0	0	21.54
36	2	0	0	0	21.55

3.2. Screening of carbon and nitrogen sources for tannase production

The selection of appropriate carbon and nitrogen source, is an important factor for tannase production. The effect of carbon and nitrogen sources of culture medium on tannase activity was studied. The optimization of the medium by the conventional research techniques like classical one factor at a time method involves changing one independent variable (such as carbon source or nitrogen source) while fixing the other at a constant level. At the first step, five different carbon sources (at concentration of 1% wv^{-1}) were examined, and the results are shown in Table 3. As shown, adding glucose instead of tannic acid in the basal medium caused no further tannase production (0.1 UmL^{-1} was the basal level). It was probably due to this fact that tannase is an inducible enzyme, and there was no inducer in the medium to induce tannase production. Furthermore, the results showed that other carbon sources had good effects on enzyme production by Penicillium sp. EZ-ZH390. As it resulted in the highest enzyme productivity; among all carbon sources tested in this study, raspberry leaves powder in the basal medium was the most suitable. The probable reason that the raspberry leaves are rich in nitrogen, carbon, phosphorous, potassium, calcium and magnesium. Also raspberry leaves contain averagely 4.5 mg tannin in dry weight [24]; so, these nutrients were probably available for fungal metabolism and tannase production .

The mechanism that stimulates tannase production in the microorganism is still under investigation; however, evidence suggests that tannic acid could not cause this induction as it cannot penetrate the cell membrane. However, it is believed that the intermediary compounds in the hydrolysis of tannic acid may have a role, but this needs to be investigated. Basal levels of tannase, as observed in the presence of glucose as a sole carbon source in the medium, might have started the initial hydrolysis of tannic acid into intermediary compounds [25].

As shown in Table 3, both raspberry leaves and tannic acid served as good substrates for tannase production. However, in view of high productivity, we used raspberry leaves powder as the substrate because the maximum yield of enzyme was obtained after 96 h of incubation at 30°C with tannic acid as carbon source, but raspberry leaves powder as carbon source caused maximum tannase yield after 72 h. Then, to study the carbon source content, different contents of raspberry leaves powder (at concentrations of 0.5-20% wv^{-1}) were used in further experiments to find the highest tannase activity (Table 3).

When different concentrations of the carbon source were tested, it was observed that increasing the concentration of raspberry leaves powder caused the increase of tannase production. The highest level of tannase production (10.74 UmL^{-1}) by Penicillium sp.

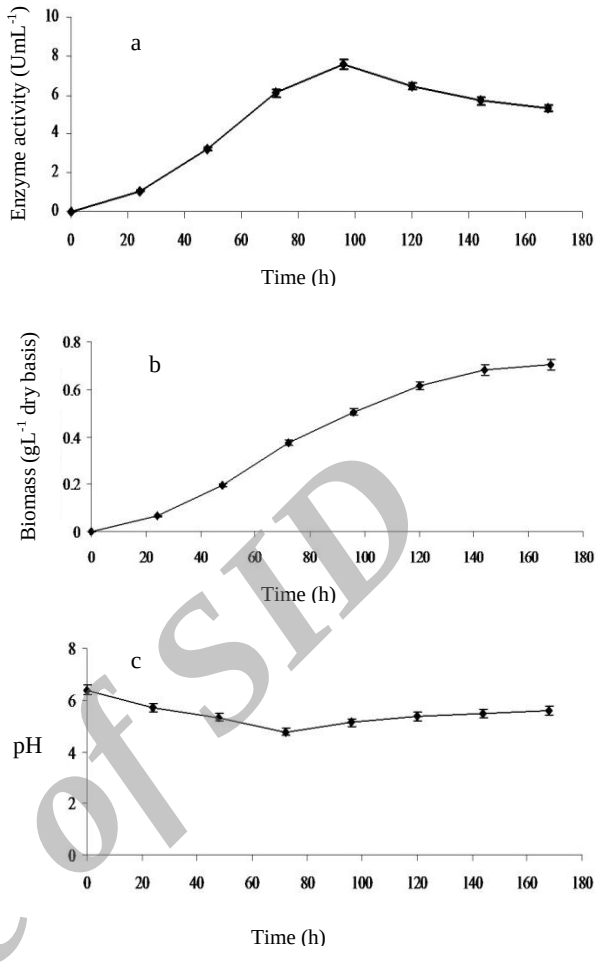

Figure 1. Time course of a) tannase activity, b) biomass production, and c) pH of the culture medium by Penicillium sp. EZ-ZH390

Table 3. Effect of different carbon sources and different contents of raspberry leaves on tannase production

Carbon source	Enzyme activity (UmL^{-1})		
	Day 3	Day 4	Day 5
Glucose	0.1 ± 0.03*	0.09 ± 0.02	0.09 ± 0.04
Tannic acid	6.11 ± 0.05	7.59 ± 0.06	6.45 ± 0.03
Green tea leaves	5.48 ± 0.06	6.29 ± 0.04	7.07 ± 0.05
Raspberry leaves	7.38 ± 0.04	5.92 ± 0.07	5.35 ± 0.06
Green husk of walnut	3.17 ± 0.08	2.21 ± 0.05	1.35 ± 0.03

Content of raspberry leaves (% wv^{-1})	Enzyme activity in day 3 (UmL^{-1})
0.5	6.74 ± 0.07 [a]
1	7.38 ± 0.04 [b]
2	7.96 ± 0.03 [c]
4	8.4 ± 0.05 [d]
8	9.06 ± 0.02 [e]
10	9.71 ± 0.04 [f]
12	10.25 ± 0.06 [g]
14	10.74 ± 0.03 [h]
16	10.8 ± 0.06 [h]
18	10.82 ± 0.05 [h]
20	10.84 ± 0.04 [h]

Experiments were performed in triplicate, and the values are expressed as mean ± standard deviation .
** Different letters indicate significant differences (P< 0.05).

EZ-ZH390 was obtained in the basal medium added with 14% wv^{-1} raspberry leaves powder and maintained under the rotation rate of 100 rpm at 30°C for 72 h. Under these conditions, tannase production increased by 1.45 fold as long as the basal medium was added with 1% wv^{-1} raspberry leaves powder. As shown in Table 3, increasing the concentration of carbon source to more than 14% did not significantly increase tannase production, probably because the excessive tannin source in the medium could react irreversibly with the proteins in the cell surface, inhibiting growth and enzymatic synthesis. Riul et al. observed that a reduction in tannase production by *Asergillus awamori* with high tannic acid concentration was the result of gallic acid deposition on the cell surface [25].

At the second step, three different nitrogen sources were used at a concentration of 0.2% wv^{-1} in a medium containing 14% wv^{-1} raspberry leaves powder as carbon source, and the results are shown in Table 4.Among all the nitrogen sources used, ammonium nitrate was the most effective in tannase biosynthesis with the relative activity of 16.11 UmL^{-1}. By the contribution of ammonium ions, ammonium nitrate stimulates the synthesis of proteins, and is a source of readily utilizable nitrogen [26]. Other nitrogen sources such as sodium nitrate and ammonium chloride are comparatively less significant for tannase production, presumably because of the less availability of nitrogen in the culture medium.

Naidu et al. observed that tannic acid by itself could produce only a moderate increase in tannase production whereas by interacting with $NaNO_3$, tannase activity could be increased significantly [18]. Bardoo et al. also reported $NaNO_3$ as the preferred nitrogen source for both growth and tannase production by *Asergillus japonicas* [21]. This is while Battestin and Macedo observed maximum tannase production by *Paecilomyces variotii* in the medium containing 1.2% NH_4NO_3 [27].

Table 4. Effect of different nitrogen sources and different concentration of ammonium nitrate on tannase production

Nitrogen source	Enzyme activity (UmL^{-1})		
	Day 3	Day 4	Day 5
$NaNO_3$	10.74±0.03*	8.59±0.05	7.98±0.06
NH_4Cl	12.89±0.05	10.31±0.04	9.66±0.07
NH_4NO_3	16.11±0.06	12.88±0.03	12.08±0.05

Ammonium nitrate concentration (% wv^{-1})	Enzyme activity in day 3 (UmL^{-1})
0.1	10.32 ± 0.04 [a]
0.2	16.11 ± 0.06 [b]
0.4	17.08 ± 0.05 [c]
0.8	19.4 ± 0.03 [d]
1	20.51 ± 0.06 [e]
1.2	21.43 ± 0.04 [f]
1.4	21.45 ± 0.05 [f]
1.6	21.5 ± 0.04 [f]

*Experiments were performed in triplicate, and the values expressed as mean ± standard deviation.
** Different letters indicate significant differences (P< 0.05).

To optimize the nitrogen source content, different concentrations of ammonium nitrate (0.1- 1.6% wv^{-1}) were used in further experiments to find the highest tannase activity (Table 4).

It was observed that increasing the concentration of ammonium nitrate caused the increase of tannase production. The optimum concentration of ammonium nitrate for the highest tannase production by Penicillium sp. EZ-ZH390 was recorded as 1.2% wv^{-1}.

Tannase production was increased by 1.33 fold (21.43 UmL^{-1} against 16.11 UmL^{-1} for 0.2% wv^{-1} ammonium nitrate) at 14% wv^{-1} raspberry leaves powder (as carbon source) and 1.2% wv^{-1} ammonium nitrate (as nitrogen source) in the basal medium and the rotation rate of 100 rpm and the incubation at 30°C for 72 h. As shown in Table 4, higher concentrations of ammonium nitrate in the fermentation medium did not lead to significant increase in tannase production.

3.3. Optimization of the culture condition variables using RSM

The design matrix and the corresponding results of RSM experiments to determine the effects of three independent variables (time and temperature of incubation and rotation rate) are shown in Table 2. The data obtained from CCD were fitted to a second-order polynomial with multiple regressions using the following equation (Eq. 1):

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC$$

Where, Y is the predicted response, β_0 is the intercept, β_1, β_2 and β_3 are linear coefficients, β_{11}, β_{22} and β^{33} are squared coefficients, and β_{12}, β_{13} and β_{23} are interaction coefficients. The fitting resulted in the following regression equation, representing an empirical relationship between the logarithmic values of enzyme yields and test variables in the coded unit (Eq. 2):

$$Y = 21.53 - 0.24A - 0.86B + 0.81C - 1.79A^2 - 1.79B^2 - 1.19C^2$$

Where, Y is tannase activity, A is incubation temperature, B is rotation rate, and C is incubation time. Analysis of variance (ANOVA) was then employed to determine the significant variables (data not shown). The model terms of A, B, C, and A^2, B^2 and C^2 were found to be significant ("p value >F" less than 0.05).

The regression equation obtained by ANOVA showed that the R^2 value (multiple correlation coefficient) was 0.9705. This is an estimate of the fraction of overall variation in the data evaluated by the model; thus the model is capable of explaining 97.05% of variation in the response. The "adjusted R^2" was 0.9603 and the predicted R^2 was 0.9371, indicating that the model was good. The "adequate precision value" of the present model was 27.444. This value also suggests that the model can be used to navigate the design space. The "precision value" is an index of the signal-

to-noise ratio, and values greater than 4.0 are prerequisites for a model to enjoy a good fit. The model showed mean, standard deviation, and predicted residual sum of squares (PRESS) values of 17.91, 0.46, and 11.74, respectively. The lower value of the coefficient of variation (2.57) indicates that the experiments were precise and reliable.

Experimental data on the effect of three culture condition variables on tannase production of Penicillium sp. EZ-ZH390 in a total of 36 experiments (Table 2) revealed strong dependence on the presence and the levels of selected variables since the enzyme production varied between 14.64 UmL^{-1} and 21.58 UmL^{-1} under the experimental conditions studied. It was observed that in the experimental runs 15-18 and the replicated runs 33-36, when the variables were at the central point "0", the enzyme yield varied between 21.5 UmL^{-1} and 21.58 UmL^{-1} with a mean value of 21.54 UmL^{-1}.

In order to determine the optimal levels of each variable for maximum tannase production, the three-dimensional plots were constructed by plotting the response against each of the two independent variables, while maintaining the third variable at fixed (zero) level. Figure 2a shows the response surface plot of the effects of incubation temperature and rotation rate on tannase activity at a constant incubation time (72 h). The contour indicated that the increase of both temperature and rotation rate nearly to 30°C and 90 rpm, respectively, can increase tannase production to its maximum level, and thus further increase can reverse this trend. Figure 2b shows the three-dimensional plot of the effects of incubation temperature and time on tannase activity at a constant rotation rate (100 rpm). The effects of rotation rate and incubation time on tannase activity at a constant incubation temperature (30°C) are shown in Figure 2c. The figure indicates that the best incubation temperature, rotation rate and incubation time ranges were 28-32 °C, 70-90 rpm, and 72-88 h, respectively.

The contours (Figs. 2b and 2c) further revealed that tannase production is sensitive to the changes in fermentation time within the defined levels. For instance, longer times have negative effect on tannase production. Therefore, timing should be kept in a reasonable way to guarantee sufficient enzyme production and lower fermentation costs as much as possible.

3.4. Validation of the response model

According to the optimization process, the best conditions for tannase production by Penicillium sp. EZ-ZH390 were temperature of 30°C, rotation rate of 85 rpm, and fermentation time of 84 h (enzyme activity was predicted 21.73 UmL^{-1}). Validation of the response surface model based on the previous experiments was carried out in shake flasks under the conditions predicted by the model.

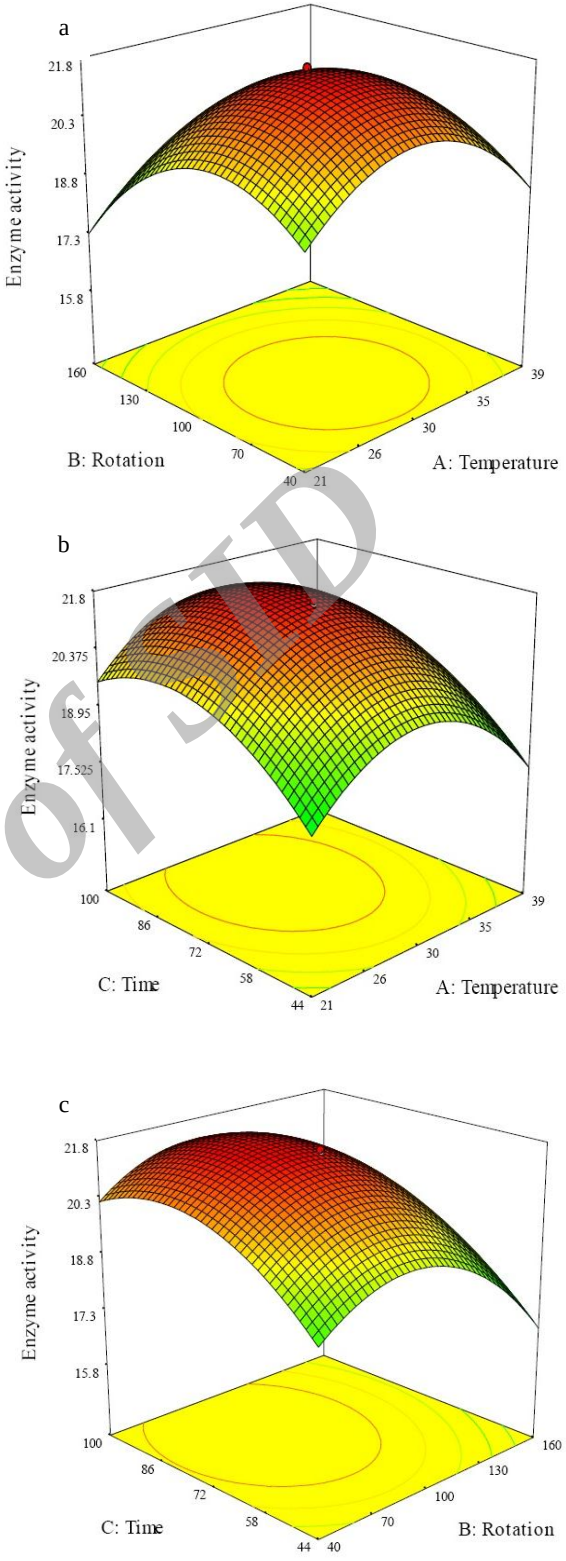

Figure 2. response surface plot of tannase activity (UmL^{-1}) as a function of a) incubation temperature and rotation rate, b) incubation temperature and incubation time, and c) rotation rate and incubation time based on the central composite experimental result.

The experimental value (21.77 UmL^{-1}) was found very close to the predicted value; hence, the model was

successfully validated. Under the optimized conditions, tannase productivity was found to be 0.26 $UmL^{-1}h^{-1}$.

In accordance with the findings of Zhong et al., the tannase from Aspergillus oryzae produced from recombinant Pichia pastoris showed 7 UmL^{-1} activity after 96 h of fed batch culture (productivity of 0.073 $UmL^{-1}h^{-1}$) [28].Trevino-Cueto et al. observed that the maximum tannase activity of *Aspergillus niger* Aa-20 (1.042 UmL^{-1}) was obtained after 43.5 h fermentation at the solid state culture of a tannin-rich desert plant (productivity of 0.024 $UmL^{-1}h^{-1}$) [29]. Enemuor and Odibo reported the maximum tannase production of 0.9 UmL^{-1} by *Aspergillus tamarii* IMI388810 after 144 h of incubation (productivity of 0.006 $UmL^{-1}h^{-1}$) [30]. Based on the findings of the present study, it can be concluded that at the optimized conditions, tannase productivity is at least 3.55 times (0.26 $UmL^{-1}h^{-1}$) the maximum tannase productivity in the reported literature.

4. Conclusion

According to the optimization process, the best conditions for tannase production by Penicillium sp. EZ-ZH390 with both one factor at a time method and RSM design were: 14% raspberry leaves powder as carbon source, 1.2% ammonium nitrate as nitrogen source, temperature of 30°C, rotation rate of 85 rpm, and fermentation time of 84 h. Using the optimized process, the best set resulted in 2.87 fold more enzyme production than that obtained before optimization (21.77 UmL^{-1} at optimum conditions against 7.59 UmL^{-1} with 1% tannic acid as carbon source, 0.2% sodium nitrate as nitrogen source, temperature of 30°C, rotation rate of 100 rpm and at 72 h fermentation time). The present study suggests that at the optimized conditions, tannase productivity is at least 3.55 times (0.26 $UmL^{-1}h^{-1}$) the maximum tannase productivity in the reported literature. Thus, we conclude that Penicillium sp. EZ-ZH390 at the optimum conditions is an excellent strain for use in the efficient production of tannase.

5. Acknowledgement

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6. Conflict of interest

The authors declare that there is no conflict of interest.

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