

ENZYMES USED FOR SUSTAINABLE WET PROCESSING IN TEXTILE INDUSTRY

Ms. Anju Kushwaha¹, Dr. Priyanka Kesarwani², Rashi kushwaha³

^{1&3}Research Scholar & ²Assistant Professor

Department of Family and Community Sciences, University of Allahabad, Prayagraj, U.P. India-211002

Abstract

Today, enzymes have become an integral part of the textile processing. With the increasing awareness of environmental pollution and the extensive consideration of mankind health, chemical processes used in textile industries are being replaced by enzymatic processes. Usages of enzymes in textile industry will be the best possible alternative of chemicals used in textile industry. Enzymes like amylase, cellulase, catalase, protease, pectinase, laccase, and lipase are widely used in textile manufacturing and processing industries. Use of enzymatic treatments in textile industries is very promising approach as they are eco-friendly, produce high-quality products, and lead to the reduction of energy, water, and time. It is observed that enzymes can replace harsh chemicals, catalyze reaction and operate under mild conditions. These are biodegradable, safe to use and easy to control. In this review environment-friendly uses of various enzymes in different textile processing steps have been discussed.

Keywords: enzymes, oxidoreductases, transferases, hydrolases, cellulase, protease,

Introduction

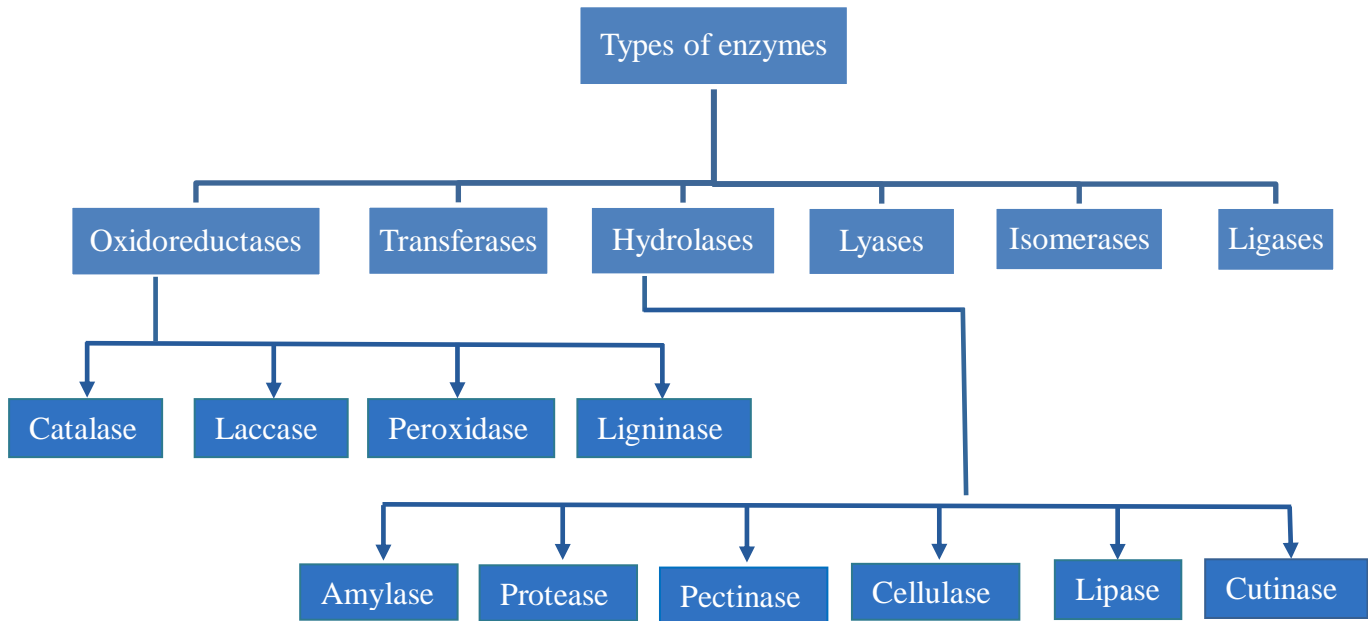
Enzymes:-

The term Enzyme came from the ‘Greek’ word ‘Enzymos’ means ‘In or From the Cells’. Enzymes are discovered in the second half of the nineteenth century. Enzymes are biological catalysts that accelerate the rate of chemical reactions. All enzymes are made up of protein and they each have a very specific three dimensional shape. The shape is different for each enzyme and each enzyme only works on a specific substrate such as amylase speeds up the breakdown of starch and cellulase speeds up the breakdown of cellulose (Mojsov, 2012; Mojsov, 2011; Bharathi, V., & Kanaka, M. 2015).

Without being consumed in the process, enzymes can speed up chemical reactions. Usually most enzymes are used only once and discarded after their catalytic action. There are mainly three primary sources from where enzymes are obtained i.e., animal tissues, plants and microorganisms (Mojsov, n.d.). There are large numbers of microorganisms like fungi, moulds, yeasts, bacteria etc. which produces a variety of enzymes. Most of the industrial enzymes are produced by few selected microorganisms like *Aspergillus*, *Bacillus*, *Trichoderma*, *Streptomyces*, etc. (Boyer, 1971; Fersht, 2007).

Types of enzymes:-

There are mainly six different classes of enzymes, namely Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases. Classification of enzymes is shown below in the **Figure 1**.



Enzyme and their type of reactions:-

Enzymes are highly substrate specific; they react with their specific substrates at a region within the protein molecule which is called active site. The active site of the enzymes must have the necessary structural characteristic to recognize the right substrate and the proper chemical environment to occur the reaction. The International Commission on Enzymes (EC) was established in 1956 by the International Union of Biochemistry (IUB), in consultation with the International Union of Pure and Applied Chemistry (IUPAC), to put the enzymes in order that had been discovered by that point and establish a standardized terminology that could be used to systematically name newly discovered enzymes.

The International Commission on Enzymes (EC) classification system is divided into six categories on basic function (Mojsov, K. 2011). According to Choudhury, R. A. K. (2014) enzymes and their types of reaction are shown in **Table 1**.

Table 1 Enzyme and their type of reactions

Class	Name of enzyme	Type of reactions
EC 1	Oxidoreductases	Catalyze oxidation/reduction reactions
EC 2	Transferases	Transfer a functional group (e.g., a methyl or phosphate group)
EC 3	Hydrolases	Catalyze the hydrolysis of various bonds
EC 4	Lyases	Cleave various bonds by means other than hydrolysis and oxidation
EC 5	Isomerases	Catalyze isomerisation changes within a single molecule
EC 6	Ligases	Join two molecules with covalent bonds usually at the expense of an energy source (usually ATP)

Major enzymes used in textile industry

Most of the enzymes used in textile industry belong to the class hydrolases and oxidoreductases (Roy Choudhury, A. K. 2014; Doshi & Shelke, n.d.). Hydrolases enzymes can be further classified into five broad groups: - Proteases, Pectinase, Cellulase, Lipases, Amylase and Cutinases. The group of oxidoreductases includes catalase, laccase, peroxidase, and ligninase. Major enzymes which are used in textile industries are shown in **Table 2** (Doshi & Shelke, n.d.; Mojsov, K. 2011; Mojsov et al., 2018).

Table 2 Major enzymes used in textile industry

Type of Enzyme		Application in Textile Industry
Hydrolases	Proteases	Bio-scouring, Bio-desizing
	Pectinase	Bio-scouring
	Cellulase	Bio-scouring, Bio-polishing, stone washing
	Lipases	Scouring, desizing
	Amylase	Bio-desizing
	Cutinase	Bio-scouring
Oxidoreductases	Ligninase	Wool finishing
	Laccase	Bio-bleaching of indigo in denim, Discoloration of coloured effluent
	Catalase	Bleaching
	Peroxidase	Decolouration of dyes

Proteases

Proteases are one of the most important groups of enzyme, used in textile industry for the manufacturing of shrink proof wool, degumming of silk fabric, bio-scouring and bio-desizing because proteases are used to break down non-collagenous skin constituents and remove non-fibril proteins (Solanki et al., 2021; Saha et al., 2011; Ghasemi et al., 2011). These enzymes can be also used for the preparation of leather and many other textiles (Saha et al., 2011; Ghasemi et al., 2011). In leather processing, proteases enzymes enhance its quality and give stronger and softer leather with less spots (Fang et al., 2017).

Proteases enzymes from microbial sources are preferred over the enzymes derived from plant and animals since they possess almost all characteristics desired for their biotechnological applications (Padmapriya et al., 2012). Thus proteases enzymes account for about 60% of the total industrial enzyme sale in the world (Hamza, T. A. 2017). These enzymes have been categorized on the basis of various parameters like the site of action, the type of substrate, active pH range, mechanism of action involving particular amino acid present in the active site (Guleria et al., 2016a, b). Depending on the site of action, these enzymes can be broadly classified as endopeptidase and exopeptidase (Solanki et al., 2021).

Pectinases

Pectinases are one of the upcoming enzymes of textile industries. Pectinases are one of the first enzymes to be used in homes. Their commercial application was first observed in 1930 in the textile industry. Primarily, these enzymes are responsible for the degradation of the long and complex molecules called pectin. Alkaline pectinases are mainly used in the degumming and retting of fiber crops (Kashyap et al., 2001). These enzymes are also used for bio-scouring in the textile wet processing industries.

Cellulases

Cellulase is the third largest industrial enzyme as it can degrade cellulose the common natural polymer. They are also involved in the conversion of lignocelluloses into glucose units which are further used in the bio-stoning of denim, bio-polishing, increasing softness and lustre of textile fibers (Kakkar, P., & Wadhwa, N. 2021). The treatment with cellulase enzyme is carried out under mild conditions so as to minimize the degradation of the fabric (Rasel et al., 2018; Ibrahim et al., 2011). Cellulase is produced by a wide variety of bacteria (e.g. *Clostridium*, *Cellulomonas*) and fungi (e.g. *Humicola*, *Trichoderma*, *Penicillium*). Among these, the most important commercial bacteria are the *Trichoderma reesei* (Heikinheimo et. al., 1998).

Lipases

The presence of lipases has been observed in *Bacillus prodigiosus*, *B. pyocyaneus* and *B. fluorescens* in the year 1901 (Eijkman, C. 1901). Lipase enzyme hydrolyses insoluble oil droplets and converted them to soluble products. Traditionally lipases have been obtained from animal pancreas but nowadays numerous species of bacteria, yeasts and molds are used to produce lipases.

Lipases are used in the textile industry to assist in the removal of sizing materials (bio-desizing), natural triglycerides (bio-scouring) and lubricants, in order to provide a fabric with greater absorbency for improved levelness in dyeing. Usage of lipases enzymes also reduces the frequency of streaks and cracks in the denim abrasion systems. Commercial preparations used for the bio-desizing of denim and other cotton fabrics, contains both alpha amylase and lipase enzymes (Hasan et al., 2006).

Amylases

The amylase can be found in microorganisms, plants and animals. The amylase is a hydrolase enzyme which belongs to a family of endo-amylases that catalyse the initial hydrolysis of internal α -1, 4-glycosidic linkages in starch in low molecular weight products. Amylases derived from bacteria and fungi are quite stable over a wide range of pH from 4 to 11 (Mojsov, K. 2011).

Amylases are used in textile industry for desizing process. Sizing agents like starch are applied to yarn before fabric production to ensure a fast and secure weaving process. Desizing involves the removal of starch from the fabric. Amylase is employed to cleave starch particles randomly into water soluble components that can be removed by washing. This also reduced the discharge of waste chemicals to the environment. The α -amylases remove selectively the size and do not attack the fibres (Gupta et al., 2003; Souza, P. M. D., & Magalhães, P. D. O. 2010; Ahlawat et al., 2009).

Cutinases

The cuticle layer of a cellulosic fiber has a complicated composition that includes cutin, wax, pectin and protein. Both the wax and cutin can be hydrolysed by the cutinases from *Pseudomonas mendocina*, *F. solani pisi* and *T. fusca*. When these cutinases are combined with pectinase, the wettability of cellulosic fiber can be improved efficiently, at low temperature, without the addition of alkali (Agrawal et al., 2008; Degani et al., 2002; Yan et al., 2011; Zhang et al., 2010, 2011).

Cutinases are multifunctional enzymes that belong to the α/β hydrolase family. Cutinases can catalyze hydrolysis reactions, esterifications and transesterification reactions. As a result, they have substantial potential to be widely used in the textile industries.

Laccases

Laccases are extracellular enzymes that use molecular oxygen to oxidize phenols, and various aromatic and non-aromatic compounds by a radical-catalysed reaction mechanism (Thurston 1994). They belong to a larger group of enzymes termed the blue-multicopper oxidase family. Laccases have been found in plants, insect, bacteria, but are most predominant in fungi (Benfield et al., 1964; Claus, H. 2004; Baldrian, P. 2006). Fungal laccase is a protein of approximately 60-70 KDa. It works at the temperature range between 50°C-70°C and in the optimal acidic range pH.

Laccases are widely used for the decolourization of textile effluents because of their ability to degrade dyes of diverse structures, including synthetic dyes (Abadulla et al., 2000; Hou et al., 2004; Couto et al., 2006; Mishra, S., & Bisaria, V. S. 2006; Hao et al., 2007). This enzyme is very important in the treatment and finishing of denim fabrics. According to Doshi, R., & Shelke, V. (2001) laccase enzyme is able to decolourise indigo dyes. Laccases can also be used for the degradation of complex natural polymers, such as lignin (Claus, H. 2004). The range of substrates with which laccases can react is very broad, showing a remarkable lack of specificity towards their reducing substrate (Araujo et al., 2008). Shin et al. (2001) reported in their study that laccase was able to colour wool fabric that was previously padded with hydroquinone.

Catalases

Catalases catalyse the degradation of H_2O_2 into H_2O and O_2 . They are produced by a variety of different micro organisms including bacteria and fungi (Mueller et al., 1997) and works at moderate temperatures (20-50°C) and neutral pH. Catalases from animal sources (bovine liver) are generally cheap and therefore, the production of microbial Catalases will only be economically advantageous when recombinant strains and cheap technology is used. In the textile industry, Catalases are used to decompose excess H_2O_2 (Fraser, J.L. 1986). This enzyme eliminates the need of reducing agent

and minimizes the need for rinse water, resulting in less polluted wastewater and lower water consumption.

Peroxidases

Peroxidases are widely distributed in nature. These enzymes are produced by a variety of sources including plants, animals, and microorganisms. Peroxidases produced from microbial sources such as bacteria (*Bacillus sphaericus*, *Bacillus subtilis*, *Pseudomonas sp.*, *Citrobacter sp.*), fungi (*Candida krusei*, *Coprinopsis cinerea*, *Phanerochaete chrysosporium*), and yeast are used in textile dye degradation and decomposition of pollutants. Peroxidases have been reported as excellent oxidant agents to degrade dyes. Several bacterial peroxidases have been used for decolourization of synthetic textile dyes. Removal of chromate Cr (VI), acid orange 7 (AO7) and azo dye using peroxidases under nutrient-limiting conditions has been studied (Bansal, N., & Kanwar, S. S. 2013). An edible macroscopic fungi *Pleurotus ostreatus* produces an extracellular peroxidase that can decolorize remazol brilliant blue and other structurally different groups including triarylmethane, heterocyclic azo, and polymeric dyes. Bromophenol blue was decolorized best (98%) by peroxidases, while methylene blue and toluidine blue O were least decolorized 10% (Shin, J. S., & Kim, B. G. 1997). Many plant sources for peroxidases production have been reported such as horseradish, papaya (*Carica papaya*), banana (*Musa paradisiacal*), and bare (*Acorus calamus*).

Conclusions

Keeping in view the increasing environmental concerns and constraints being imposed on textile industry, enzyme treatment of textiles is an environmental friendly way of improving different properties. Environmental friendly processes in the textile industry are gaining ground all over the world. In this scenario, enzymes can be used in order to develop environmental friendly alternatives of the polluting textile chemical processes in almost all steps of textile processing.

There were various uses of enzymes in the early stages of the development, but their innovative applications are increasing and spreading rapidly into all areas of textile processing. The textile industry can greatly benefit from the expanded use of these enzymes as non-toxic, environmentally friendly compound. Enzymes are not only beneficial from ecological point of view but the uses of enzymes in textile processing would save time, energy, raw materials, water, and cost and would make the entire process viable, sustainable, and eco-friendly.

References

- [1] Abadulla, E., Tzanov, T., Costa, S., Robra, K. H., Cavaco-Paulo, A., & Gübitz, G. M. 2000. Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta*. *Applied and environmental microbiology*, 66(8): 3357-3362.
- [2] Agrawal, P. B., Agrawal, P. B., Nierstrasz, V. A., Bouwhuis, G. H., & Warmoeskerken, M. M. C. G. 2008. Cutinase and pectinase in cotton bioscouring: an innovative and fast bioscouring process. *Biocatalysis and biotransformation*, 26(5): 412-421.
- [3] Ahlawat, S., Dhiman, S. S., Battan, B., Mandhan, R. P., & Sharma, J. 2009. Pectinase production by *Bacillus subtilis* and its potential application in biopreparation of cotton and micropoly fabric. *Process Biochemistry*, 44(5): 521-526.
- [4] Araujo, R., Casal, M., & Cavaco-Paulo, A. 2008. Application of enzymes for textile fibres processing. *Biocatalysis and Biotransformation*, 26(5): 332-349.
- [5] Baldrian, P. 2006. Fungal laccases—occurrence and properties. *FEMS microbiology reviews*, 30(2): 215-242.
- [6] Bansal, N., & Kanwar, S. S. 2013. Peroxidase (s) in environment protection. *The Scientific World Journal*, 2013.
- [7] Barrett, A. J., Woessner, J. F., & Rawlings, N. D. (Eds.). 2012. *Handbook of Proteolytic Enzymes*, Volume 1 (Vol. 1). Elsevier.

- [8] Benfield, G., Bocks, S. M., Bromley, K., & Brown, B. R. 1964. Studies of fungal and plant laccases. *Phytochemistry*, 3(1): 79-88.
- [9] Bryce, C. F., & Balasubramanian, D. 2004. *Concepts in biotechnology*. Universities Press.
- [10] Chen, S., Tong, X., Woodard, R. W., Du, G., Wu, J., & Chen, J. 2008. Identification and characterization of bacterial cutinase. *Journal of biological chemistry*, 283(38): 25854-25862.
- [11] Chen, S., Su, L., Chen, J., & Wu, J. 2013. Cutinase: characteristics, preparation, and application. *Biotechnology advances*, 31(8):1754-1767.
- [12] Ciroth, A., Finkbeiner, M., Traverso, M., Hildenbrand, J., Kloepffer, W., Mazijn, B., ... & Vickery-Niederman, G. 2011. Towards a life cycle sustainability assessment: making informed choices on products.
- [13] Claus, H. 2004. Laccases: structure, reactions, distribution. *Micron*, 35(1-2): 93-96.
- [14] Couto, S. R., Rosales, E., & Sanromán, M. A. 2006. Decolourization of synthetic dyes by *Trametes hirsuta* in expanded-bed reactors. *Chemosphere*, 62(9): 1558-1563.
- [15] Dandekar, S.P. (2010). *Concise Medical Biochemistry. Elsevier, a division of Reed Elsevier India Private Limited.* pp 9-10.
- [16] Degani, O., Gepstein, S., & Dosoretz, C. G. 2002. Potential use of cutinase in enzymatic scouring of cotton fiber cuticle. *Applied Biochemistry and Biotechnology*, 102(1): 277-289.
- [17] Doshi, R., & Shelke, V. 2001. Enzymes in textile industry-An environment-friendly approach.
- [18] Duncan, M., Lippiatt, B. C., Haq, Z., Wang, M., & Conway, R. K. 2008. Metrics to support informed decision making for consumers of biobased products. *Agriculture Information Bulletin*, 803.
- [19] Eijkman, C. 1901. Ueber Enzyme bei bakterien und Schimmelpilzen. *Cbl Bakt Parasitenk Infektionskr*, 29: 841-8.
- [20] Fang, Z., Yong, Y. C., Zhang, J., Du, G., & Chen, J. 2017. Keratinolytic protease: a green biocatalyst for leather industry. *Applied microbiology and biotechnology*, 101(21): 7771-7779.
- [21] FRASER, J. L. 1986. Peroxygens in environmental protection. *Effluent & water treatment journal*, 26(5-6): 186-199.
- [22] Ghasemi, Y., RSOUL, A. S., Ebrahiminezhad, A., Kazemi, A., Shahbazi, M., & Talebnia, N. 2011. Screening and isolation of extracellular protease producing bacteria from the Maharloo Salt Lake.
- [23] Gupta, R., Gigras, P., Mohapatra, H., Goswami, V. K., Chauhan, B. 2003. Microbial α -amylases: a bio- technological perspective. *Process Biochemistry*, 38: 1599-1616
- [24] Guleria, S., Walia, A., Chauhan, A., & Shirkot, C. K. 2016a. Immobilization of *Bacillus amyloliquefaciens* SP1 and its alkaline protease in various matrices for effective hydrolysis of casein. *3 Biotech*, 6(2): 1-12.
- [25] Guleria, S., Walia, A., Chauhan, A., & Shirkot, C. K. 2016b. Molecular characterization of alkaline protease of *Bacillus amyloliquefaciens* SP1 involved in biocontrol of *Fusarium oxysporum*. *International Journal of Food Microbiology*, 232: 134-143.
- [26] Hamza, T. A. 2017. Bacterial protease enzyme: safe and good alternative for industrial and commercial use. *Int J Chem Biomol Sci*, 3(1): 1-0.
- [27] Hasan, F., Shah, A. A., & Hameed, A. 2006. Industrial applications of microbial lipases. *Enzyme and Microbial technology*, 39(2): 235-251.
- [28] Heikinheimo, L., Cavaco-Paulo, A., Nousiainen, P., Siika-aho, M., & Buchert, J. 1998. Treatment of cotton fabrics with purified *Trichoderma reesei* cellulases. *Journal of the Society of Dyers and Colourists*, 114(7-8): 216-220.
- [29] Hao, J., Song, F., Huang, F., Yang, C., Zhang, Z., Zheng, Y., & Tian, X. 2007. Production of laccase by a newly isolated deuteromycete fungus *Pestalotiopsis* sp. and its decolorization of azo dye. *Journal of industrial microbiology and biotechnology*, 34(3):233.
- [30] Hou, H., Zhou, J., Wang, J., Du, C., & Yan, B. 2004. Enhancement of laccase production by *Pleurotus ostreatus* and its use for the decolorization of anthraquinone dye. *Process Biochemistry*, 39(11): 1415-1419.

- [31] Ibrahim, N. A., EL-Badry, K., Eid, B. M., & Hassan, T. M. 2011. A new approach for biofinishing of cellulose-containing fabrics using acid cellulases. *Carbohydrate Polymers*, 83(1): 116–121. <https://doi.org/10.1016/j.carbpol.2010.07.025>
- [32] International Resource Panel, United Nations Environment Programme. Sustainable Consumption, & Production Branch. 2011. Decoupling natural resource use and environmental impacts from economic growth. UNEP/Earthprint.
- [33] Josephine, F. S., Ramya, V. S., Devi, N., Ganapa, S. B., Siddalingeshwara, K. G., Venugopal, N., & Vishwanatha, T. 2012. Isolation, production and characterization of protease from *Bacillus* sp. isolated from soil sample. *Journal of Microbiology and Biotechnology Research*, 2(1): 163-168.
- [34] Kakkar, P., & Wadhwa, N. 2021. Extremozymes used in textile industry. *The Journal of The Textile Institute*, 1-9.
- [35] Kashyap, D. R., Vohra, P. K., Chopra, S., & Tewari, R. 2001. Applications of pectinases in the commercial sector: a review. *Bioresource technology*, 77(3): 215-227.
- [36] Li, M. 2012. An Environmentally Benign Approach to Cotton Preparation: One Bath Enzymatic Desizing, Scouring and Activated Bleaching.
- [37] Marcher, D., Hagen, H. A., & Castelli, S. 1993. Entschlichten mit enzymen. *ITB Veredlung*, 39(3): 20-32.
- [38] Masaki, K., Kamini, N. R., Ikeda, H., & Iefuji, H. 2005. Cutinase-like enzyme from the yeast *Cryptococcus* sp. strain S-2 hydrolyzes polylactic acid and other biodegradable plastics. *Applied and environmental microbiology*, 71(11): 7548-7550.
- [39] Mishra, S., & Bisaria, V. S. 2006. Production and characterization of laccase from *Cyathus bulleri* and its use in decolourization of recalcitrant textile dyes. *Applied microbiology and biotechnology*, 71(5): 646-653.
- [40] Mojsov, K. 2011. Application of enzymes in the textile industry: a review.
- [41] Mojsov, K. 2012. Enzyme scouring of cotton fabrics: a review. *International Journal of Marketing and Technology (IJMT)*, 2(9):256-275.
- [42] Mojsov, K., Andronikov, D., Janevski, A., Jordeva, S., Kertakova, M., Golomeova, S., ... & Ignjatov, I. 2018. Production and application of α -amylase enzyme in textile industry. *Tekstilna industrija*, 66(1): 23-28.
- [43] Mueller, S., Riedel, H. D., & Stremmel, W. 1997. Determination of catalase activity at physiological hydrogen peroxide concentrations. *Analytical biochemistry*, 245(1): 55-60.
- [44] Padmapriya, B., Rajeswari, T., Nandita, R., & Raj, F. 2012. Production and purification of alkaline serine protease from marine *Bacillus* species and its application in detergent industry. *European Journal of Applied Sciences*, 4(1): 21-26.
- [45] R. Murali, A. K. Rakshit, C. N. Murthy, Studies on the different colloids in the water bodies of Narmada, *J. Ind. Chem. Soc.*, 9(4):477, 2014.
- [46] Radhakrishnan, S. 2014. Application of biotechnology in the processing of textile fabrics. In *Roadmap to Sustainable Textiles and Clothing* (pp. 277-325). Springer, Singapore.
- [47] Roy Choudhury, A. K. 2014. Sustainable textile wet processing: Applications of enzymes. In *Roadmap to sustainable textiles and clothing* (pp. 203-238). Springer, Singapore.
- [48] Saha, M. L., Begum, K. H., Khan, M. R., & Gomes, D. J. 2011. Bacteria associated with the tannery effluent and their alkaline protease activities. *Plant Tissue Culture and Biotechnology*, 21(1): 53-61.
- [49] Sarmiento, F., Peralta, R., & Blamey, J. M. 2015. Cold and hot extremozymes: industrial relevance and current trends. *Frontiers in bioengineering and biotechnology*, 3: 148.
- [50] Shaikh, M. A. 2010. Enzymes: a revaluation in textile processing. *Pakistan Textile J*, 48-51.
- [51] Shin, H., Guebitz, G., & Cavaco-Paulo, A. 2001. "In situ" enzymatically prepared polymers for wool coloration. *Macromolecular materials and engineering*, 286(11): 691-694.
- [52] Shin, J. S., & Kim, B. G. 1997. Kinetic resolution of α -methylbenzylamine with o-transaminase screened from soil microorganisms: Application of a biphasic system to overcome product inhibition. *Biotechnology and bioengineering*, 55(2): 348-358.



- [53] Solanki, P., Putatunda, C., Kumar, A., Bhatia, R., & Walia, A. 2021. Microbial proteases: ubiquitous enzymes with innumerable uses. *3 Biotech*, 11(10): 1-25.
- [54] Souza, P. M. D., & Magalhães, P. D. O. 2010. Application of microbial α -amylase in industry- A review. *Brazilian journal of microbiology*, 41: 850-861.
- [55] Sundarram, A., & Murthy, T. P. K. 2014. α -amylase production and applications: a review. *Journal of Applied & Environmental Microbiology*, 2(4): 166-175.
- [56] Thurston, C. F. 1994. The structure and function of fungal laccases. *Microbiology*, 140(1): 19-26.
- [57] Vadlamani, S., & Parcha, S. R. 2011. Studies on industrially important alkaline protease production from locally isolated superior microbial strain from soil microorganisms. *Int. J. Biotechnol. Appl*, 3(3): 102-105.
- [58] W. Atkins, "The Elements of Physical Chemistry", 3rd Ed., Oxford University Press, 2001.
- [59] Yan, H. J., Du, G. C., & Chen, J. 2011. Enhancement of cotton waxes removal with *Thermobifida fusca* cutinase by temperature control process. In *Advanced Materials Research* (Vol. 332, pp. 81-86). Trans Tech Publications Ltd.
- [60] Zhang, Y., Chen, S., Xu, M., Cavoco-Paulo, A., Wu, J., & Chen, J. 2010. Characterization of *Thermobifida fusca* cutinase-carbohydrate-binding module fusion proteins and their potential application in bioscouring. *Applied and environmental microbiology*, 76(20): 6870-6876.
- [61] Zhang, Y., Chen, S., He, M., Wu, J., Chen, J., & Wang, Q. (2011). Effects of *Thermobifida fusca* cutinase-carbohydrate-binding module fusion proteins on cotton bioscouring. *Biotechnology and Bioprocess Engineering*, 16(4): 645-653.