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Production and Application of Amylase Enzyme for Bio-desizing

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Abstract

In this study, an attempt has been made to investigate the effect of enzyme on desizing of cotton. Cotton fabric was subjected to desizing using commercially available enzyme. An attempt has also been made, to synthesize amylase enzyme from microbe and evaluate their suitability for desizing of cotton fabrics. The performance of enzyme treated fabric has been compared with that of the acid desized sample. The desized samples were subjected to various tests like residual starch, iodine test, absorbency, shrinkage, breaking strength and fabric weight. The effluent after the process was analysed for pH, BOD, COD, TDS and TSS.

Keywrods : *Amylase; Cotton fabrics; Desizing; Enzyme.*

1. INTRODUCTION

World's ever increasing population and its progressive adoption of an industrial based lifestyle have inevitably led to an increased anthropogenic impact on the biosphere. Man is facing one of the most horrible ecological crises – the problem of pollution of his environment. Textile wet processing is one of the largest and oldest industries worldwide, responsible for the substantial resource consumption and pollution especially in terms of water.

Numerous researchers in textiles are embracing enzymatic processing more and more in an attempt to minimize contribution to the effluent from conventional, harsh chemicals. Enzymes are safe to use, easy to control and biodegradable and hence, can be an alternative for harsh chemicals. The area that can have the most dramatic impact on reducing environmental pollution is preparation of cotton, as 75 % of the organic pollutant level arising from textile finishing is derived from the preparation of cotton goods.

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Sizing is the process in which certain compounds are applied to yarns to bind the fibres together and stiffen it to provide abrasion resistance and strength to withstand the stress and strain during weaving. Cotton yarn is coated with a paste composed of starch, adhesive, lubricants and other additives to give better weavability .Once the yarn reaches the cloth, these compounds have no useful role to play and causes serious problem during dyeing and printing and this finish has to be removed prior to further processing.

In the desizing process the sizing compounds are rendered water soluble and removed from the cloth to make it suitable for further processing. In conventional desizing, grey cloth is soaked in dilute sulphuric acid or hydrochloric acid for 4-12 hours at room temperature and then rinsed in water(Manivasakam, 1995). Desizing of cotton using oxidizing agents or acids has the draw back that while acting on starch size, these do act on cellulose as well there by decreasing the tensile strength of fabric (Shukla et al., 2000).

Enzymatic desizing is the most popular and most effective method. Starches are hydrolysed to

water soluble compounds by enzymes (Storti et al. 2005) amylases a bacterial enzyme capable of doing the hydrolysis of starch into water soluble sugars is popularly used for desizing (Nalankalli, 1998). These amylolytic enzymes in their natural or modified state destroy any type of starch, turning into water soluble products without affecting cellulose. The destruction occurs stepwise, starch molecules hydrolyse to dextrin (a polysaccharide), maltose (a disaccaride) and finally to the monosaccarideglucose (Sekar, 1998).

The advantage of enzymatic desizing over traditional desizing are 1) Due to very specific reaction of enzyme, there is no adverse effect on cellulose, resulting in strength retention 2) Process time of desizing can be reduced 3) Neutralisation is not required, therefore zero salt formation in ETP 4) Saving of energy, as desizing takes place at moderate temperature 5) Feel of fabric is much softer, with less hairiness further there is no mildew growth during storage 6) The use of acid in the conventional method increases the BOD (800-1000) and COD of the process considerably compared to that of bio desizing (Khanna and Maheshwari, 2004).

An investigation is made to evaluate the relative merits and demerits of the above mentioned desizing techniques by considering the change in properties after desizing with each method.

2. METHODOLOGY

2.1 Material

Plain weave 100 % cotton (95x42), 40'S count.

2.2 Enzyme production from bacterial source

From literature survey, it was observed that amylase could be used for desizing. Hence, efforts were taken to synthesize the same from bacterial source.

2.2.1. Bacterial media

Tryptone-0.1 g, Sodium chloride-0.1g and Yeast extract-0.5 g were dissolved in ten ml of distilled water, pH was adjusted to 7.2 and made up to 100ml.1.5 % agar and anti fungal agent were added (Ravikumar et al., 2002). The contents were dispensed into sterile petridishes and test tubes, plugged with cotton and autoclaved at 121° C for 15 minutes. It was cooled, allowed to solidify and preserved.

2.2.2. Sampling and Collection

Enzymes are commercially produced by soil dwelling fungi and bacteria. Strains of enzyme producing microorganisms selected for the study were isolated from the composting soil, collected near the institution cafeteria. The soil samples were collected by using sterile gloves, aseptically placed in sterile containers and were used immediately.

2.2.3. Culture preparation

One gram of soil sample was added to 1 ml of sterile water blank. The sample was serially diluted up to the dilution of 10^{-8} for each dilution. From the above liquor 10^{-5}dilutent was selected and 0.1ml of the appropriate dilution was taken and inoculated uniformly using a sterile L–rod throughout the bacterial medium (Ravikumar et al., 2002). The inoculated culture plates and test tubes were incubated at 28 °C for 24 hours and examined for new colony formation.

2.2.4. Identification of bacterial strain

Once colonies develop, agar plates were sorted according to types. Initial observations were made by using a dissecting microscope, to identify the different bacteria. It was noted that there were a number of bacteria in the medium. Bacterial strain producing amylase were isolated after primary screening of different isolates and the bacterial strain *Bacillus lincheniformis* was then chosen for the study.

2.2.5. Preparation of culture media for *Bacillus licheniformis* for production of amylase

The culture media was prepared using Calf brain infusion- 200g, Beef heart infusion - 250g,

Peptone- 10g , Glucose -2g , Sodium chloride -5g, Di sodium hydrogen phosphate -2.5g, Agar -15g, Starch-0.5g along with anti fungal agent and the pH was adjusted to 7.4. The medium was dispensed into the test tubes and petridishes. They were sterilized in an autoclave at 121°C for 15 minutes and allowed to cool before storing.

The streak plate technique was adopted for the study. A single colony of *Bacillus licheniformis* from the bacterial culture plate was taken by using a sterile inoculation needle and was inoculated in the culture media for sub culturing. The streaked plates and test tubes were incubated for 24 hours at 37°C.

2.2.6. Submerged fermentation for amylase extraction

The submerged fermentation was prepared by mixing the media as mentioned in media preparation without agar (Solidifying agent) and 30ml was distributed in 250ml conical flasks. In the microbial production of amylase, the selection of carbon source is an important factor. Lactose, dextrin and soluble starch were found suitable for enzyme production, but higher enzyme activities were obtained from starch (Fabiana et al. 2001).

Starch solution was prepared separately by boiling 2 gm powder in 40 ml distilled water. It was then added to 60 ml of other dissolved components. The pH was adjusted using 0.1N solution of sodium hydroxide or hydrochloric acid. All the chemicals used in the experiment were of analytical grade. The organism from cultured plates were cut and transferred to the submerging medium in the flasks, which was kept in a shaker and agitated for 144 hours (six days) to provide proper aeration to the inoculums. The enzyme was extracted by centrifugation and ultrafiltration.

2.2.7. Measurement of amylase activity

Assay method for the enzyme activity was followed. Diluted crude enzyme (0.2ml) was added to 0.4ml of 2% soluble starch substrate along with the addition of 0.4ml acetate buffer (pH5) and the mixture was incubated at 55ºC for 30 minutes. The reaction was stopped by the addition of 1ml dinitro - salicylic acid reagent and the mixture was boiled for 10 minutes. 10 ml of distilled water was added and the absorbance was measured at a wavelength of 540nm, which gives the amount of reducing sugar produced during the reaction. (Benefield, 1980).

2.2.8. Purification of enzyme

The crude broth was purified by ammonium sulphate precipitation method. The crude broth was kept in an ice bath and ammonium sulphate was added slowly with constant stirring. The precipitate was dissolved with minimum amount of distilled water and kept in a beaker for dialysis for 12-18 hours against distilled water(Shukla et al.,2005).

2.2.9.Optimisation of amylase production

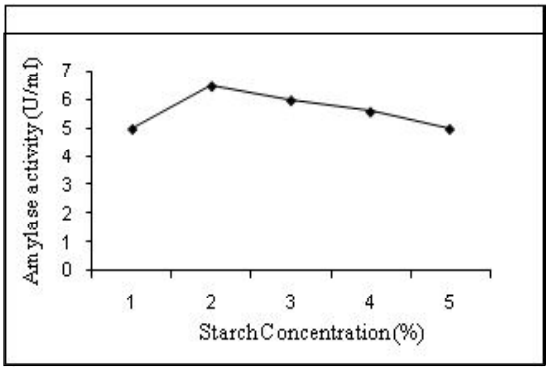

Fig.1 Effect of starch concentration on production of amylase

The effect of concentration of starch in nutrient medium on production of bacterial amylase was studied. Starch concentration in the medium was varied from 1-5% and the flasks were incubated for 96 hours. The amylase yield is graphically shown in figure – 1. From the figure, it is evident that the maximum production of amylase in terms of activity, is obtained with 2% starch concentration.

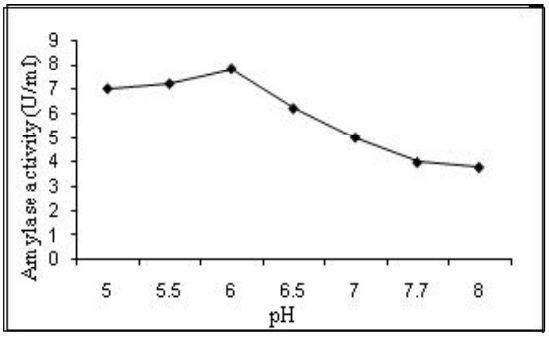

Fig.2 Effect of pH on production of amylase

The effect of pH on amylase activity was studied between pH 5 and 8. The same is shown in figure 2. From the figure it is obvious that the optimum pH for production of amylase is 6, at which the enzyme activity is maximum.

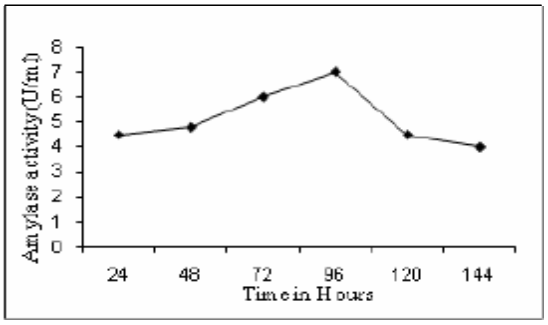

Fig3. Effect of time of incubation on production of amylase

The time of incubation of culture was onitored upto 144 hours as indicated in figure - 3. It is clear that the enzyme activity increased upto 96 hours and then decreased. Hence 96 hours was chosen as the optimum time for enzyme production.

2.3.Desizing

To suit the objectives of the study, it was decided to give the desizing in three different ways, namely, using commercially available enzymes, synthesizing similar enzymes from microbes and utilizing them for desizing and to desize with hydrochloric acid which is in practice today in the processing units for purpose of comparison.

2.3.1.Desizing with commercial enzyme

RexsizeLHT 100 liquid, an amylase based highly concentrated enzyme was selected for desizing. As per the instructions in the manual for Bio desizing RexsizeLHT, wetting agent and pre dissolved sodium chloride were added to water at 75°C and mixed thoroughly. (Salt is necessary in enzymatic treatment as it improves heat stability of the enzyme. Wetting agent help in quick wetting and better penetration). The pH was adjusted to 7.5. The prewetted fabric was treated in this liquor for about an hour and thoroughly rinsed in soft water. The temperature of the liquor was raised to deactivate the enzyme. Later the fabric was rinsed in soft water and dried.

2.3.2.Desizing with synthesized enzyme

2.3.2.1.Pilot study

A pilot study was carried out to choose the most efficient and effective concentration and temperature at which the enzymes are most effective. Each process was carried out in the temperature range of 60, 70 and 80°C. Concentration was varied as 0.5,1 and 2%. For the pilot study, each samples weighing 10 gms were taken with the additives, material liquor ratio (1:15) and time (45-60minutes) constant.

2.3.2.2.Actual Study

Grey cotton samples were desized using synthesized amylase enzyme at pH 6 at 60°C, Material liquor ratio was kept as 1:15. Fabric along with amylase enzyme 1%, Wetting agent-0.1% and sodium chloride-0.5% were kept in a water bath for 30 minutes. After treatment, the temperature of the bath was raised to 100°C to deactivate the enzyme. Finally samples were given one hot wash at boil for 10-15 minutes and cold wash at room temperature in soft water before drying.

2.3.3.Conventional Method or Acid desizing

Conventional desizing can be done by hydrolytic method, oxidative method, chlorine, chlorite and bromite treatment. The investigator selected the hydrolytic method as it is suitable for both chemical and enzyme method. Dilute hydrochloric acid was used to hydrolyse starch from the sized fabric, by steeping in acid solution at room temperature. Desizing was carried out by dissolving dilute hydrochloric acid2%, Sodium chloride 1%,wetting agent 0.2% in 1:15 liquor ratio at pH 7. Fabric was run in the solution for 8 hours. The fabric was then rinsed thoroughly in soft water and dried.

2.4.Analysis of Sample

Residual starch, Iodine test, Shrinkage, Fabric Weight , Tensile Strength and Absorbency were analysed.

2.5. Analysis of Effluent Water

The characteristics of the effluent with respect to pH, total dissolved solids, suspended solids, biological oxygen demand and chemical oxygen demand were analyzed.

3.RESULTS AND DISCUSSION

3.1.Residual starch

Table 1. Residual Starch in Desized Sample

Samples	Residual starch %	F value
Acid desized	0.95	
Biodesized-commercial	0.58	2072.5616**
Biodesized-synthesised	0.54	

** - significant at 1 per cent

The use of enzymes for desizing reduced the percentage of residual starch. Though the individual difference was very meagre, the synthesized enzyme faired better than commercial enzyme. Statistical analysis reveals that the amount of residual starch in the desized sample varies significantly based on the type of treatment.

3.2.Iodine test

Table 2. Iodine test for original and desized samples

Samples	Colour
Original sample	Dark blue colour
Acid desized	Light blue colour
Biodesized-commercial	Absence of blue colour
Biodesized-synthesised	Absence of blue colour

Absence of blue colour on the samples desized with enzymes indicate their superiority over the conventional method.

3.3 Absorbency

Table 3. Absorbency value of the samples

Samples	Drop penetration test mean in sec	Capillary rise mean in sec	Sinking test mean in sec
Original sample	50	57	53
Acid desized	23	21	20
Biodesized-commercial	19	18	14
Biodesized-synthesised	12	11	9

The absorbency of the samples were determined in terms of drop penetration test,capillary rise and sinking time, All desized samples gave good results as far as absorbency is concerned. It is obvious that enzymes faired better than acid.

3.4.Shrinkage

It is clear that the use of enzymes have reduced the shrinkage in both warp and weft directions when compared to the use of chemicals.

Table 4. Shrinkage percent of the desized samples

Samples	Shrinkage per cent	
	Warp	Weft
Acid desized	6.4	6.8
Biodesized-commercial	5	5.8
Biodesized-synthesised	5	5.6

3.5 Fabric weight

Table 5. Fabric Weight of the original and desized samples

Samples	Mean fabric weight in GSM	Gain or loss over previous treatment	Percentage gain or loss
Original	106.1	-	-
Acid desized	101.1	-5	-4.71
Biodesized-commercial	105.3	-0.8	-0.750
Biodesized-synthesised	104.9	-1.2	-1.13

Desizing has caused a reduction in fabric weight of samples, irrespective of the method. The loss percentage is nearly five in the case of Acid desizing. Enzymatic desizing has caused a loss of only one percent.

3.6 Tensile strength

Table 6. Tensile Strength of the original and desized samples

Samples	Warp			Weft		
	Mean strength in kgs	Gain or loss over previous treatment	% gain or loss	Mean strength in kgs	Gain or loss over previous treatment	% gain or loss
Original	36.31	-	-	29	-	-
Acid desized	35.06	-1.25	-3.442	25	-4	-13.79
Biodesized-commercial	35.77	-0.54	-1.49	26.34	-2.66	-9.17
Biodesized-synthesised	35.99	-0.32	-0.881	26.74	-2.26	-7.79

Desizing causes a reduction in the tensile strength of samples in the warp and weft direction. But the reduction rate is minimum when the enzymes are used. The percentage of reduction is found to be less than one per cent in the warp direction and eight in the weft direction in sample desized with synthesized enzyme.

3.7 Analysis of Effluent Water

Table 7. Analysis of Effluent Water

Samples\Effluent water	Parameters				
	pH	BOD (mg/l)	COD (mg/l)	TDS (mg/l)	TSS (mg/l)
Acid desized liquor	8.6	1050	2780	2850	1775
Commercial Enzyme- desized liquor	8.5	560	976	2083	1500
Synthesized Enzyme- desized liquor	8.5	476	1004	2097	1500

Effluent analysis reveals that desizing with enzymes has no effect on the pH level of the effluent. Though the BOD and COD level of the effluent is much higher than the tolerance limit, it is obvious that the BOD and COD level can be reduced noticeably with the help of enzymes. Use of enzymes

either reduces the TDS level of the effluent almost nearer to the tolerance limit. The TSS level of the effluent is found to be much higher than the tolerance limit but a comparison between chemical and enzymatic pretreatment reveal that the former has much adverse effect on the effluent.

4. CONCLUSION

Bio deisizing may be a valuable and environment friendly alternative to harsh chemicals. Use of enzymes have reduced the percent of residual starch. All three methods improved the absorbency. The advantages of biodesizing over acid desizing are reduced effluent load, less weight loss, shrinkage and less damage since it is specific to starch besides increased soft, smooth hand on the fabric. It provides a safe working environment as the chemical reaction occur under mild conditions.

Rapid changes in technology and a direct need to conserve water and energy have forced the textile industry to give up the old conventional processes and try out new methods, which enable not only cost reduction, but also savings in terms of water and energy. Utilization of biotechnological applications like enzymatic procedures in the industrial sectors not only reduces load on the effluents by avoiding chemical usage but also improves quality apart from providing a safe working atmosphere. Ecofriendly procedures and products have always been and will always create a niche in business and society.

REFERENCES

Benefield.P., Methods of enzymology, Academic press, *London*, 149 (1980).

Fabianna.G.M.,Lenartoriez.V., Rams.E.P. and Peralta.R., *Brazilian journal of microbiology*, 32, 15 (2001)

Khanna.A. and Maheshwari.R.C., Wet processing of denim garments, *Colourage*, LI (9), 47 (2004).

Manivasakam.N., Water used in textile processing-Quality, treatment and analysis, *Sakthi Publication*, Coimbatore, 155, 160 (1995).

Nalankilli.G.,Application of enzymes in eco-friendly wet processing of cotton, *Colourage*, XLV (10), 17-19 (1998).

Ravikumar.K, Prabhakaran.R. andPrema.K., Basic techniques in molecular biology, *Published by school of biotechnology*, Coimbatore, 12 (2002).

Sekar.N., Green technology in textile processing, *Colorage*, XLVI, 11, 20 (1998).

Shukla.S.R, Sharma.U. andKulkarini.K.S.,Enzymes and their use in textile processes, Colourage, XLVII (2), 19-20, 22 (2000).

Shukla.S.R.,Ananthanarayan.L. and Damle.A.J., Production and application of amylase enzymes, *Colourage*, LII (11), 43-48 (2005).

Storti.S., Gava.P., Mackay.L.,,The biological treatment of waste water for textile and dyeing industries, *Colrage*, LII (12), 40 (2005).