Summary and Conclusion
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α-Amylases are among the most important hydrolytic enzymes having great industrial significance. It is a key enzyme in the production of starch derivatives such as dextrins, oligosaccharides and glucose molecules. It is used in various industries like textile, baking, pharmaceuticals and detergents. Microbial amylases generally meet industrial demand.

The industrial use of amylases is expected to grow tremendously in the coming decade. Therefore, efforts have been directed to explore the means to reduce the production cost of amylase through improving the yield and the use of either cost-free or low-cost agricultural by-products as substrate for α-amylase production. In recent years, solid-state fermentation involving growth of microbes on moist solid substrates in the absence of free-flowing water, has gained tremendous momentum owing to certain advantages over the conventional submerged fermentation, namely, low production cost, saving of water and energy, less waste effluent problem and stability of the product due to less dilution in the medium.

The use of enzymes in the processing of textiles is gaining wider recognition because of their non-toxic, eco-friendly and biodegradable characteristics, coupled with specificity of action. They can be safely used in various textile processes such as desizing, scouring, degumming, bio-polishing, bleaching, dyeing and finishing, where the conventional processes make use of harsh chemicals whose disposal into the environment leads to many problems. Due to the globalization of the textile industry and increasing awareness towards the polluting nature of the textile effluent, social pressure is increasing on the textile processing industries to use environment-friendly processing techniques. Desizing process involves the removal of starch from the fabric which serves as the strengthening agent to prevent breaking of the warp thread during the weaving process. Removal of starch is necessary for obtaining absorbent fabric suitable for further processing.
Hence the present study was undertaken to isolate α-amylase producing bacterial and fungal strains and optimize their production. The study was also focused to characterize and assess the suitability of α-amylase as desizing agent in textile processing.

**The study was conducted in four phases:**

In the first phase, a total of 22 different sources, namely, soils, effluents, waste water discharged soils and spoiled food sources were selected. The sources were collected in and around Coimbatore district. The α-amylase producing bacterial and fungal strains were isolated based on their amylolytic ratio on starch agar medium. The hydrolysis zone formed on starch agar plates were visualized by flooding the plates with Gram’s Iodine solution. Isolates having higher hydrolysis zone were selected and the α-amylase activity was determined in these strains. The bacterial and fungal isolates that produced maximum activity were selected and identified based on morphological and biochemical tests. The selected bacterial and fungal strains were identified as *Bacillus* sp. and *Aspergillus* sp. and confirmed as *Bacillus cereus* and *Aspergillus awamori* by Institute of Microbial Technology (IMTECH), Chandigarh.

The second phase of the study included the optimization of various physical and chemical parameters for the maximum production of α-amylase by selected bacterial and fungal strains. Various factors, namely, fermentation technique, agro-residues as solid substrates, inoculum level, incubation period, moistening agents, moisture level, pH, temperature, supplementary carbon and nitrogen sources were optimized for the maximum production of α-amylase. The effect of amount of substrate to flask volume, metal salts and different sized vessels on α-amylase production was determined. The influence of extraction medium, solid to solvent ratio and extraction time on α-amylase recovery from fermented solid media were also studied.

In the third phase, the characterization of bacterial and fungal α-amylases was carried out. The effect of pH and temperature on bacterial and fungal α-amylase activity and stability were analyzed. The effect of incubation
period, metal ions and EDTA on α-amylase activity was studied. The kinetic parameters Km and Vmax were determined. Adsorption, hydrolysis of raw starch and analysis of its hydrolytic product using thin layer chromatography were also done.

The fourth phase of the study included the application of crude bacterial and fungal α-amylases extracted from *Bacillus cereus* and *Aspergillus awamori* in desizing the selected cotton fabrics. Various parameters, namely, α-amylase concentration, treatment time, temperature and pH of the desizing bath, were optimized for each of the selected cotton fabric. Desizing by conventional and commercial enzymatic method was carried out to compare the efficiency of the bacterial and fungal α-amylases produced. The desired fabrics were evaluated for fabric weight, tensile strength, thickness, stiffness, elongation, drapability, crease recovery and absorbency tests. All the desired fabrics except denim were dyed with natural dye source, annatto seed powder to analyze the absorption of the desized fabrics. The dyed fabrics were evaluated visually for appearance, brightness of shade and evenness of dyeing and checked for colour fastness to sun light, crocking, pressing and washing.

**Salient findings of the study are summarized as follows:**

**Phase I**

A total of 433 bacterial strains and 186 fungal strains were isolated from the selected sources. Among them, 42 bacterial strains and 17 fungal strains were selected which showed amylolytic ratio greater than 3. From these strains, a bacterial isolate from sago factory effluent and a fungal strain from spoiled cooked rice which showed the highest α-amylase activity were selected for further experiments. Based on the morphological and biochemical characteristics, the selected bacterial and fungal isolates were identified as *Bacillus cereus* MTCC 10202 and *Aspergillus awamori* MTCC 9997 by the Institute of Microbial Technology (IMTECH), Chandigarh.

**Phase II**

Experiments on fermentation techniques revealed solid-state fermentation as the suitable technique for the production of α-amylase by both
Bacillus cereus and Aspergillus awamori. Screening of different agro-residues showed that wheat bran and cassava peel powder were the best substrates for the production of α-amylase by Bacillus cereus and Aspergillus awamori, respectively. An increase in inoculum level improved the growth and growth related activities. α-Amylase production increased with increase in inoculum concentration and reached the maximum at 10 per cent (v/w) for both Bacillus cereus (29.74 U/gds) and Aspergillus awamori (34.20 U/gds).

The incubation period for achieving the maximum production of α-amylase depends on the growth rate of the microorganisms and its enzyme production pattern. Maximum α-amylase production was recorded at an incubation period of 72 h for Bacillus cereus and 96 h for Aspergillus awamori. Among the different moistening agents used, moistening agent I (MA I (g/L) - MgSO₄ 7H₂O - 0.5g; K₂HPO₄ -1.5 g – pH 7.2) was found to produce the maximum α-amylase with Bacillus cereus (34.93 U/gds) and Aspergillus awamori (40.06 U/gds). The optimum α-amylase production by both Bacillus cereus and Aspergillus awamori were observed with MA I in the ratio of 1:2 (w/v).

The initial pH of the medium had an influence on the growth and enzyme secretion of microorganisms. The maximum α-amylase production was recorded at pH 7 (34.93 U/gds) for Bacillus cereus and pH 6 (42.13 U/gds) for Aspergillus awamori. α-Amylase production increased with increasing temperature up to 50°C for Bacillus cereus (31.77 U/gds) and 40°C for Aspergillus awamori (33.63 U/gds) after which the activities decreased.

Enzyme secretion depended on the nutrients available in the medium. α-Amylase production by both Bacillus cereus and Aspergillus awamori were increased by the supplementation of starch at 1 per cent concentration for Bacillus cereus and 2 per cent for Aspergillus awamori. Among the different nitrogen sources, supplementation of yeast extract showed significant increase in α-amylase production in Bacillus cereus and beef extract in Aspergillus awamori. Yeast extract at a concentration of 1.5 per cent and beef extract at 2 per cent recorded the maximum α-amylase yield.
The maximum production of bacterial and fungal α-amylases were recorded when the ratio of substrate weight to flask volume was maintained at 1:50. From the study, it is evident that α-amylase production was strongly influenced by the presence of 1 per cent calcium chloride for Bacillus cereus and 0.8 per cent calcium chloride with Aspergillus awamori. Among the different sized vessels used, 250 ml Erlenmeyer flask was found be suitable with both Bacillus cereus and Aspergillus awamori for the growth and enzyme secretion.

Phosphate buffer was found to be the best extraction medium for the recovery of α-amylase from the fermented biomass of Bacillus cereus and Aspergillus awamori in a ratio of 1:10 (w/v). There was no significant difference noticed on α-amylase recovery when the extraction time was increased above 30 minutes for both Bacillus cereus and Aspergillus awamori.

**Phase III**

Bacterial α-amylase activity was found to increase with increasing pH and reached a maximum at pH 10 (44.44 U/ml) and fungal α-amylase at pH 9 (53.53 U/ml), indicating that both the α-amylases extracted were active at alkaline pH. The bacterial α-amylase was stable over a pH range of 9-11 and retained 92.3 per cent of its initial activity at pH 10 up to 180 minutes. α-amylase from Aspergillus awamori recorded stability in the pH range of 8-10 and the enzyme retained 94.4 per cent of its initial activity up to 180 minutes.

α-Amylase activity was found to be maximum at 50°C for both Bacillus cereus and Aspergillus awamori and retained 87.3 and 80.2 per cent of initial activities, respectively after incubation for 180 minutes at 50°C. α-Amylase activities increased with increasing starch concentration and reached a maximum at 2 per cent, after which the activities remained stable in both. The Km value was recorded as 3.3 mg/ml for bacterial α-amylase and 1.9 mg/ml for fungal α-amylase. The Vmax value was found to be 0.25 and 0.12 mg/ml/min for bacterial and fungal α-amylase, respectively.
The optimum enzyme-substrate reaction time was found to be 30 minutes for both *Bacillus cereus* and *Aspergillus awamori* and the activities were found to be activated by calcium chloride, barium chloride, potassium chloride, magnesium sulfate, sodium chloride and lithium sulphate. Among these, calcium chloride showed the maximum increase in the activity. EDTA inhibited both bacterial and fungal α-amylases, confirming the role of metal ions in the stabilization of α-amylase.

The results of raw starch adsorption and its hydrolysis by bacterial and fungal α-amylases revealed that fungal α-amylase showed a higher adsorption (87 per cent) and hydrolysis (48 per cent) when compared with bacterial α-amylase, which showed 76 per cent adsorption and 35 per cent hydrolysis. Analysis of hydrolytic products of starch by α-amylases of *Bacillus cereus* and *Aspergillus awamori* revealed that both the α-amylases degraded starch randomly. Spots for maltose and glucose became more visible, indicating that the enzyme is endo in action and it is α-amylase.

**Phase IV**

The results of optimization of different parameters for desizing revealed that the fabrics ghada and denim required 80 per cent bacterial α-amylase concentration for desizing. The fabrics khadi cotton, long cloth, voile, silk cotton, bamboo, modal and tencel required an optimum bacterial α-amylase concentration of 60 per cent. However, poly cotton required only 40 per cent of bacterial α-amylase concentration. The optimal fungal α-amylase concentration for the fabrics ghada, long cloth and bamboo was found to be 60 per cent. For the fabrics khadi cotton, voile, poly cotton, silk cotton, modal and tencel, the optimum fungal α-amylase concentration was found to be 40 per cent. However, denim required a higher fungal α-amylase concentration of 80 per cent.

A treatment time of 9 h was found to be optimum for the fabrics ghada, khadi cotton and long cloth using bacterial α-amylase, whereas for the fabrics voile, poly cotton, silk cotton, bamboo, modal and tencel, a treatment time of 6 h was found to be optimum. Denim required a treatment time of 12 h for
desizing using bacterial α-amylase. For the fabrics long cloth and denim, a treatment time of 9 h was found to be optimum and for all the other fabrics, 6 h was found to be optimum for desizing using fungal α-amylase.

The optimum temperature for desizing all the selected cotton fabrics using bacterial and fungal α-amylases was found to be 50°C. A pH of 10 and 9 in the desizing bath was found to be optimum for bacterial and fungal α-amylases, respectively. The results of visual evaluation of all the original and desized fabrics revealed that all the desized fabrics, irrespective of the treatment methods, were rated to be good in appearance by 92 per cent of the judges. All the desized fabrics were rated to be soft in texture by 82 per cent of the judges. 84 per cent of the judges rated all the desized fabrics to be low in stiffness. This indicates that there was no difference between the treatment methods and the enzymatic desizing was effective.

The fabric weight of all the desized fabrics decreased irrespective of the treatment methods when compared with their respective originals. There was no significant decrease in tensile strength in bacterial and fungal α-amylases treated fabrics when compared with their respective originals. Significant increase in elongation was observed in all the desized fabrics in both warp and weft direction when compared with their respective originals. Fabric thickness and stiffness of all the desized fabrics decreased irrespective of the treatment methods when compared with their respective originals. All the desized fabrics showed increase in drape co-efficient. The crease recovery was found to be increased in fabrics khadi cotton, long cloth, voile, silk cotton, bamboo, modal and tencel in warp direction, ghada, voile, silk cotton, bamboo and modal fabrics in weft direction.

The absorbency of all the desized fabrics increased significantly when compared with their respective originals. The results of drop test revealed that the time taken to absorb a droplet of water decreased, irrespective of the desizing method and there was no significant difference among the treatments.

All the desized fabrics were found to show increase in capillary rise. The time taken by all the desized fabrics for sinking was found to be significantly
reduced when compared with their respective originals and there was no significant difference among the treatments.

The results of visual evaluation of dyed fabrics revealed that all the dyed fabrics, irrespective of the treatment method used were good in appearance, brighter in shade and evenly dyed. With regard to colour fastness property to sun light, pressing, crocking and washing, all the dyed fabrics were rated as good or excellent. All these results indicated that desizing of fabrics using bacterial and fungal α-amylases is on par with conventional chemical and commercial enzymatic methods. Hence, the extracted α-amylases from Bacillus cereus and Aspergillus awamori could be used to replace the chemical treatment and can be taken as one of the measures to overcome the pollution problem.

The following conclusions are drawn from the present investigation:

- *Bacillus cereus* and *Aspergillus awamori* were found to be the potential producers of α-amylase.

- Solid-state fermentation proved to be efficient for the production of α-amylase using *Bacillus cereus* and *Aspergillus awamori*.

- Wheat bran and cassava peel powder were found to be the suitable substrates for the production of high titres of α-amylase using *Bacillus cereus* and *Aspergillus awamori* with the supplementation of simple nutrients such as starch, yeast extract / beef extract and calcium chloride and they were also less expensive and economically viable.

- A pH of 7 and 6 were found to be optimum for the production of α-amylase using *Bacillus cereus* and *Aspergillus awamori*, respectively at 50°C.

- α-Amylases from *Bacillus cereus* and *Aspergillus awamori* were found to be alkaline in nature and their optimum pH was found to be 10 and 9, respectively and had an optimum temperature of 50°C.
Both the α-amylases proved to be efficient in the removal of starch in desizing processes and their efficiency was comparable with that of conventional and commercial enzymatic method.

*Bacillus cereus* and *Aspergillus awamori* could be effectively exploited for the commercial production of α-amylases due to their constitutive and less catabolically repressive nature, alkalophilicity, thermostability (upto 50°C), lower Km and high starch hydrolysis percentage.

These studies clearly hold promise for the effective, economical and eco-friendly production of α-amylase from *Bacillus cereus* and *Aspergillus awamori* for industrial exploitation and creating a pollution free environment. Due to the alkalophilic nature and stability over a wide range of pH and temperature, these amylases have potential applications in various industries.

Thus, the use of α-amylases extracted from *Bacillus cereus* and *Aspergillus awamori* could lead to reactions that can reduce the negative environmental impact apart from improving the physical properties of the fabric. The present work has proven to be convincingly reproducible and environmentally friendly which can be easily adopted by the textile industry.

**Scope for further studies:**

- To make α-amylase production economically attractive, over-producing mutant and recombinant strains can be developed.

- *Bacillus cereus* and *Aspergillus awamori* strains can be immobilized into suitable matrices and studied for their efficiency in the removal of starch and to find the suitability of reuse.

- Large scale production of α-amylases using the strains *Bacillus cereus* and *Aspergillus awamori* can be studied.

- Purification of α-amylases can be carried out and its application in other fields such as food, baking etc. can be studied.
- Optimization studies can be carried out, using response surface methodology.
- Strains producing thermostable α-amylase can be exploited and studied for their efficiency in starch removal.