Studies on the Production of Pectinase from Tamarind Kernel Powder by Submerged Fermentation using *Aspergillus* Species, and Optimization of Medium Using Design Expert

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Six strains of filamentous fungi (*Aspergillus foetidus* NCIM 505, *Aspergillus foetidus* NCIM 510, *Aspergillus foetidus* NCIM 1027, *Aspergillus niger* NCIM 548, *Aspergillus niger* NCIM 616 and *Aspergillus awamorii* NCIM 885) were compared for their capacity to produce exo-pectinase in submerged fermentation (SMF) from a new substrate (tamarind kernel powder; TKP). Maximum pectinolytic activity was reached at 72 h of growth, the best fungal strain being *A. foetidus* NCIM 505. Different types of carbon sources (glucose, fructose and sucrose) both in individual (20 and 40 g L^{-1}) and in combined (10 g L^{-1} each) form were used to assess the effect of carbon sources on the production of exo-pectinase. Commercially available purified pectin and polygalacturonic acid (20 g L^{-1}) were used to assess the effect of pectin and polygalacturonic acid on the production of exo-pectinase. Further, the combination of pectin and polygalacturonic acid (10 g L^{-1} each) was used to assess the degree of production of exo-pectinase. Design-Expert was used to optimize the medium concentration (glucose, TKP, ammonium sulphate) and the response surface method was used to analyze the counter plot to enhance the production of pectinase.

Key words:
Aspergillus, pectinase, tamarind, submerged fermentation, design-expert

Introduction

The Tamarind (*Tamarindus indica*) is the only species of the genus *Tamarindus* in the family Febaceae. It is a tropical tree, native to eastern Africa, including parts of the Madagascar dry deciduous forests. India is a semi-arid tropical region, well known for the production of pulses (red gram, bengal gram and green gram), oil seeds (sunflower) and tamarind. Tamarind seeds have been used in a limited way as emergency food. It can be used in fruit preserving with or without acids and gelatinizes with sugar concentrates even in cold water or milk. It is recommended as a stabilizer in ice cream, mayonnaise and cheese and as an ingredient or agent in a number of pharmaceutical products. Tamarind seed is one of the cheap available sources which contain value added product pectin.

Microbial enzymes are now being used in a large number of industries. They have a wide range of application, in pharmaceuticals, in detergents, in the manufacture of a variety of food products, as reagents for the treatment of chemicals in the leather industry and in the treatment of industrial wastes. The increasing availability of enzymes will allow their exploitation as potent catalysts to introduce chirality and specificity into compounds in chemical processes. Pectinase has wide application starting from the processing of fruit juices, vegetables juices and fruit macerates, where the pectin molecules are reduced to simple galacturonic acid.[1,2,3]

Pectin is a generic name for high molecular mass polysaccharides present in the cell wall of higher plants. Their common property is the 1–4 glycosidic linkage of D-galacturonic acid unit.[4,5] Pectinase enzymes belong to the group of hydrolytic enzymes and are utilized to eliminate pectin and pectin-like collides in fruit juices and thus facilitate clarification of the juice and as a means of preventing the gelling of the juice during the concentration step of the process. Pectinases catalyze the breakdown of pectin-containing substances and can be produced by various fermentation methods like solid state fermentation (SSF)[2,6,7,8,9] or by submerged fermentation (SMF)[10,11] methods.

Pectinases are a group of hydrolyzing enzyme that catalyses the breakdown of pectin-containing substances.[12] The main four groups that act on the pectin molecule are[13]

Pectin lyases

– Depolymerizes pectin by transelimination.

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Polygalacturonase

– Hydrolyzes pectin with a degree of esterification.

Rhamnogalacturonans

– Hydrolyzes the Rhamnogalacturonic acid parts of the molecules.

Pectine esterase

– Catalyzes the deesterification of pectin.

For the commercial production of pectinases, the amount of pectin in the raw material and the type of microorganism used are the two main factors. Pectinases are produced by various bacteria and fungi, although commercial production utilizes species of *Penicillium* or Aspergillus (*Aspergillus*). Pectinase production in these fungi is stimulated by the presence of pectin or pectin-containing compounds in the fermentation medium. Since pectinase in part is retained in the cells and in part is excreted into the medium, the enzyme is recovered from both the sources. The regulatory production of pectinases by fungi have been little studied in spite of its importance at the industrial level.

The present work deals with the production of 'Pectinases' by submerged fermentation using Tamarind kernel powder as substrate with the selection of better organism from different fungal species and further optimizing the medium components (Design-Expert) to enhance the synthesis of pectinase. Tamarind kernel is a naturally occurring source for the production of pectinase, which is abundantly found in the tropical regions of India. The present work involves the study of the effect of various carbon sources, pectin and polygalacturonic acid and various combinations of these on the production of pectinases by submerged fermentation and optimization of the process.

Materials and methods

Inoculum and substrate

The following six fungal species (*Aspergillus foetidus* NCIM 505, *Aspergillus foetidus* NCIM 510, *Aspergillus foetidus* NCIM 1027, *Aspergillus niger* NCIM 548, *Aspergillus niger* NCIM 616 and *Aspergillus awamorii* NCIM 885) were obtained from National Chemical Laboratory, Pune, India for the present work. The strains were maintained on potato dextrose agar slant which was maintained at 4 °C and periodic subculture. The inoculums were prepared by growing the organisms in potato dextrose agar slants for about 72 h at 30 °C. The conidia were dispersed in 10 mL of dilute Tween 80 solution. Fresh tamarind seeds with testa were soaked in water for about 5 h and then the testa were removed. Testa-free tamarind seeds were ground and the fine powder was used as substrate for submerged fermentation.[14]

Fermentation medium

The medium required for the submerged fermentation contained 20 g L^{-1} of TKP, 10 g L^{-1} of ammonium sulphate and 2 g L^{-1} of potassium dihydrogen orthophosphate. The effect of glucose, fructose and sucrose on the production of pectinase were analysed individually for the selected strains by adding 20–40 g L^{-1}. Apart from the individual addition of carbon sources, studies were carried out by using combination of the carbon sources (glucose and fructose). The effect of addition of pectin and polygalacturonic acid was studied by adding 10 and 20 g L^{-1} respectively. Fermentation duration was examined from 60 to 96 h. In all the fermentations 50 mL of fermentation medium was dispersed into 250 mL Erlenmeyer flasks. The medium used in every study was autoclaved at 15 bar for 20 min and inoculated after cooling to 30 °C, on a rotary shaker for fermentation to take place.

Enzyme assay

The method of Miller[15] has been employed by several research scientists using galacturonic acid as the reference.[16,17,18] A known quantity of suitably diluted enzyme extract was added to 0.42 mL of (9 g L^{-1}) substrate solution (pectine) and mixed with 0.7 mL of 0.1 mol L^{-1} acetate buffer, pH 5.2, incubated at 45 °C for 30 min. After incubation the reaction was completed by adding alkaline sodium potassium tartarate reagent. The sample was estimated spectrophotometrically at 540 nm.

Result and discussion

Evaluation of age of slant

Experiments were conducted taking four sets of 250 mL Erlenmeyer flask of 50 mL sterilized medium containing TKP-20 g L^{-1}, ammonium sulphate-10 g L^{-1}, potassium dihydrogen orthophosphate-2 g L^{-1}. The flasks were inoculated aseptically with 1 mL spores of two day old slant, three day old slant, four day old slant and five day old slant of *Aspergillus awamorii* NCIM 885 for a period of four days.[8] The same procedure was repeated for the other five species of *Aspergillus* namely *Aspergillus niger* NCIM 548, *Aspergillus niger* NCIM 616, *Aspergillus foetidus* NCIM 505, *Aspergillus foetidus* NCIM 510 and *Aspergillus foetidus* NCIM 1027. From the experimental analysis it was observed that the three day old slant of *Aspergillus awamorii* NCIM 885, *Aspergillus niger* NCIM 616 and *Aspergillus foetidus* NCIM 510 syn-

thesised maximum level of pectinases of 0.3375 U, 0.696 U and 0.924 U after 60 h of fermentation. Also it was observed that the three day old culture of *Aspergillus niger* NCIM 548, *Aspergillus foetdius* NCIM 505, *Aspergillus foetidus* NCIM 1027 produced a maximum pectinase level of 0.576 U, 1.207 U and 1.029 U after 72 h of fermentation. Based on the comparative analysis *Aspergillus foetidus* NCIM 505 was found to be a better organism. Experiments were conducted to evaluate the inoculum level of the selected organism. It was observed that out of four chosen inoculum levels of 0.5, 1.0, 1.5 and 2.0 mL, 1.0 mL of inoculum which contains 10^5–10^7 spores mL^{-1} gave a maximum level of pectinase (Figs. 1 and 2).

Evaluation of fermentation period

Production of pectinase was evaluated up to 96 h irrespective of the organism. From the analysis it was observed that a gradual increase in the production of pectinase was observed over a period of 24–72 h, after which the activity declined.

Effect of glucose on the production of pectinase

Effect of glucose and sucrose as one of the carbon sources on the production of pectinase from Tamarind kernel powder was examined for different mass concentrations of the carbon sources (20 and 40 g L^{-1}). Fig. 3 shows the synthesis of pectinase using 20 g L^{-1} of glucose apart from the other common constituents. Maximum synthesis of pectinase of 1.25 U and 0.92 U were obtained with *Aspergillus foetidus* NCIM 505 and *Aspergillus foetidus* NCIM 510 after 60 h and 72 h of cultivation. *Aspergillus foetidus* NCIM 1027 synthesized 0.89 U of pectinase for about a period of 72 h. The rest of the organism *Aspergillus niger* NCIM 548, *Aspergillus niger* NCIM 616 gave a maximum synthesis of 0.75 U and 0.56 U while *Aspergillus awamorii* NCIM 885 gave synthesis level of 0.55 U. To study the effect of higher concentrations of carbon source, pectinase was synthesized in the presence of 40 g L^{-1} of glucose where the results were shown in Fig. 4. From the analysis it was observed that the *Aspergillus foetidus* NCIM 505 and *Aspergillus foetidus* NCIM 1027 showed a maximum yield when compared with the 20 g L^{-1} of glucose. The rest of the organism synthesized below 0.5 U of pectinase. This shows that higher glucose has repressive action.[19,20,21]

Effect of glucose-fructose and sucrose on the production of pectinase

Equal amount of carbon sources 20 g L^{-1} of glucose and fructose each was added to the fermen-

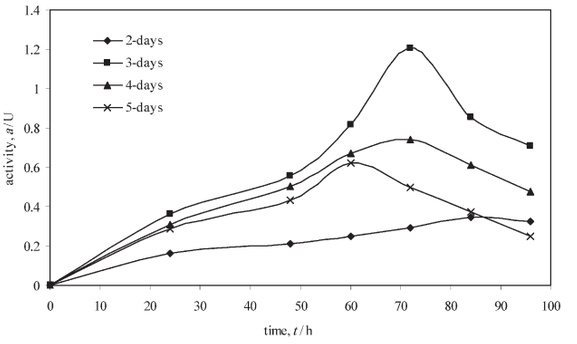

Fig. 1 – *Determination of slant age of A. foetidus NCIM 505*

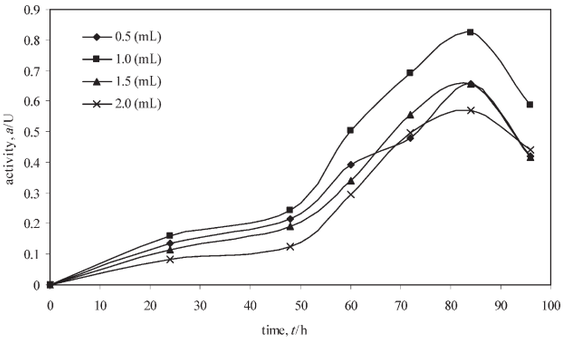

Fig. 2 – *Determination of inoculum level of A. foetidus NCIM 505*

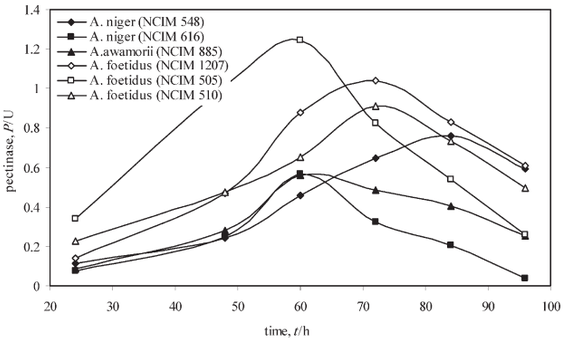

Fig. 3 – *Effect of glucose (20 g L^{-1}) on the production of pectinase*

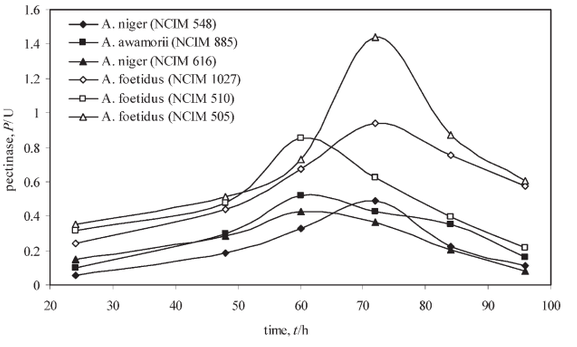

Fig. 4 – *Effect of glucose (40 g L^{-1}) on the production of pectinase*

tation broth along with the other constituents.[16,22] Fig. 5 shows the profile of synthesis of pectinase. The highest level of 1.28 U was obtained with *Aspergillus foetidus* NCIM 505. *Aspergillus niger* NCIM 548, *Aspergillus awamorii* NCIM 885 and *Aspergillus foetidus* NCIM 510 showed a similar result of 1.18, 0.91 and 1.10 U. The rest of the organism showed a lesser level of pectinase synthesis. To avoid direct repressive action of glucose, glucose was substituted with sucrose where little accumulation of glucose might result during the fermentation.[4,23] The synthesis of pectinase profile at 40 g L^{-1} of sucrose in the fermentation medium is shown in Fig. 6. Among the six organisms *Aspergillus foetidus* NCIM 505, *Aspergillus foetidus* NCIM 510 and *Aspergillus awamorii* NCIM 885 were found more favorable, while the other organism produced a comparatively low amount of pectinase.

Effect of pectin on the production of pectinase

Since pectinases were assumed an induced enzyme, pectinase synthesis was studied in the presence of pectin. In the experimental analysis 20 g L^{-1} of pectin was added along with the other constituent in the fermentation medium. Fig. 7 shows the amount of pectinase synthesized in the presence of pectin. Highest activity of pectinase was obtained with *Asperigillus niger* NCIM 548, 1.16 U after 60 h of cultivation. The next higher synthesis was obtained with *Aspergillus awamorii* NCIM 885, 0.68 U after 72 h of fermentation. The rest of the organism synthesized below 0.5 U of pectinase. This appears to suggest that the induction effect of pectin is different for different organisms. *Aspergillus foetidus* NCIM 505 secreted higher synthesis of pectinase with glucose, glucose plus fructose and sucrose whereas the synthesis of pectinase by *Aspergillus foetidus* NCIM 505 with the additional amount of synthetic pectin was minimal.

Effect of pectin and polygalacturonic acid on the production of pectinase

The profile of pectinase synthesis when pectin and polygalcturonic acid were taken at 10 g L^{-1} each keeping the contents of other common constituents constant which is shown in the Fig. 8. Highest level of pectinase was obtained with *Aspergillus niger* NCIM 548 of 1.12 U, the next higher level of pectinase was obtained in *Aspergillus awamorii* NCIM 885 and *Aspergillus foetidus* NCIM 505. Almost the same result of pectinase synthesis was obtained with *Aspergillus foetidus* NCIM 510, 0.56 U. *Aspergillus foetidus* NCIM 1027 yield low level of pectinase of about 0.46 U.

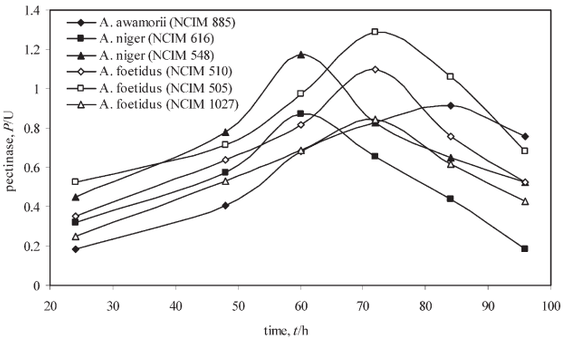

Fig. 5 – *Effect of glucose-fructose (20 g L^{-1}-each) on the production of pectinase*

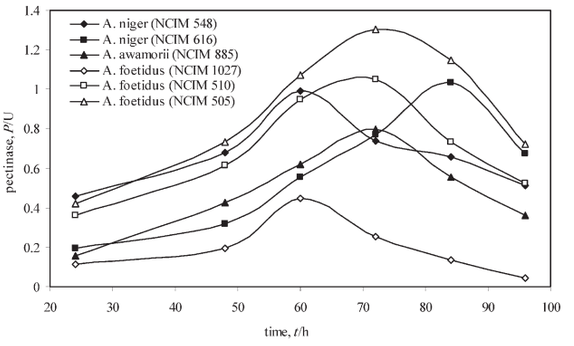

Fig. 6 – *Effect of sucrose (40 g L^{-1}) on the production of pectinase*

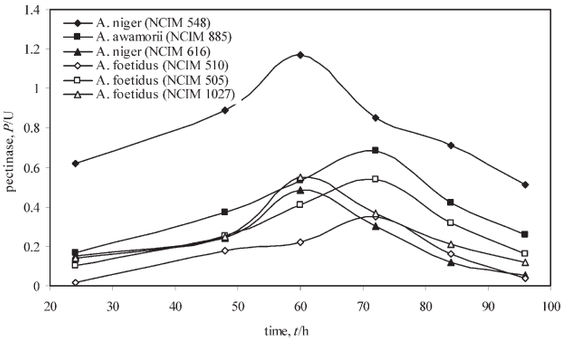

Fig. 7 – *Effect of pectin (20 g L^{-1}) on the production of pectinase*

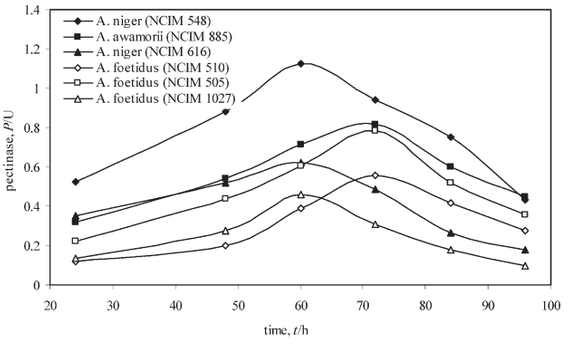

Fig. 8 – *Effect of pectin and polygalacturonic acid (10 g L^{-1} each) on the production of pectinase*

Table 1 – *Comparison of production of pectinase by different organism*

Organism	glucose 20 g L^{-1}	glucose 40 g L^{-1}	glucose + fructose 20 g L^{-1} each	sucrose 40 g L^{-1}	pectin 20 g L^{-1}	pectin + PGA 10 g L^{-1} each	PGA 20 g L^{-1}
A. awamorii NCIM 885	0.55	0.51	0.91	1.02	0.68	0.82	0.88
A. niger NCIM 548	0.75	0.48	1.16	0.98	1.16	1.12	1.2
A. niger NCIM 616	0.56	0.42	0.86	0.78	0.48	0.62	0.73
A. foetidus NCIM 505	1.25	1.44	1.28	1.30	0.54	0.78	1.11
A. foetidus NCIM 510	0.92	0.86	1.10	1.05	0.35	0.56	0.70
A. foetidus NCIM 1027	0.89	0.94	0.85	0.45	0.55	0.46	0.91

Effect of polygalacturonic acid on the production of pectinase

The profile of pectinase synthesis in the presence of polygalacturonic acid 20 g L^{-1} in the fermentation medium is shown in the Fig. 9. There was a slight inductive effect of polygalacturonic acid on the synthesis of pectinases but the maximum synthesis was incomparable with that of sugars. Higher pectinase synthesis was obtained with *Aspergillus niger* NCIM 548 of 1.2 U and *Aspergillus foetidus* NCIM 505 of 1.11 U. Almost similar levels of synthesis were obtained with *Aspergillus niger* NCIM 616, 0.73 U, *Aspergillus foetidus* NCIM 510, 0.70 U and *Aspergillus awamorii* NCIM 885, 0.88 U.

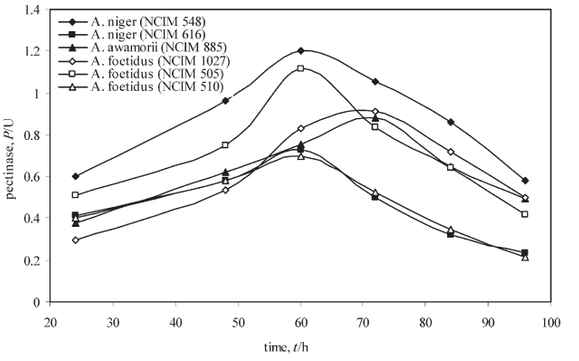

Fig. 9 – *Effect of polygalacturonic acid (20 g L^{-1}) on the production of pectinase*

Optimization of medium components for enhanced synthesis of pectinase by *Aspergillus foetidus* NCIM 505

From the above analysis of different *Aspergillus* species for the production of pectinase it was observed that the *Aspergillus foetidus* NCIM 505 gave a higher yield of pectinase (1.44 U). Further, we aimed to optimize the medium for the chosen species (*Aspergillus foetidus* NCIM 505) using mathematical tool Design-Expert-Response Surface Method (Design-Expert, Stat-Ease, Inc., Mineapolis, Minnesota, USA).

The medium chosen from the screening studies contained mixed carbon sources (TKP and glucose) and nitrogen sources (ammonium sulphate). It was observed from the literature that the effect of carbon and nitrogen sources is predominant in governing the synthesis of pectinase in *Aspergili*.[16,24] In addition the common sources of phosphorous (KH_2PO_4 (2 g L^{-1})) was used in the media for pectin production. The optimization of carbon and nitrogen sources in the above condition was aimed at enhancing the synthesis of pectinase using the chosen organism.

Response surface methodology

The statistical optimization method chosen was the central composite design procedure for optimizing the level of TKP, ammonium sulphate and glucose which were the three independent variables to be optimized in the medium. The range of TKP, ammonium sulphate and glucose are given in Table 2.

Table 2 – *Independent variables (carbon and nitrogen sources in the medium) and level studied in the optimization design. $X_{i\ (i = 1,2,3)}$-real values, (–α and +α)-lowest and highest values, (–1 and +1)- intermediate values, (0) – center values.*

Variables	Components	Range studied	Levels				
			–α	–1	0	+1	+α
X_1	TKP	0.5 – 3.0	0.5	1	1.75	2.5	3
X_2	Ammonium sulphate	0 – 10	0	2	5	8	10
X_3	Glucose	0 – 40	0	8	20	32	40

The second-degree factorial central composite design was chose to optimize the medium components.[25] This design had six star points (α), six replicates at the centre points, and eight cube points. According to this design there were 20 experiments, performed in duplicate, to minimize experimental error. In this case, with three variables, α was equal to 1.682.

Further in regression analysis the coded values of the independent variables were used instead of the real values. The variables are coded as follows:

$$x_i = (X_i - \overline{X}_i)/\Delta X_i \quad (1)$$

Regression analysis was performed on the results. The results of the central composite design polynomial are more adequately represented by a second order polynomial than the first order model.

A second order polynomial of the following form was used:

$$\hat{Y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \\ + b_{33} X_3^2 + b_{12} X_1 X_2 + b_{23} X_2 X_3 + b_{31} X_3 X_1 \quad (2)$$

The coefficient of $\beta_{i\ (i = 1,2,3)}$, $\beta_{ii\ (i = 1,2,3)}$, $\beta_{ij\ (i = 1,2,3 \text{ and } j = 1,2,3)}$ are evaluated using Analysis of Variance (ANOVA) which are listed in the Table 3 and the ANOVA results are listed in Table 4. In each 20 sets the required variable or the response was taken to be the average maximum pectinase activity obtained ($\hat{Y}_1$) in units.

Counter plot shows the relative effect of the variables when the third variable was maintained constant. The constant concentrations of the variables were the central levels of the variables in the respective ranges considered. For ammonium sulphate, the central level was 5.0 g L^{-1}, while for TKP and glucose the levels were 1.75 % (17.5 g L^{-1}) and 20 g L^{-1} respectively. The coordinate of the center point gives an approximate idea of the optimal concentration of the variables taken on the axes of the plot.

Table 3 – *Coefficient of polynomial model for synthesis of pectinase*

Coefficient	Values
β_0	2.0036
β_1	0.2704
β_2	0.3185
β_3	0.1600
β_{11}	–0.5737
β_{22}	–0.3906
β_{33}	–0.1391
β_{12}	–0.1168
β_{31}	0.0241
β_{23}	0.3662

Fig. 10 shows the relative effect of TKP and glucose on pectinase synthesis when ammonium sulphate was taken at 5 g L^{-1}. The highest counter level in this case corresponds to 2.03 U, for which the level of TKP was about 1.8 %. A comparatively wide range of concentration between 20 g L^{-1} and

Table 4 – *Analysis of variance (ANOVA)*

Source of variance	Degree of freedom	Sum of squares	Mean squares	*F* value	Prob > *F*
Regression Model	$(k^2 + 3k)/2$	SSR	SSR/(P – 1)		
Blocks	b – 1			MSR/MSE	
Residual Error	$N - (K^2 + 3K)/(2 - b)$	SSE	SSE/(N – P)		
Total	N – 1	SST			

33 g L^{-1} of glucose were observed to correspond to the highest contour level of 2.03 U. From these observations the synthesis of pectinase was influenced more strongly by the level of TKP than by the level of glucose.

The relative effect of ammonium sulphate and TKP was considered at a constant glucose concentration of 32 g L^{-1} (Fig. 11). The highest counter level was of 2.11 U. The central point in this contour corresponds to concentrations of ammonium sulphate between 5 g L^{-1} and 7 g L^{-1} and to about 1.8 % of TKP.

At constant TKP concentration of 17.5 g L^{-1} (i.e. 1.75 %) the synthesis of pectinase was examined as a function of ammonium sulphate and glucose (Fig. 12). The highest contour corresponds to 2.0 U. The central point in this contour corresponds to about 5.5 g L^{-1} of ammonium sulphate and about 30 g L^{-1} of glucose.

A collective assessment of all the counter plots was done by examining the coordinate of the central point within the highest contour in Figs. 10, 11 and 12. These observations indicate that glucose at 30 g L^{-1}, ammonium sulphate at 6 g L^{-1} and TKP at 17.5 g L^{-1} are close to optimal concentration. Optimized concentration of all the sources result in increased yield of pectinase synthesis by 0.8 U compared to the unoptimised concentration.

The development strategies that are employed in finding the substrate and microorganisms for economic value and their potentials in the fermentation process are most critical and important. The successful approach to achieve this objective is to understand the multifaceted interactions of the substrate and organism in the prevailing conditions. Several researchers have reported the production of a wide range of fungal pectinases from a variety of substrates under optimum conditions. Hours *et al.*[26] recorded 10.62–13.00 U g^{-1} pectinase production from apple pomace with an adequate addition of organic nitrogen using *Aspergillus foetidus*. Blandino *et al.*[27] recorded 2.0–2.5 U g^{-1} of pectinase from wheat employing *Aspergillus awamorii*. Galiotou-Panayotou and Kapantai[28] observed the production of 14.5 U mL^{-1} pectinase production by *A. niger* on supplemented citrus pectin. But all the sources used for the production of pectinase were off expensive and frequently used. So to substitute these sources with a cheap one, TKP was selected. However, no literature as per our survey indicated the production of fungal pectinase from tamarind kernel powder. An attempt was made here for the production of pectinases from the substrate tamarind kernel powder under submerged conditions employing *A. foetidus* NCIM 505, *A. foetidus* NCIM 510, *A. foetidus* NCIM 1027, *A. niger* NCIM

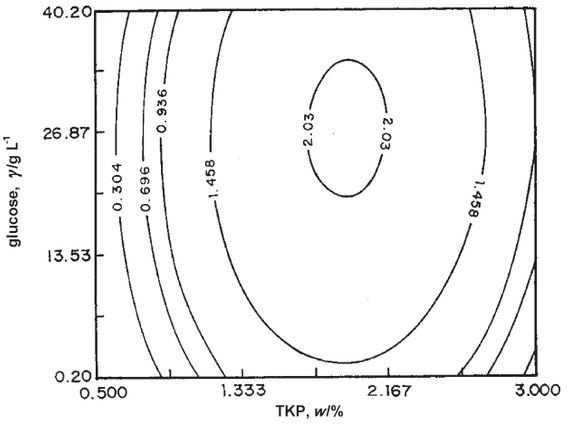

Fig. 10 – *Iso response contour plot showing synthesis of pectinase as a function of TKP and glucose at ammonium sulphate concentration of 5 g L^{-1} (A. foetidus NCIM 505)*

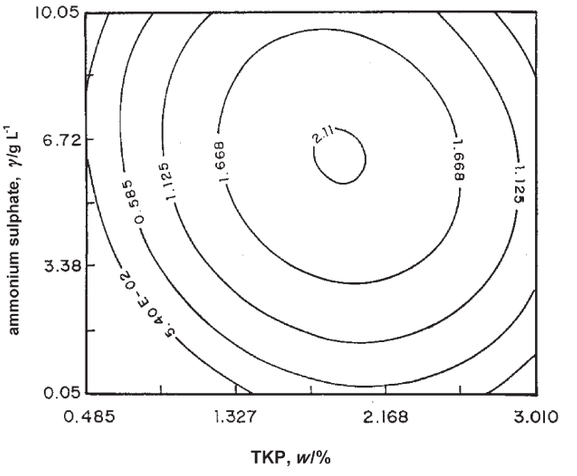

Fig. 11 – *Iso response contour plot showing synthesis of pectinase as a function of TKP and ammonium sulphate at glucose concentration of 32 g L^{-1} (A. foetidus NCIM 505)*

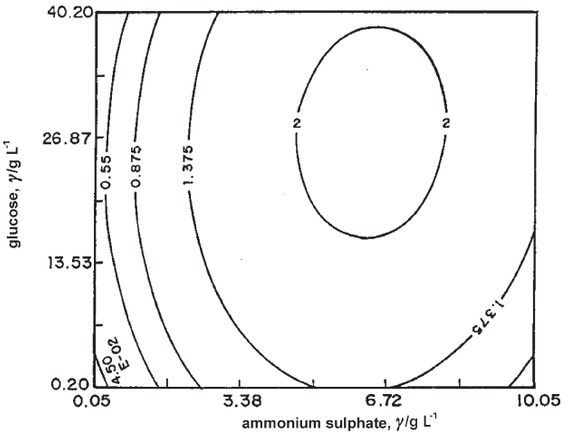

Fig. 12 – *Iso response contour plot showing synthesis of pectinase as a function of glucose and ammonium sulphate at TKP concentration of 1.75 g L^{-1} (A. foetidus NCIM 505)*

Table 5 – *Optimal concentration of carbon and nitrogen sources obtained using central composite experimental design*

Components	Unoptimized	Optimized	Pectinase synthesized/U		
			unoptimized	optimized	predicted
TKP *w*/%	2	1.75			
Ammonium sulphate/g L^{-1}	10	6.0	1.44	2.211	2.033
Glucose/g L^{-1}	40	32			

548, *A. niger* NCIM 616 and *A. awamorii* NCIM 885 strains (Table 1). Standardized bioprocess with all optimized conditions resulted in maximum production of exo-pectinase (1.44 U) by *A. foetidus* NCIM 505 in SMF. Further, to enhance the production of pectinase using the chosen organism (*A. foetidus* NCIM 505) the composition of the medium was optimized using Design-Expert and the results were tabulated (Table 5).

Conclusion

Six *Aspergillus* species were studied for the synthesis of pectinase using tamarind kernel powder which is a cheap alternate and abundant source compared to apple, wheat, corn and citrus. Tamarind kernel powder could be an attractive and promising substrate especially in submerged fermentation for the production of pectinases by *A. foetidus* NCIM 505. Further, the optimum slant age and inoculums level were found to be 72 h and 1.0 mL respectively (Fig. 1 and Fig. 2). The synthesis of pectinase was studied when each organism was separately cultivated in media differing in carbon sources. The highest synthesis of pectinase (1.44 U) was obtained with *A. foetidus* NCIM 505 when cultivated in the medium containing TKP of 20 g L^{-1}, glucose 40 g L^{-1}, ammonium sulphate 10 g L^{-1}, potassium dihydrogen phosphate 2 g L^{-1}. Further, the physical factors pH and temperature responsible for higher synthesis of pectinase were optimized and were found to be 4.5 and 30 °C respectively.

The optimal concentration of the medium, which was evaluated using Design-Expert, was found to be 32 g L^{-1} of glucose, 6 g L^{-1} of ammonium sulphate and 17.5 g L^{-1} of TKP and potassium dihydrogen phosphate 2 g L^{-1}. The synthesis of pectinase increased by 45 % after optimization of physical and chemical parameters.

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Nomenclature

A – *Aspergillus*
ANOVA – analysis of variance
NCIM – National Collection of Industrial Microorganism
SSF – solid-state fermentation
SMF – submerged fermentation
TKP – tamarind kernel powder
PGA – polygalacturonic acid
Y_I – observed maximum mean response for the design number I in the central composite design
$\hat{Y}$ – predicted response (pectinase synthesis)
β_o – offset term
$\beta_1, \beta_2, \beta_3$ – linear effect
$\beta_{11}, \beta_{22}, \beta_{33}$ – squared effect
$\beta_{12}, \beta_{23}, \beta_{31}$ – interaction effect
γ – mass concentration, g L^{-1}
w – mass fraction, %

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