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Evaluation of Uses of Some Enzymes in Removal of Plant Impurities from Raw Wool Fabrics

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Highlights:

- The efficiency of cellulase and phytase enzyme obtained from new *Bacillus* isolates and commercial acid pectinase were determined.
- Enzymes were applied alone and in mixtures at different pHs, volumes and incubation times.
- Enzymatic effect was demonstrated by light microscopy

ABSTRACT:

In this study, phytase from *Bacillus megaterium* EBD9-1 and cellulase from *Bacillus subtilis* 171ES obtained from isolated from different provinces soils, and commercial pectinase enzymes were investigated for the removal potential of plant impurities from raw wool fabrics. Enzymatic removal of plant impurities is usually carried out at pH 4.0. The efficiency of the enzyme was shown at pH 7.0, but the best effect was obtained with pH 4.0. Enzymes were effective alone, but the effects of double and triple enzyme mixtures on plant impurities were more. It was determined that the effect decreased in case the enzyme amount was used low, and the effect increased even more in the presence of the concentrated enzyme. With this study, which was carried out for the first time with local isolate, the operability of more environmentally friendly enzymes in carbonization processes traditionally performed with chemical substances was demonstrated.

Keywords:

- Cellulase
- Phytase
- Pectinase
- Plant impurities
- Wool fabric

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INTRODUCTION

Enzymes are biological catalysts that catalyze biochemical reactions that occur in living things and are generally protein-based. Enzymes, which have important metabolic functions in cells, have now entered daily and economic life to be used for different purposes in many fields. They are used in many industrial areas such as food, textile, medicine, cosmetics, animal feed, waste treatment, agriculture, and detergent, etc. (Sharma et al., 2020). Because, they have many advantages such as being economically produced, being biodegradable, being specific, not creating unwanted by-products, producing less waste than conventional methods, and being safe for employee and environmental health (Choi et al., 2015, Seager et al., 2018). Enzymatic treatments have gained popularity in the textile industry due to their environmentally friendly and energy-saving alternatives.

Enzymatic processes have gained popularity in the textile industry due to their environmental, water, and energy-saving alternatives, and nowadays enzymes have become an integral part of the textile process. Enzymes are a sustainable alternative to harsh toxic chemicals in the textile industry. Many enzymes such as cellulases, laccases, amylases, catalases, and peroxidases are used in various stages of textile processing such as desizing, biostoning of denim and non-denim, biological polishing, peroxide removal, bio-carbonization of wool and waste treatment, etc. (Chelikani et al., 2004; Cavaco-Paulo & Gubitz, 2003; Barrett et al., 2012). The use of enzymes in the textile industry is one of the fastest-growing areas of industrial enzymology.

Raw wool, which has an important place in textiles, is a truly impure component, often contaminated with 40% to 70% of unnecessary matter (Fong et al., 1951). These pollutants found in wool are mostly plant-derived impurities consisting of thorns, straw, grasses, leaves, burrs, branches, seeds, and forage residues. (El-Sayed et al., 2010). These impurities must be removed efficiently for the wool to be processed.

Traditionally, a chemical method called carbonization is used to remove plant impurities The most commonly used chemical for this purpose is acid sulfuric acid, which is a strong acid. However, this method causes weakening and discoloration of the fibers, weight loss during subsequent processes, and negatively affect dye uptake, a lot of water is used in this process, and it is laborious and time-consuming. Due to these disadvantages, various environmentally friendly methods such as the use of plasma, ultrasound, and enzymes have been studied in various studies (Sun & Stylios, 2004; Das & Ramaswamy, 2006; Zheng et al., 2012; Bahtiyari & Duran, 2013). Enzymes gained importance from these methods and plant impurities content of wool fiber was removed at various rates with cellulase, pectinase, or xylanase enzymes (Rahman & Nur, 2014). The first attempts to carbonize wool with the use of enzymatic preparations in the process of removing from the wool of plant impurities were made in Poland in the 1980s. This process is known as "BIOCARBO of wool" or bio-carbonization (Sedelnik, 1993; El-Sayed et al., 2010).

Although enzymes are also produced by animals and plants, microorganisms are the main source of enzyme production because they are obtained in a short time under controlled conditions. Approximately 96% of enzymes used for industrial purposes are produced from microorganisms (Lowe, 2001). The main sources of microbial enzymes are fungi such as *Aspergillus* and *Trichoderma* and bacteria such as *Streptomyces* and *Bacillus* (Fersht, 2007; Mojsov, 2011).

In this study, the efficiency of *Bacillus subtilis* 171ES cellulase, *Bacillus megaterium* EBD9-1 phytase, previously isolated from different provincial soils, and commercial pectinase enzymes in removing plant impurities from untreated raw wool fabric at pH 4.0 and pH 7.0 were investigated.

Enzymes were tested alone or in mixtures. The effect of enzymatic incubation time, enzyme amount, and lyophilized enzyme were also tested.

MATERIALS AND METHODS

Materials

The microbial phytase and cellulase used in this study were produced from *Bacillus megaterium* EBD9-1 and *Bacillus subtilis* 171ES isolated from the soils and identified in our previous studies. GenBank accession numbers for *Bacillus megaterium* EBD9-1 and *Bacillus subtilis* 171ES was OM004562 and OM00456, respectively. The commercial pectinase used in the study was obtained from NUY Kimya company. 100% wool fabrics contaminated with plant impurities were obtained from YÜNSA A.Ş (Türkiye). The fabric properties are given in Table 1.

Table 1. Raw Wool Fabric Properties

Raw Material	Weight (g/m^2)	Thread Density		Yarn Count	
		Warp Thread Density (thread/cm)	Weft Thread Density (thread/cm)	Warp Yarn Count (Ne)	Weft Yarn Count (Ne)
100% Wool Fabric	321	16	14	6/1	6/1

Methods

Cultivation and media

For cellulase production, medium containing (%w/v) 1 CMC (Carboxymethyl cellulose), 1.6 skim milk powder, 1 maltose, 0.03 $LiSO_4$, 0.2 K_2HPO4 was used. Bacteria growth conditions were temperature 37°C, pH 7.0, incubation time 40 h, aeration 150 rpm and inoculation size of 1% (Msanki, 2020).

The growth medium used for phytase production was composed of 5 g/L lactose, 8 g/L meat extract, 1 g/L $CaCl_2 \cdot 2H_2O$, and 5 g/L Na-phytate, and the sample was rotated at 150 rpm for 48 h at 37°C, pH 7.5 and inoculation size of 1% (Demirkan et al., 2017).

Enzyme activity assays of cellulase and phytase

Since the activity of commercial acid pectinase enzyme is known, only the activity values of cellulase and phytase enzymes obtained from new isolates were determined.

Cellulase

The method by Miller (1959) was used for the determination of cellulase enzyme activity. 0.5 mL of 1% carboxyl methyl cellulose substrate solution was added to 0.5 mL of crude enzyme solution and was incubated at 37 °C for 30 minutes in a water bath. To stop the reaction, 1 mL of dinitrosalicylic acid (DNS) reagent (Garriga et al., 2017) was put and tubes were incubated in boiling water for 5 min., the absorbance was measured at 540 nm after cooling. One unit (U) of enzyme activity was defined as the enzyme amount that releases 1 μmol glucose per minute. The glucose standard curve was obtained by preparing glucose at different concentrations.

Phytase

Phytase activity was assayed according to the method described by Choi et al. (2001). 0.1 mL of crude enzyme solution and 0.9 mL of 2 mM Na-phytate was mixed in the test tubes containing 0.1 M Tris-HCl buffer (pH 7.0). The reaction mixture were incubated in a water bath at 37 °C for 10 min and then the reaction was stopped by adding 0.75 mL of 5% (w/v) TCA. Freshly prepared 1,5 mL of color reagent [four volumes of 2.5% (w/v) ammonium molybdate in 5.5% (v/v) sulfuric acid and one volume of 2.5% ferrous sulfate (w/v)] was added to the test tube and the released phosphate was

measured at 700 nm. One enzyme unit was defined as the amount of phytase liberating 1 μmol of inorganic phosphate in a minute under the assay conditions. A phosphate calibration curve was made by treating standard phosphate solutions of 0-100 μM KH_2PO4.

Lyophilization of enzymes

Cellulase and phytase crude enzymes produced in 1 liter of medium were lyophilized. The lyophilization process was performed with the LAB312 brand TOPT-10 Model brand at -55°C for 48 hours. Lyophilized enzymes were stored in sterile falcon tubes at +4°C. Commercial pectinase enzyme is not lyophilized because it has high activity.

The removal of the cellulosic or plant impurities in wool fabrics

Crude cellulase, phytase, and commercial pectinase enzymes were applied to raw wool (100%) alone or in combination. For this purpose, 5 cm diameter wool fabric pieces were placed in sterile Petri dishes and incubated with 15 mL enzymes (alone and mixed enzyme treatment) at pH 4.0 and 7.0 at 37°C for 1 hour (pH 7.0 was not tested for the enzyme since acid pectinase was used in the study). After incubation, the fabric pieces were dried at room temperature. Whether or not the cellulosic or plant impurities in wool fabrics were removed was determined by light microscope (Nikon Eclipse E1000) imaging and photographing. It was compared with the plant impurities on the fabrics photographed under the light microscope before being treated with the enzyme (control). In addition, 5 and 10 mL of each enzyme and enzymes concentrated by lyophilization (10 mL of each) were also included in the experiment. The size of the wool fabric containing plant impurities not treated with the enzyme used in the experiments is given in Figure 1.

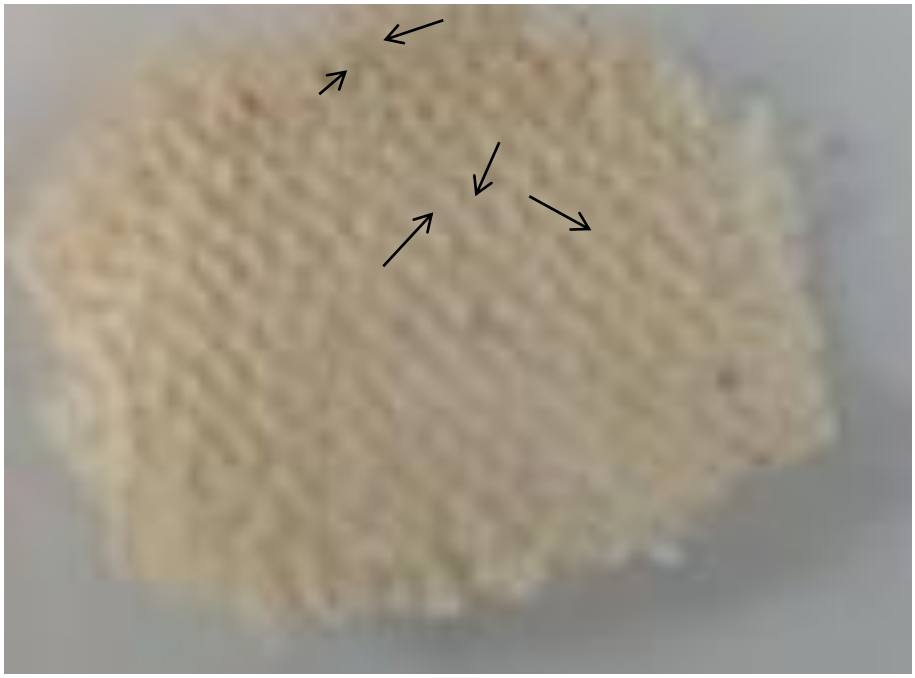

Figure 1. 100% Wool Fabric Containing Cellulosic or Plant Impurities

RESULTS AND DISCUSSION

Raw wool fabrics contain high levels of plant impurities as well as some impurities. These impurities are removed by a process called “wool carbonization” in the industry. This process is based on the principle of converting cellulose into an easily removable hydro cellulose using a strong acid, sulfuric acid. Instead of processing using strong and dangerous chemicals, the use of enzymes reduces wool fiber damage, waste load, and energy consumption (Gouveia et al., 2008).

In this study, the effects of crude cellulase from the new isolate *Bacillus subtilis* 171ES and crude phytase enzymes from *Bacillus megaterium* EBD9-1 and commercial pectinase enzyme on the removal of cellulosic or plant impurities in wool fabrics were investigated.

The activities of all enzymes used in the study were adjusted to 445 U/mL. Enzymes were used singly or in enzyme combination. The effects of enzymes on the removal of plant impurities from fabrics were evaluated by light microscopy (10x100 magnification), and compared with the control. The enzymes were effective on the impurities when used alone, but it was observed that the effect increased when used in combination. In industrial carbonization applications, plant impurities are generally removed at pH 4.0. In this study, better results were obtained at pH 4.0. However, cellulase and phytase were effective in removing plant impurities at an acceptable level at pH 7.0.

While cellulase and phytase enzymes were effective in the breakdown of plant impurities at pH 4.0 and 7.0, cellulase and phytase were more effective at 4.0. Commercial pectinase alone was also effective (Figure 2).

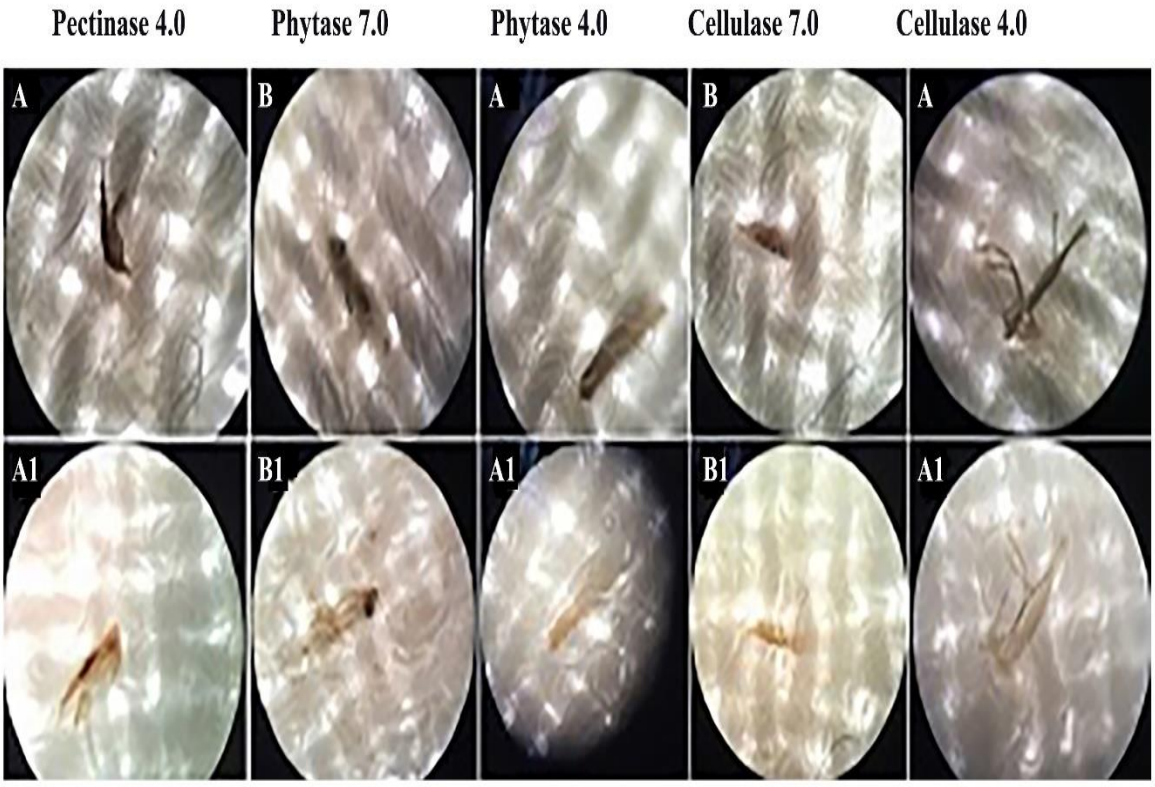

Figure 2. Light Microscope İmages Taken After Working with Different Enzyme Structures at and at Different pH on the Wool Fabric. A and B: Without Applying Enzyme (Control), A1: İncubation with Enzyme at pH 4.0, B1: İncubation with Enzyme at pH 7.0. 15 mL Crude Enzyme, 1 h İncubation at 37°C

The effect of treating the wool with the enzyme mixture was also examined. Since cellulase is an important enzyme in biocarbonization, in experiments with cellulase + pectinase and cellulase + phytase in the dual enzyme combination, cellulase + pectinase media had a greater effect on plant impurities (Figure 3). In the study made with a triple enzyme mixture, woolen fabrics were treated with cellulase + pectinase + phytase.

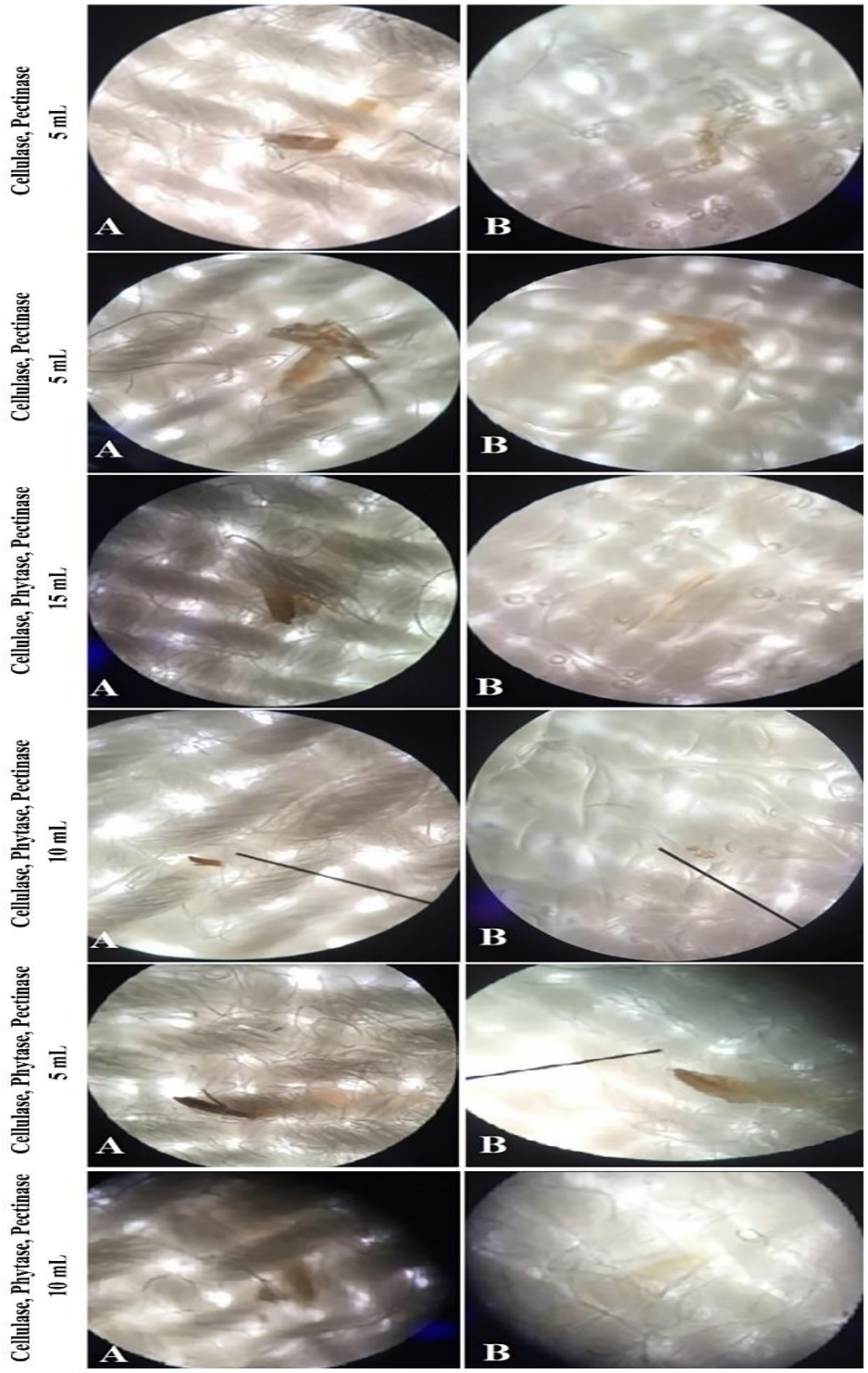

Figure 3. Light Microscope İmages of Wool Fabrics After Combined Enzyme Use on Plant İmpurities. A: Without Applying Enzyme (control), B: İncubation with Enzymes at pH 4.0, 37°C for 1h

When cellulase and phytase enzymes obtained from local isolates were concentrated by lyophilization and treated with commercial pectinase on raw wool fabric, the effect on plant impurities was better than in other experiments. This showed that the enzyme density was quite effective.

On the other hand, the amount of enzyme can also be effective. In the study for this purpose, in the case of using less amount of raw enzyme (5 mL), the enzyme had a small effect, but it was observed that the effect increased as the amount of enzyme increased (10 mL). Even more efficient

results were obtained in the amount of 15 mL enzyme used in the experiments. However, it was determined that it was better to apply the enzyme in concentrated form.

As a result of the studies, in all experiments, as seen in the controls, the colors of the plant impurities, which were dark in color, showed a clear opening, fragmentation and deformation with enzymatic treatment. The synergistic effect of the three enzymes mixture was found to be much more effective at removing cellulosic or plant impurities from raw wool than using an individual or dual enzyme system. Cellulase enzyme hydrolyzes cellulose and hemicellulose, pectinase enzyme hydrolyzes pectin, phytase enzyme hydrolyzes phytate. The effect of phytase enzyme on plant impurities was reported for the first time with this study. Enzyme treatment of woolen fabrics removes plant impurities from wool to varying degrees depending on the activity of the enzyme used.

On the other hand, it has been observed that enzymes break down small particles more easily. Therefore, the shape, thickness and size of the wastes are important. For thick and large wastes, a chemical treatment may need to be applied beforehand.

There are not enough studies in the literature on the removal of plant impurities from wool by using only enzymes. El-Sayed has been reported that a mixture of acid cellulase, acid pectinase and xylanase has been utilized for removing plant impurities from lightly contaminated Egyptian wool fleece (El-Sayed et al., 2010). On the other hand, the pretreatment of wool with an enzyme bath in the carbonization process has been reported to help reduce the sulfuric acid concentration from 6% to 1.5% (Sedelnik et al., 2003).

CONCLUSION

In this study, the effects on the removal of plant impurities by using enzymes were investigated and it was determined that the enzymes were effective alone, but the concentrated enzyme mixtures were more effective on plant impurities. Since the size and thickness of the plant impurities are very effective, the enzyme incubation period may need to be extended. It is thought that after 1 hour of enzymatic incubation, plant impurities are weakened by breaking down by enzymes, but weakened wastes can be removed as a result of mechanical rubbing. The potential power of enzymes will be revealed with detailed studies on this subject. Since enzymatic removal is new, there is not much study in the literature. This study will shed light on the scientific literature.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

Author E. Demirkan and D.Kut: The study was planned and designed.

Author E.Demirkan, D.Kut and N.Aladağ Tanik: Collected and analyzed data.

Author E.Demirkan, D.Kut and N.Aladağ Tanik: Wrote the article.

REFERENCES

Bahtiyari, M. I. & Duran, K. (2013). A Study on the usability of ultrasound in scouring of raw wool. *Journal of Cleaner Production*, 41, 283–290.

Barrett, A. J., Woessner, J. F., & Rawlings N. D. (Eds.). (2012). *Handbook of Proteolytic Enzymes*, (Vol. 1) Elsevier.

Cavaco-Paulo, A., & Gubitz, G. (Eds.). (2003). *Textile processing with enzymes* (Vol. 29). Woodhead publishing.

Chelikani, P., Fita, I., & Loewen, P. C. (2004). Diversity of structures and properties among catalases, *Cellular and Molecular Life Sciences*, 61 (2), 192–208.

Choi, J. M., Han, S. S. & Kim, H. S. (2015). Industrial applications of enzyme biocatalysis:current status and future aspects. *Biotechnology Advances*, 33, 1443–1454.

Choi, Y. M., Suh, H. J. & Kim, J. M. (2001). Purification and properties of extracellular phytase from *Bacillus* sp. KHU-10. *Journal of Protein Chemistry*, 20, 287–292.

Das, T. & Ramaswamy, G. N. (2006). Enzyme treatment of wool and specialty hair fibers. *Textile Research Journal*, 76(2), 126–133.

Demirkan, E., Sevgi, T., Akcakoca, D. & Ersoy, F. (2017). Partial purification, characterization and wheat bran degradation studies of a new phytase from the *Bacillus megaterium* EBD 9-1 strain. *Turkish Journal of Biochemistry*, 42, 329–337.

El-Sayed, H., El-Gabry, L. K. & Kantouch F. (2010). Effect of bio-carbonization of coarse wool on its dyeability. *Indian Journal of Fibre and Textile Research*, 35, 330–36.

Fersht, A. (2007), Enzyme structure and mechanism, San Francisco: Brenda, WH, *The comprehensive enzyme information system*, 50(2).

Fong, W., Yeiser, A. & Lundgren, H. (1951). A new method for raw-wool scouring and grease recovery. *Textile Research Journal*, 21, 540–555.

Garriga, M., Almaraz, M. & Marchiaro, A. (2017). Determination of reducing sugars in extracts of *Undaria pinnatifida* (harvey) algae by UV-visible spectrophotometry (DNS method). *Actas de Ingeniería*, 3, 173–179.

Gouveia, I. C., Fiadeiro, J. M. & Queiroz, J. A. (2008). Combined bio-carbonization and dyeing of wool: a possibility using cell wall-degrading enzymes and 1:1 metal–complex dyes. *Engineering in Life Sciences*, 8, 250–259.

Lowe, D. A. (2001). Production of Enzymes. In C. Ratledge & B. Kristionsen (Eds.), *Basic Biotechnology* (2 ed., pp. 679). Cambridge University Press.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426–428.

Mojsov, K. (2011, March 09-11) Application of enzymes in the textile industry: a review. *II International Congress Engineering/Ecology and Materials in the Processing Industry*, Jahorina, Bosnia and Hercegovina.

Msanki S. (2020). Screening of cellulase enzyme production by *Bacillus* sp. isolated from Turkish soils, nutritional optimization, partial purification of enzyme, characterization and effectiveness of plant waste removal in wool fabric. Master Thesis. Bursa Uludag University, Institute of Science, Department of Biology, Bursa

Rahman, M. & Nur, G. (2014). Feasible application of modern eco-friendly treatment of wool fabric before coloration. *International Journal of Science Research Publication*, 4(7), 1–7.

Seager, S. L., Slabaugh, M. R. & Hansen, M. S. (2018). *Chemistry for Today: General, Organic, and Biochemistry* (9 ed., pp. 886). Cengage Learning.

Sedelnik, N. (1993). BIOCARBO-Method of removal of plant impurities from the carded woollen textiles. *Textile Overview*, 12, 294–97.

Sedelnik, N. (2003), Biotechnology to improve the quality of wool. *Research Journal of Textile and Apparel*, 7(2), 1–10.

Sharma, N., Angural, S., Rana, M., Puri, N., Kondepudi, K. K. & Gupta, N. (2020). Phytase producing lactic acid bacteria: Cell factories for enhancing micronutrient bioavailability of phytate rich foods, *Trends in Food Science & Technology*, 96(2020), 1–12.

Sun, D. & Stylios, G. K. (2004). Effect of low temperature plasma treatment on the scouring and dyeing of natural fibers. *Textile Research Journal*, 74(9), 751–756.

Zheng, L., Du, B. & Wang L. (2012). Bio-scouring process optimization of wool fiber and wastewater utilization. *The Journal of Textile Institute*, 103(2), 159–165.