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Synthesising and Evaluating the Performance of Enzyme for Scouring of Cotton

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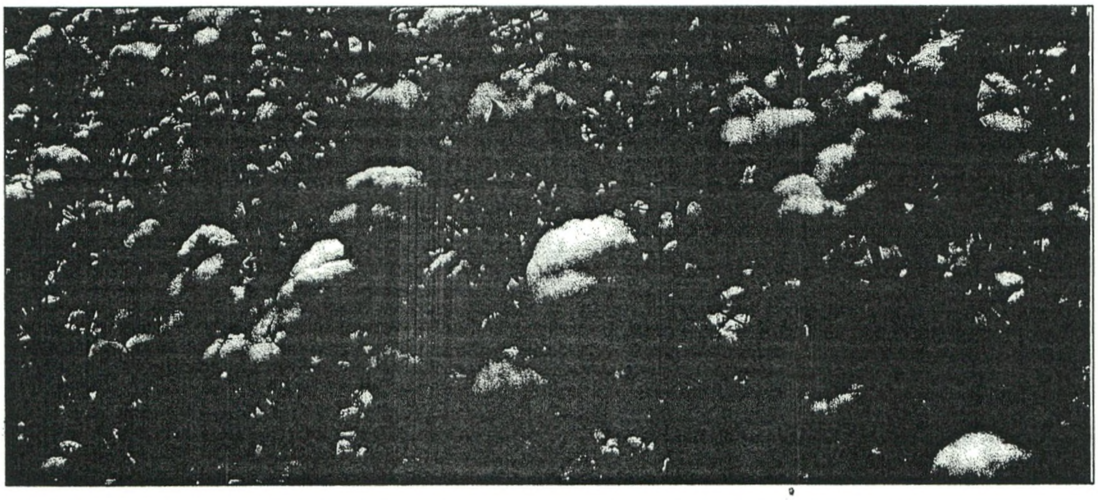

The world's ever increasing population and its progressive adoption of an industrial based lifestyle have inevitably led to an increased anthropogenic impact on the biosphere. Man is facing one of the most horrible ecological crises - the problem of pollution of his environment. Textile wet processing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

Utilisation 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.

Numerous researchers in textiles are embracing enzymatic processing more and more in an attempt to minimise 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.

Cotton consists of a thick secondary wall that is composed of cellulose and an external primary wall of cellulose and non-cellulosics. The non-cellulosics include waxes, proteins, pectins, inorganics and other substances. It is the primary wall that is responsible for protection of fibre during growth and non absorptive nature of unprepared cotton, as the matters it contains are hydrophobic in nature and interfere with further aqueous chemical processing. Hence, they need to be removed before dyeing and finishing to make the fabric more receptive and hydrophilic. Scouring is the removal of non-cellulosic material from the surface of cotton fibre.

Boiling in sodium hydroxide is the most widely practised conventional and effective process to remove impurities in the primary wall. It makes the fabric absorbent and ready for subsequent processes. However, it is a major source of environmental pollution. It is a non-specific reaction leading to damage of the fibre causing excessive weight loss, reduced elongation, extensive shrinkage, low strength and develops a harsh hand. It is useful to examine an alternative to this conventional, harsh chemical preparation.

Bioscouring is a bio-technological scouring method that uses enzyme instead of sodium hydroxide. A lot of research activities have been directed to find the ability of enzymes such as pectinase, protease, cellulase and lipase to remove the non-cellulosic material. Pectinase enzymes can break the pectins and related components into water-soluble fragments, which can be washed out in subsequent working. While doing so, the other impurities, which are held by the pectins with the fibre, are also removed. Lipases are used in the removal of lubricants to provide a fabric with good absorbency. The enzymes are non-toxic and eco-friendly. They can be deactivated easily by raising the temperature or changing the pH.

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

Methodology

Material

Desized, Plain weave 100 % cotton (95x42), 40s count.

Enzyme production from fungal source

From literature survey, it was observed that pectinase could be used for decomposition of pectin substances and lipases for fats. Hence, efforts were taken to synthesise the same from fungal source.

Fungal media

Fungal agar weighing 3.5 g was mixed thoroughly in 100 ml of sterile water. The pH was adjusted to 6.5. The contents were autoclaved for 45 minutes and cooled. 0.2 microgram of antibacterial antibiotic was added for preservation and dispensed into sterile Petri dishes and test tubes.

Isolation of fungus from sample source

Soil was collected from local canteen. One gram of soil sample was added to 1 ml of sterile water blank. The sample was serially diluted upto the dilution of 10^{-8} for each dilution. From the above liquor, 10^{-5} dilutent was selected.

Culture preparation

Diluted soil was inoculated on fungal media plates using streak plate method and incubated at 28°C for 48 hours and examined everyday for colony formation.

Identification of fungal strain

From the fungal media, it was noted that there were many species present. After preliminary screening, the following fungi were selected for the study based on their activity :

Organism	**Enzyme**
Aspergillus foetidus	Pectinase
Aspergillus japonicus	Lipase.

Preparation of culture media for Aspergillus foetidus for pectinase production

The growth media for Aspergillus foetidus was prepared using Tryptone - 3 g, Sodium nitrate - 30 g, Potassium chloride - 5 g, Magnesium sulphate - 5 g, Ferrous sulphate - 0.1 g, Dipotassium hydrogen phosphate - 1 g, Yeast extract - 5 g, Sucrose - 30 g, Agar - 15 g, and Pectin - 10 g. The pH was maintained at 5 along with an antibiotic tetracyclin and was autoclaved. The strain was sub cultured. The inoculated cultures were incubated at 50°C for 144 hours (6 days) and were examined every day for colony growth.

Submerged fermentation and pectinase extraction

The submerged fermentation was prepared by mixing the media as mentioned in media preparation without agar (solidifying agent) and 30 ml was distributed in 250 ml conical flasks along with pectin. 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). The enzyme was extracted by centrifugation and ultra filtration.

Measurement of pectinase activity

The pectinase activity was determined using pectin and polygalactronic acid as substrates. The reaction mixture containing equal amount of substrate prepared in citrate buffer (0.05 M, pH 4.4) and suitably diluted enzyme was incubated at 50°C for 30 minutes in water bath. After incubation, 3 ml dinitro salicylic acid solution was added to stop the reaction and tubes were kept in boiling water for 10 minutes. On cooling, the developed colour was read at 575 nm using UV-visible spectrophotometer. The amount of released reducing sugar was quantified using galactronic acid as standard.

Purification of enzyme

The crude broth was purified by ammonium sulphate precipitation method.

Optimisation of pectinase production

- Effect of pectin concentration on production of pectinase : The effect of concentration of pectin in nutrient medium on production of fungal pectinase was studied. Pectin concentration in the medium was varied from 0.5-2% and the flasks were incubated for 72 hours. From *Fig 1* it may be observed that the maximum production of pectinase in terms of activity was obtained with 1% pectin concentration.

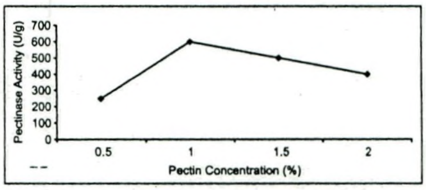

Fig 1 : Optimisation of pectin concentration for pectinase production

- Effect of pH on production of pectinase : The effect of pH on

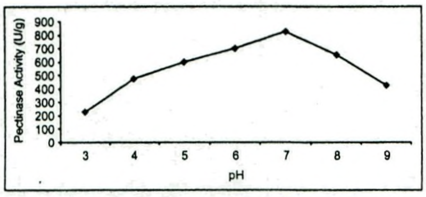

Fig 2 : Optimisation of pH for pectinase production

pectinase activity was studied between 3-9. Results indicate that pH 7 was more suitable for pectinase. Increase or decrease in pH beyond the maximum value showed decline in enzyme activity *(Fig 2)*.

- Effect of time of incubation on production of pectinase : The production profile of pectinase on pectin containing

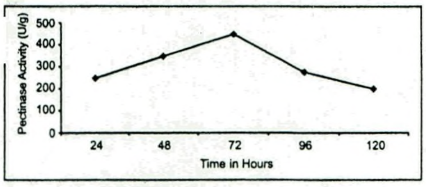

Fig 3 : Optimisation of reaction time for pectinase production

basal medium was studied. The time of incubation of culture was monitored up to 120 hours. It was found that maximal production of pectinase was obtained after 72 hours of incubation. Further incubation resulted in decline in enzyme activity *(Fig 3)*.

Preparation of culture media for Aspergillus japonicus for production of lipase

The growth media for Aspergillus japonicus was prepared using Malt extract - 20 g, Agar - 20 g, Peptone - 30 g, Olive oil - 5 ml along with an antibiotic tetracyclin with pH adjusted to 6.5 and autoclaved.

The strain was sub cultured. The inoculated cultures were incubated at 50°C for 144 hours (6 days) and were examined every day for colony growth.

Submerged fermentation and lipase extraction

The submerged fermentation was prepared by mixing the media as mentioned in media preparation without agar (solidifying agent) and 30 ml was distributed in 250 ml conical flasks along with olive oil. The organisms 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). The enzyme was extracted by centrifugation and ultra filtration.

Measurement of lipase enzyme activity

Lipase activity was assayed in the enzyme containing supernatants with p-nitro phenyl palmitate as substrate and the absorbance determined spectrophotometrically at 410 nm.

Purification of enzyme

The crude broth was purified by ammonium sulphate precipitation method.

Optimisation of lipase production

- Effect of olive oil concentration on production of lipase : The effect of different concentrations of olive oil in

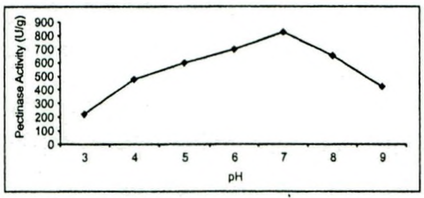

Fig 4 : Optimisation of olive oil concentration for lipase production

nutrient medium on production of fungal lipase was evaluated as shown in *Fig 4*. Olive oil concentration in the medium was varied from 0.5-2% and the flasks were incubated for 96 hours. From the *Fig 4*, it is clear that the maximum production of lipase in terms of activity is obtained with 1% olive oil concentration.

- Effect of pH on production of lipase : The pH values of the mixture were

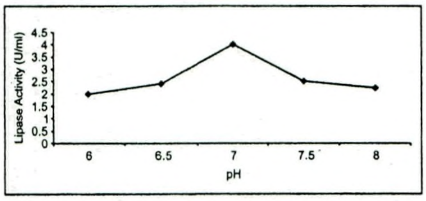

Fig 5 : Optimisation of pH for lipase production

varied from 6 to 8 and lipase activity was measured as indicated in *Fig 5*. The optimum was reached at pH 7 and pH values below or above declined lipase activity.

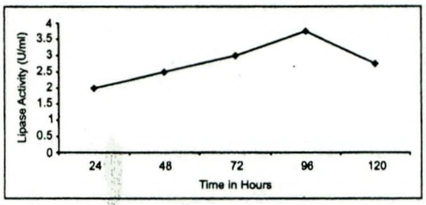

Fig 6 : Optimisation of reaction time for lipase production

- Effect of time of incubation on production of lipase : The production profile of lipase was studied. The time of incubation of culture was monitored up to 126 hours as shown in *Fig 6*. It is clear that 96 hours of incubation is needed for maximum production of lipase. Further incubation resulted in decline in enzyme activity.

Scouring

To suit the objectives of the study, it was decided to give the scouring in three different ways, namely, using commercially available enzymes, synthesising similar enzymes from microbes and utilising them for scouring, and to scour with sodium hydroxide which is in practice today in the processing units for purpose of comparison.

Scouring with commercial enzyme

Descour New, a unique blend of several enzymes especially pectinase and lipase supplied by Rossari Biotech was selected for scouring. The bioscour liquor was prepared by dissolving Descour enzyme (0.5%) and Zywet NIS, a non-ionic wetting agent (1%), in soft water, with the material liquor ratio as 1:15 and pH adjusted to 9. The desized fabric was immersed in it, maintaining the temperature as 55°C for 30 minutes. The temperature was raised to 90°C and left for 10 minutes to deactivate the enzyme. The sample was then rinsed thoroughly in soft water and dried.

Scouring with synthesised enzyme

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 10g were taken with the additives, material liquor ratio (1:15) and time (45-60 minutes) constant.

Actual study

The fabric was scoured with fungal pectinase (0.5%) and lipase (0.5%) at pH 7, for 30-45 minutes at 70°C in the presence of Zywet NIS as a wetting agent (1%). The temperature was raised to deactivate the enzyme activity. The fabric was thoroughly rinsed in soft water and dried.

Conventional method or Caustic scouring

The desized cotton fabric was weighed accurately. The fabric was then steeped in a solution containing sodium hydroxide (4%), soda ash (2%) and wetting agent (0.2%) at 40°C with the liquor ratio as 1:15. The temperature of the solution was increased to boiling point and the fabric was run in the solution at boil for 4 hours. The pH was maintained as 8.5. It was then washed first with hot water and then with cold water and dried.

Analysis of sample

Shrinkage, fabric weight, tensile strength, thickness and absorbency 'e analysed.

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 analysed.

Results and discussion

Absorbency

The absorbency of scoured samples indicates the effectiveness of scouring, as it is indicative of the effective removal of hydrophobic matter. The absorbency of the samples was determined in terms of drop penetration test, capillary rise and sinking time *(Table 1)*.

All scoured samples gave very good results as far as absorbency is concerned.

Samples	Drop penetration test mean in sec	Capillary rise mean in sec	Sinking test mean in sec
Unscoured	23	21	20
NaOH	3	5	4
Bioscouring commercial	4	5	5
Bioscouring synthesised	4	5	5

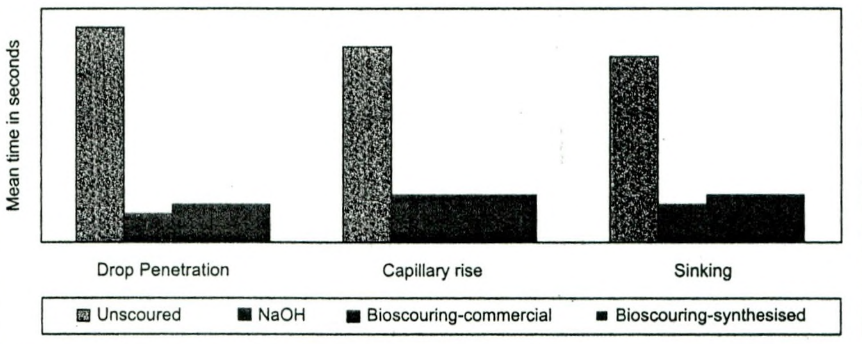

Table 1 : Absorbency

Shrinkage

NaoH causes more shrinkage in warp where it is 2.08, and in weft, it is 3. Enzymatic scouring causes minimum shrinkage than NaOH scouring, in both warp and weft directions *(Table 2)*.

Samples	Shrinkage percent: Warp	Shrinkage percent: Weft
Unscoured	4	6
NaOH	2.08	3
Bioscouring commercial	2	2.5
Bioscouring synthesised	2	2.5

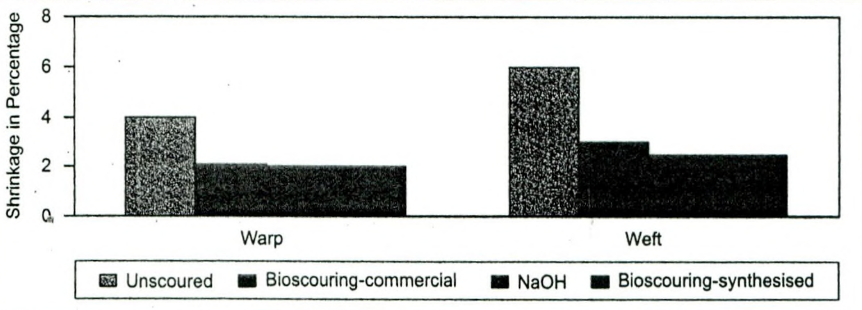

Table 2 : Shrinkage

Fabric weight

Scoured samples have shown a reduction in fabric weight irrespective of the scouring agent. While the NaoH scouring had caused a loss of 4.6%, enzymes have caused only 2.6% and 2.7% loss *(Table 3)*.

Tensile strength

Scouring has brought about a reduction in tensile strength of

Samples	Mean weight in GSM	Loss over original	Percentage weight loss
Unscoured	101.1	-	-
NaOH	96.5	-4.6	-4.550
Bioscouring commercial	98.5	-2.6	-2.57
Bioscouring synthesised	98.4	-2.7	-2.67

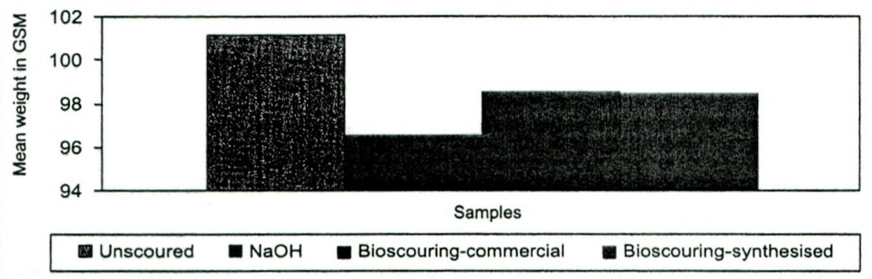

Table 3 : Fabric weight

Samples	Warp			Weft		
	Mean strength in Kgs	Loss over original	% strength loss	mean strength in kg	Loss over original	% strength loss
Unscoured	35.06	-	-	25	-	-
NaOH	32.79	-2.27	-6.475	23.4	-1.6	-6.4
Bioscouring-commercial	33.87	-1.19	-3.394	24.17	-0.83	-3.32
Bioscouring-synthesised	34.2	-0.86	-2.453	24.23	-0.77	-3.08

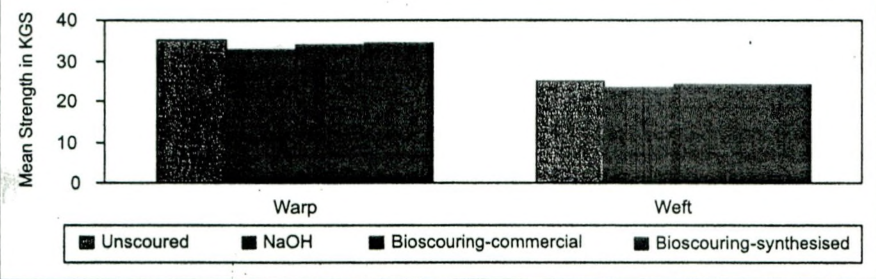

Table 4 : Tensile strength

Samples	Mean thickness in mm	Gain or loss over original	% gain or loss in thickness
Unscoured	29.65	-	-
NaOH	26.8	-2.85	-9.61
Bioscouring commerical	32.4	2.75	9.27
Bioscouring synthesised	30.88	1.23	4.10

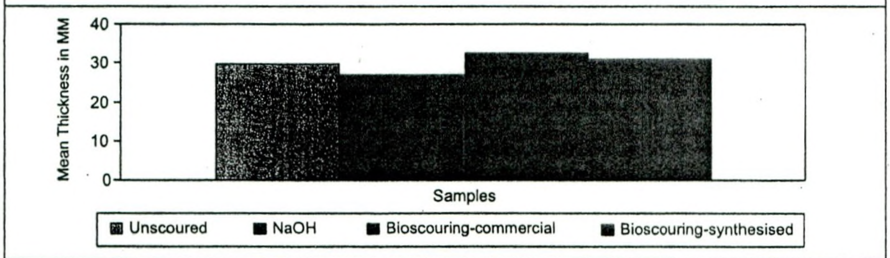

Table 5 : Thickness

samples, irrespective of the method. While the NaoH scouring has caused maximum reduction of 6%, the enzyme has caused a minimum reduction in both warp and weft directions *(Table 4)*.

Thickness

While the NaoH scouring has caused a reduction in fabric thickness, enzymes have increased the thickness *(Table 5)*.

Analysis of effluent water

Effluent analysis reveals that scouring with enzymes can bring down the pH level of the effluent well within the tolerance limit. 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 reduces the TDS level of the effluent almost nearer to the tolerance limit. The TSS level of the effluent at various stages is found to be much higher than the tolerance limit, but a comparison between chemical and enzymatic pretreatment reveals that the former has much adverse effect on the effluent *(Table 6)*.

Conclusion

Bioscouring may be a valuable and environment friendly alternative to harsh chemicals. All the three methods have improved the absorbency. The advantages of bioscouring over alkali scouring are reduced effluent load, less weight loss, less shrinkage and less damage since it is specific to pectin and waxes, besides increased soft, smooth hand on the fabric. It provides a safe working environment as the chemical reaction occurs under mild conditions.

Rapid changes in technology and a dire 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. Utilisation of biotechnological

Table 6 : Analysis of effluent water

Samples/Effluent water	Parameters				
	pH	BOD (mg/l)	COD (mg/l)	TDS (mg/l)	TSS (mg/l)
NaOH-scoured liquor	10.9	2480	9840	3350	1380
Commercial enzyme scoured liquor	8	368	1920	1006	1208
Synthesised enzyme scoured liquor	7.9	360	1940	1083	1197

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 thus create a niche in the business and the society.

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Advanced Academy for Textile Technologists by DyStar and Alok

DyStar India Private Limited, a leading provider of dyestuffs, auxiliaries and services to the textile industry and Alok Industries Limited, India's most diversified and vertically integrated textile manufacturer are jointly setting up the Advanced Academy for Development of Textile Technologists (AADTT).

The Academy will be the first-of-its-kind in the country and an unparalleled knowledge based training platform to facilitate skill development in the textile industry. AADTT will provide the platform for identification, training, placement and career development of textile professionals to raise standards of textile manufacturing in India.

Candidates will be hand picked from premier partnering institutes, trained for a period of one year and offered back to the textile industry in the interest of the development of the industry. Candidates will be paid 'market level' stipends and trained through a well designed curriculum combining theory, specific application expertise and practice relevant and adapted to modern industrial needs. Renowned and experienced industrial experts will be working with the team as faculties.

Mr Dilip B Jiwrajka, Managing Director of Alok is upbeat about this unique initiative, 'The fast growing textile industry in India is in urgent need of competent professionals who have a sound technical base and can be developed into leaders of tomorrow. Every journey begins with a single step. The AADTT is but one small step by two leaders in their respective fields for the benefit of the industry at large'.

Mr Rajesh Balakrishnan, Managing Director of DyStar India Private Limited, stresses the importance of this co-operation for all partners. 'The AADTT will be a wonderful avenue for competent students graduating out of textile institutes to upgrade their skills and knowledge. DyStar with its expertise in technology, application and standards alongwith Alok which has an extremely modern textile manufacturing set up will provide this lifetime opportunity to institutes and students aspiring to develop a career in the textile industry'.

DyStar is a leading complete solution provider for the textile industry has an unparalleled global presence with textile standards, sales, technical service, laboratories, distribution centres, expert solutions and production facilities. DyStar helps its customers to reduce the cost, reliably meet quality and environmental standards.