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Enhancement of α -amylase production in pelleted *Aspergillus tamarii* through optimization for desizing of cotton fabric

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Abstract

Aim : The present study was carried out to enhance the production of α -amylase by pelleted *Aspergillus tamarii* through optimization of various media compositions and cultivation conditions using shake flask and bioreactor experiments.

Methodology : Shake flask experiments were conducted to study the effect of pH, temperature and starch concentration using Response Surface Methodology (RSM) and other parameters, namely, nitrogen source, inoculum size and incubation days using single variable optimization technique for the pelleted growth of microorganism and amylase production. Scale up study was carried out for the assessment of results obtained from shake flask experiments using a laboratory scale bioreactor. In the bioreactor study, parameters, namely, pH control, agitation and aeration were considered.

Results : Maximum amylase production using pelleted *Aspergillus tamarii* was achieved at initial pH 6.7, temperature 30.5 °C, 0.5% w/v starch, 0.1% w/v urea, 1.5% v/v inoculum size and 4 days of fermentation in the shake flask experiments. Filamentous growth was observed when the concentration of starch used was above 2%. The specific enzyme activity increased to 2.77 fold after partial purification. When enzyme was used for desizing cotton fabric, it produced 90% efficiency. The scale-up experiments revealed initial pH 6.7, agitation 300 rpm and aeration 1 vvm as the conditions suitable for pelleted growth, as well as to achieve maximum amylase production.

Interpretation : The results indicate pelleted growth of *Aspergillus tamarii* and in turn achievement of maximum amylase activity depends on media composition and fermentation conditions used at the time of enzyme production. Efficient desizing of cotton fabric by amylase showed its effectiveness towards hydrolysis of starch and converting it to soluble products for easy removal.

Key words: *Aspergillus tamarii*, Cotton fabric, Desizing, Optimization, α -amylase

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Introduction

Submerged fermentation (SmF) has many advantages since it offers a good control over process parameters and recovery of enzyme in addition to easy availability of industrial equipment (Rao, 2010; Irfan *et al.*, 2012; Benjamin *et al.*, 2013; Monteiro, 2010). The major drawback with fungal fermentation is filamentous morphology leading to non-Newtonian broth rheology which, in turn, limits the biomass concentration during fermentation. Fungal growth, when it takes place in pelleted form as self-immobilized and bioactive particles, offers many advantages, namely, Newtonian broth rheology, quick settlement rates, efficient oxygen transfer, easy solid-liquid separation and high productivity for many industrial applications over mycelium (Nair *et al.*, 2016; Espinosa-Ortiz *et al.*, 2016). Mycelial pellets are used mostly in the fermentation industry for the production of citric acid, enzymes and antibiotics (Lu *et al.*, 2015). The nutrients and the environmental conditions play an important role in the morphological development of the fungi (Shuler and Kargi, 1992). Nair *et al.* (2016) reported many cultivation factors such as pH (especially acidic pH between 3 and 4), temperature, carbon source, inoculum size, additives, trace metals and reactor types influence pellet formation in the case of *Neurospora intermedia*. Nyman *et al.* (2013) successfully manipulated the growth of *Rhizopus* sp. as pellets through optimization of medium parameters, namely, pH, temperature, agitation, calcium ion concentration and particle concentration. In addition to various adverse conditions like high temperature and extreme pH, additive forces can also influence *Rhizopus oryzae* to undergo transformation from filamentous to pellet form of growth (Karmakar *et al.*, 2012). Hence, fungal pellet formation depends on cultivation conditions specific to individual species (Nair *et al.*, 2016).

α -amylases (EC 3.2.1.1) has a market share of around 65% and fulfills the need of many industries manufacturing food, paper, pharmaceutical, leather, textile etc. (Nimkar *et al.*, 2010; Chand *et al.*, 2012). In textile industry, desizing of cotton fabrics using α -amylases is widely used for the hydrolysis of starch applied to yarn prior to weaving of fabrics, as it leads to a sustainable process. Amylases from fungi have gained much attention due to their availability and high productivity. Among various fungi, *Aspergillus* species, mainly *Aspergillus niger* and *Aspergillus oryzae* produce a considerable quantity of amylases (Tiwari *et al.*, 2015; Slivinski *et al.*, 2011). Due to increasing demand from industries for amylases, there is always a need for new strains and processes that show better economic advantages (Mishra *et al.*, 2014). Hence, in the present study, attempts were made to achieve pellet form of growth of *Aspergillus tamarii* along with maximum amylase production through optimization of constituents of media compositions and fermentation conditions using shake flask and bioreactor experiments.

Materials and Methods

Microorganism and inoculum preparation: A subculture isolated from soil containing tannery effluent, namely, *Aspergillus*

tamarii (MTCC 5152) with Gen Bank accession number JN419193 was supplied by Central Leather Research Institute, India. It was grown and maintained in Czapekdox agar slant at 28 °C for 10 days, and subsequently stored at 4 °C with periodic sub culturing twice a month. The inoculum spore suspension was prepared by adding 10 ml of sterile distilled water containing 0.1% w/v Tween 80 to a ready to use sporulated Czapekdox agar slant culture of *A. tamarii*. The spores were dislodged using an inoculum needle and poured into non-absorbent cotton for obtaining mycelial free spore suspension under aseptic conditions. The spores were counted using a Haemocytometer and the number was found to be 1.67×10^6 spores ml^{-1} .

Amylase production using shake flask: Preliminary trials conducted revealed variations in pH from 4 to 9, temperature from 26° to 34 °C and starch concentration from 0.5 to 3% play a significant role in the production of α - amylase by *A. tamarii*. Morphology of the microorganism during growth was found to be either in filament or pellet form. The complete experimental combinations with 15 experiments having 3 replicates involving the above 3 variables designated as X_1 , X_2 and X_3 , respectively and each prescribed with 3 levels $+1(x_1)$, $0(x_2)$ and $-1(x_3)$ based on Box-Behnken response surface methodology are shown in Table 1. Statistical analysis of the model was performed using analysis of variance with the help of Design Expert Software (Version 7.0). Least square method was used for fitting the second- order polynomial equation.

SmF was carried out in a 250 ml conical flask containing 50 ml of an inorganic salt solution consisting 1 g l^{-1} dipotassium hydrogen phosphate, 0.5 g l^{-1} magnesium sulphate, 0.5 g l^{-1} potassium chloride and 0.01 g l^{-1} ferrous sulphate and 5 g l^{-1} meat extract as a source for nitrogen. It was further added with a specific concentration of starch and adjusted for the desired pH either with 0.1M HCl or 0.1M NaOH and sterilized (Table 1). It was then inoculated with 1% spores and fermented at desired temperature (Table 1) for 4 days at 120 rpm. After fermentation, the medium was filtered and centrifuged at 15,000 rpm for 10 min. The supernatant crude enzyme solution was decanted and subjected to amylase assay. Optimum levels for all three variables were predicted and their validation was performed for using them in the subsequent experiments.

Nitrogen source, inoculum size and fermentation days were optimized by a single variable optimization technique. Inorganic nitrogen sources (0.5% w/v), namely, ammonium chloride, ammonium sulphate, sodium nitrate, potassium nitrate and organic nitrogen sources, namely, urea, tryptone, peptone, meat extract and yeast extract were used, and the concentration of the best nitrogen source was found. Similarly, inoculum sizes (1% to 4.5% v/v) followed by fermentation days (2 to 6 days) were optimized for achieving best amylase production.

Ammonium sulphate precipitation followed by dialysis was used for partial purification of enzyme. The required quantities of solid ammonium sulphate were slowly added to

crude enzyme for achieving 70-5-95% saturation levels. These solutions were stirred for 2 hr and left overnight at 4 °C. Precipitates were collected after centrifugation at 13,000 rpm for 10 min. A 20 mM phosphate buffer (pH 6.5) was added to each precipitate and solutions, thus, obtained were dialyzed against same buffer with repeated changes at 4 °C and stored. Amylase activity was determined before and after dialysis.

Total protein content in amylase was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. The result obtained was used for calculating specific activity and, in turn, purification folds. The molecular mass of the enzyme was determined by SDS-PAGE (Mini-PROTEAN Biorad System, USA) involving 12% resolving gel and 5% stacking gel at 85 V. Protein bands were detected by destaining the gel in a methanol-acetic acid-water solution (4:1:5) after staining with Coomassie brilliant blue R-250. Presence of enzyme activity was tested using Zymogram involving Native PAGE gel incubated in gelatinized starch (2% w/v), prepared in 0.1 M sodium acetate buffer at pH 5 for 1 hr after electrophoresis. Subsequently, the gel was stained with iodine reagent (3% w/v).

Amylase activity was assayed by measuring the amount of reducing sugar obtained on hydrolyzing soluble starch using dinitrosalicylic acid (DNSA) method following the procedure of Bernfeld (1955). The reaction mixture contained 0.3% w/v starch, 0.5 ml of 0.2 M phosphate buffer (pH 6.5) and 0.25 ml of 1% NaCl. This mixture was incubated at 55 °C for 5 min followed by another 10 min after adding 0.1 ml of enzyme. The reaction was stopped after adding 0.5 ml of 2 N NaOH. It was followed by adding 0.5 ml DNSA and boiling for 10 min. After cooling, the absorbance of the solution was measured at 540 nm and enzyme activity (U ml^{-1}) was determined. One unit of α -amylase is defined as the amount of enzyme that releases 1 micromole of reducing sugar as maltose per minute under assay conditions.

Desizing of cotton fabric: Experiments were continued on the partially purified enzyme for determining optimum pH and temperature for its application. pH varying from 4 to 7 were studied keeping the incubation temperature at 55 °C. A 20 mM acetate buffer and 20 mM phosphate buffer were used for achieving pH 4 to 5.5 and pH 6 to 7, respectively. The optimum temperature was found by varying the temperature from 45 ° to 65 °C. Cotton fabric with size content 8.49%, conditioned at 25 °C and 65% RH in an Environmental Chamber (Inlab Equipments (Madras) Pvt. Ltd., India) was used for desizing. It was carried out with a known weight of the sample in a bath having liquor to material ratio of 40:1 and enzyme concentration of 994 U g^{-1} of fabric for 30 min using Infra Colour Dyeing Machine (R.B. Electronic & Engineering Pvt. Ltd., India) set at 100 rpm. Optimum application pH and temperature were used for the treatment. Starch degradation products were removed from the fabric by thorough washing, and percentage desizing efficiency was calculated.

Amylase production using bioreactor: Pre-inoculum was prepared using optimum media composition obtained from shake flask experiments. The media components were sterilized

separately at 121 °C for 20 min. A 100 ml of the medium was added to 500 ml conical flasks, and pH was adjusted to 6.7 by 2 N H_2SO_4 or 3 M NaOH. These flasks were inoculated with 1.5% spores ($1.67 \times 10^8 \text{ spores ml}^{-1}$) prepared from slants and incubated at 30.5 °C for 24 hr using 220 rpm.

Scaling-up study was carried out using a 3l bioreactor (BioFlo/CelliGen Benchtop Fermenter, New Brunswick Scientific, USA) provided with two 6 bladed Rushton type impellers (52 mm diameter). The maximum working volume of reactor was 2.2 l. Dissolved oxygen concentration, pH and temperature were measured using respective control probes. The pH was maintained at 6.7 in case of pH-controlled batch fermentation. Temperature was maintained with the help of external heating blanket and immersed stainless steel cooling coil. Addition of acid/base, starch feed and antifoaming agent at the required rate was carried out with the help of peristaltic pumps. The reactor was filled up to maximum working volume by feeding it with the media composition mentioned above and 300 ml of pre-inoculum. The parameters considered for optimization were pH (controlled and uncontrolled), agitation and aeration.

Glucose and other metabolites like lactic acid, formic acid and acetic acid produced during batch fermentation were analyzed by HPLC (Shimadzu Analytical Asia Pacific Pvt. Ltd., Singapore), equipped with Hiplax H column (7.7 x 300, 8 μm) and refractive index detector. Dry cell weight was determined by drying the biomass at 80 °C. Amylase activity was estimated following DNSA method (Bernfeld, 1955), mentioned earlier.

Results and Discussion

The growth of *Aspergillus tamarii* was modified from filamentous morphology to pellet form to achieve maximum amylase production by optimizing critical components in the medium and process conditions. Initial experiments conducted to study effect of trace metals in the medium revealed neither metals nor their concentration had any influence on the pelleted growth of fungus. In contrast, Liao *et al.* (2007) reported that the pelleted growth of *Rhizopus oryzae* in the production of fumaric and lactic acids were affected K^+ , Mg^{2+} and Zn^{2+} by metallic ions like present in the broth. The various parameters considered in the shake flask experiments were starch concentration (0.5 to 3% w/v), pH (4-9) and temperature (26 °-34 °C). Filamentous growth was observed when the starch concentration was above 2% and all other conditions were found to be supportive to pelleted growth. Box-Behnken experimental design was used for screening variables influencing amylase production. Table 1 shows experimental combinations with three replicates which improve the accuracy of regression model and the amylase activities obtained.

Summary of data obtained from analysis of variance of experimental amylase activity is given in Table 2. The model obtained was not used for quantitative prediction of amylase activity due to its inadequacy because of low predicted R^2 value.

Table 1 : Box-Behnken design with experimental and predicted enzyme activities

Run	Variables				
	pH $X_1 (x_1)$	Temperature (°C) $X_2 (x_2)$	Starch conc. (% w/v) $X_3 (x_3)$	Amylase activity (U ml ⁻¹)	
				Experimental	Predicted*
1	9 (+1)	26 (-1)	1.75 (0)	58.73	74.00
2	4 (-1)	26 (-1)	1.75 (0)	46.47	0
3	6.5 (0)	34 (+1)	3 (+1)	382.11	426.44
4	6.5 (0)	30 (0)	1.75 (0)	1027.80	1021.98
5	6.5 (0)	30 (0)	1.75 (0)	1126.10	1021.98
6	6.5 (0)	30 (0)	1.75 (0)	1058.20	1021.98
7	9 (+1)	34 (+1)	1.75 (0)	291.74	278.51
8	6.5 (0)	34 (+1)	0.5 (-1)	1130.20	1130.00
9	6.5 (0)	26 (-1)	3 (+1)	49.05	221.92
10	4 (-1)	34 (+1)	1.75 (0)	164.59	133.69
11	4 (-1)	30 (0)	3 (+1)	33.56	25.67
12	9 (+1)	30 (0)	3 (+1)	233.65	170.49
13	9 (+1)	30 (0)	0.5 (-1)	812.93	874.05
14	6.5 (0)	26 (-1)	0.5 (-1)	996.34	925.49
15	4 (-1)	30 (0)	0.5 (-1)	573.16	729.24

Figures within the parenthesis show coded values; * Determined using regression Eqn. 1.

In order to improve the adequacy, model reduction was carried out by eliminating all the insignificant terms, except X_1 . X_1 was considered due to very low p value of its corresponding higher order term X_1^2 (Montgomery, 2001). This resulted in significant improvement of the model with good R^2 values (Table 3). The reduced model regression equation obtained is given below.

$$Y = 1021.98 + 72.41x_1 + 102.26x_2 - 351.78x_3 - 572.12x_1^2 - 346.02x_2^2 \quad (1)$$

The predicted values of amylase activity determined using Eq. 1 are given in Table 1. The contour plots shown in Fig. 1 indicate the effect of pH, temperature and starch concentration at '0' coded level (vide table 1) on amylase activity. The optimum conditions for achieving maximum amylase activity determined from the plots were as follows: pH 6.7, temperature 30.5 °C, starch concentration 0.5%. The corresponding predicted amylase activity was 1383 U ml⁻¹. Earlier studies carried out with

Table 2 : ANOVA summary obtained for amylase production

Source	Sum of Squares	Degrees of Freedom	Mean Square	F value	p-value Prob > F	Significance
Model	2.716E+006	9	3.018E+005	25.33	0.0012	Significant
X_1 -pH	41944.22	1	41944.22	3.52	0.1194	Insignificant
X_2 -Temp.	83650.73	1	83650.73	7.02	0.0454	Significant
X_3 -Starch conc.	9.900E+005	1	9.900E+005	83.10	0.0003	Significant
X_1X_2	3299.93	1	3299.00	0.28	0.6212	Insignificant
X_1X_3	393.63	1	393.63	0.033	0.8629	Insignificant
X_2X_3	9920.16	1	9920.16	0.83	0.4034	Insignificant
X_1^2	1.234E+006	1	1.234E+006	103.61	0.0002	Significant
X_2^2	4.578E+005	1	4.578E+005	38.42	0.0016	Significant
X_3^2	23140.78	1	23140.78	1.94	0.2222	Insignificant
Residual	59569.54	5	11913.91			
Lack of Fit	54503.72	3	18167.91	7.17	0.1248	Not significant
Pure Error	5065.82	2	2532.91			
Cor. Total	2.775E+006	14				

R^2 – 0.98, Adj. R^2 – 0.94, Pred. R^2 – 0.68, SD – 109.15, CV% – 20.51

Table 3 : ANOVA summary obtained after model reduction for amylase production

Source	Sum of Squares	Degrees of Freedom	Mean Square	F value	p-value Prob > F	Significance
Model	2.679E+006	5	5.358E+005	50.06	<0.0001	Significant
X ₁ -pH	41944.22	1	41944.22	3.92	0.0791	Significant
X ₂ -Temp.	83650.73	1	83650.73	7.82	0.0209	Significant
X ₃ -Starch conc.	9.900E+005	1	9.900E+005	92.50	<0.0001	Significant
X ₁ ²	1.216E+006	1	1.216E+006	113.59	<0.0001	Significant
X ₂ ²	4.447E+005	1	4.447E+005	41.55	0.0001	Significant
Residual	96324.04	9	10702.67			
Lack of Fit	91258.22	7	13036.89	5.15	0.1723	Not significant
Pure Error	5065.82	2	2532.91			
Cor. Total	2.775E+006	14				

R² – 0.97, Adj. R² – 0.95, Pred. R² – 0.90, SD – 103.45, CV% – 19.43

Fig. 1 : Contour plots of amylase production as a function of (A) pH and temperature; (B) pH and starch concentration and (C) temperature and starch concentration.

Fig. 2 : Effect of (A) nitrogen sources; (B) concentration of urea; (C) inoculum size and (D) fermentation days on amylase production (Error bars represent standard error of duplicate determination).

Aspergillus flavus (Priya and Renu, 2018) and *Aspergillus niger* (Mathew *et al.*, 2016) also showed the optimum condition of pH and temperature for achieving maximum amylase production was around 6.5 and 30 °C, respectively. Gupta *et al.* (2008) also reported 0.5% starch as the best carbon source for amylase production from *Aspergillus niger*. Fermentation was carried out using the predicted optimum conditions for validation of proposed model. The experimental amylase activity was found to be 1352 U ml⁻¹ against the predicted activity of 1383 U ml⁻¹. A good agreement between the experimental and predicted amylase activities show the adequacy of model and effectiveness of optimization.

Other factors such as nitrogen source, inoculum size and incubation days had no effect on the pelleted growth of fungus. A study conducted by Liao *et al.* (2007) also showed absence of role played by inoculum size on pellet formation in the production of organic acids by *Rhizopus oryzae*. Among the tested nitrogen sources, meat extract and urea falling under organic type showed 7-9 fold increase in amylase production followed by sodium nitrate. All other agents rendered poor support in amylase production (Fig. 2A). In the industrial scale production of enzymes, the selection of carbon and nitrogen sources with appropriate concentration is vital to bring down the cost of

Fig. 3 : (A) Molecular mass of partially purified enzyme using SDS-PAGE; Lane M - Molecular marker, Lane 4 - Enzyme and (B) Zymogram of Partially purified enzyme.

enzyme production. Growth media alone contributes around 30 to 40% towards the cost of enzyme production (Juwon and

Fig. 4 : Effect of (A) pH and (B) temperature on amylase activity (Error bars represent standard error of duplicate determination).

Fig. 5 : Batch fermentation (A) pH profiles for pH controlled and pH uncontrolled conditions; (B) DCW for pH controlled and uncontrolled conditions and (C) amylase activity for pH controlled and uncontrolled conditions (Error bars in (C) represent standard error of duplicate determination).

Fig. 6 : Concentrations of fermentative metabolites for uncontrolled pH condition.

Emmanuel, 2012). As urea is cheaper than meat extract and sodium nitrate, it was selected as nitrogen source for the subsequent experiments. Use of urea as nitrogen source was reported to give maximum yield in the production of β -glucosidase, glucoamylase and amylase from *Aspergillus species* (Zambare, 2010; He *et al.*, 2017; Sinha and Vakilwala, 2016). Fig. 2B shows 0.1% as the best concentration of urea for amylase production and drastic reduction of the activity above 1%. In a study conducted on the production of amylase from *Aspergillus terreus* higher activity obtained at lower concentration of urea was found to decrease with increase in concentration (Sethi *et al.*, 2016).

Inoculum size up to 1.5% increased amylase activity, however, further increase resulted in reduced amylase activity (Fig. 2C). It clearly indicates the need for a required initial microbial load for the given nutrient concentration to avoid extension of lag phase. Higher microbial load should be compensated with increase in nutrient concentration for preventing an earlier onset of stationary phase. This fact has also been emphasized by Shah *et al.* (2014) in their study on amylase production using *Aspergillus oryzae*. Fermentation days play a major role in achieving maximum enzyme production as extended period would result in loss of enzyme activity (Ire *et al.*, 2017). Fig. 2D shows increase in amylase activity up to 4 days (1408 U ml⁻¹) followed by a drop in enzyme activity. A drop in activity may be due to deficiency of nutrients, accumulation of toxic substances and proteolysis of amylase. A similar kind of drop in amylase activity beyond optimum incubation period taken place due to catabolite repression, caused by glucose released into the medium and proteolysis of enzyme, has been reported by Fadahunsi and Garuba (2012).

Partial purification of crude enzyme was carried out with ammonium sulphate by varying the saturation level from 75-95%. Precipitation with 90% produced maximum activity, followed by

dialysis that yielded an activity of 22,373 U ml⁻¹. The specific activity of enzyme increased considerably resulting in 2.77 fold increase in purity. The results obtained from SDS-PAGE (Fig. 3A) for the partially purified enzyme showed several protein bands but with a prominent band around 60 kDa. Zone of clearance shown by Zymogram (Fig. 3B) indicated possession of amylolytic activity by the enzyme. The molecular mass of amylases usually varies between 10-210 kDa with most of them falling in the range of 50-60 kDa (Saranraj and Stella, 2013).

The effect of pH on amylase activity is shown in Fig. 4A. Maximum activity, 26,030 U ml⁻¹, was noted at pH 5. A further increase in pH resulted in reduced enzyme activity. Fig. 4B shows the effect of various temperatures on amylase activity at pH 5. The enzyme activity increased up to 55 °C, followed by a decrease. Hence, pH 5 and 55 °C were considered as optimum conditions for enzyme application. Enzyme desizing operation is eco-friendly compared to conventional acid desizing process as the former requires lower energy because of reduction in treatment temperature, and less water consumption in washing due to ease of removal of enzyme (Athalye, 2012). Desizing of cotton fabric carried out with the enzyme produced 90% starch removal efficiency as compared with that produced from *Aspergillus flavus* (Sreelakshmi *et al.*, 2014). Hence the amylase produced by the fungus is capable of hydrolyzing starch and converting it to soluble products. This shows that *Aspergillus tamarii* is a potential organism for the industrial production of amylase.

As shake flask experiments produced promising results, fermentation was conducted in bench scale bioreactor for scaling up for the evaluation of suitable conditions for pellet formation and maximum amylase production. Initially, the effects of agitation and aeration were analyzed. These parameters when exceeded above 300 rpm and 1 vvm, respectively, resulted in filamentous growth of the culture. Nyman *et al.* (2013) also reported inverse effect between agitation intensity and pellet formation and found

that lower agitation intensity increased the possibility of pellet formation. Further batch fermentation experiments were carried out with the above levels under uncontrolled pH and controlled pH conditions using optimum parameters determined for checking the effect of pH. Fig. 5A shows a drop in pH to 4.5 after 21 hr when left uncontrolled in the batch. This was due to the secretion of acids by the culture on consumption of glucose produced from starch. The concentration of acetic acid, lactic acid and formic acid in the broth during fermentation is shown in Fig. 6. Upon exhaustion of glucose, these acids were utilized by the culture for growth resulting in an increase in the pH. Xiaolin Pei et al. (2010) reported occurrence of acid consumption by *Escherichia coli* in the production of 2-deoxyribose-5-phosphate aldolase. In both the pH uncontrolled and controlled batch experiments, pelleted morphology only was observed in the broth.

Initially, the cells grew at same rate in both the batches irrespective of the control exercised on pH. However, a higher cell growth rate was observed between 8 and 40 hr in pH uncontrolled batch. It shows acidic pH favoring cell growth. No difference in maximum cell growth yielded by both the batches was observed (Fig. 5B). Pathak and Nidhi (2013) have also reported that the suitability of pH range for α -amylase production varies with the fungal sources. There was a substantial increase in the rate of production of amylase between 24 and 40 hr of fermentation in pH uncontrolled batch compared to the pH controlled batch which resulted in an overall increase in amylase production in former case. The maximum activity of amylase obtained was 1051 U ml^{-1} for pH uncontrolled batch over 898 U ml^{-1} for pH controlled batch (Fig. 5C).

This study gives a clear insight on the role played by the nutrient concentration and fermentation conditions, required for their optimization in the production of α -amylase by the pelleted form of *Aspergillus tamarii*. As the results obtained are encouraging, efforts are taken to continue the study with fed-batch approach to enhance amylase production, to explore the possibility of industrial scale operation.

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