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# A Review on Environmental Pollution Mitigation by Fungal Proteases

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**_Abstract_**

*Proteases comprise a vital group of proteolytic enzymes with variety of functions. These enzymes possess the enormous commercial potential and have been used in several industrial processes, including food industry, leather processing, detergent industry and therapeutic applications. As well as they are used as an environmental pollution cleaning agent. Proteases are the replacement of conventional method of silver recovery from the X-ray films. Among the microbial enzymes only proteases constitute approximately 60% of the total industrial enzyme market and stand amongst the most precious commercial enzymes. Fungal proteases are more dominant for commercial application as these microorganisms are more resistant to harsh climatic circumstances and produces enzymes in their habitats. Proteases are classified according to their site of action, functional group present on active site, origin, and pH.*

***Keywords:*** *Fungal proteases, environmental pollution, proteolytic enzyme, therapeutic, food industry, leather processing*

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## INTRODUCTION

Environment pollution is the emerging greatest problem that the world is facing today and it growing each and every passing year and causing a major damage to the environment. Many factors are responsible for the environment pollution. These factors can be degraded by soil microorganism. Numerous substances with high polluting potential are present in the environment and affect soil, sediments, water, air, microorganisms, plants, animals, and humans. They may be distributed in one or all environmental compartments [1].

Microorganisms have invaluable potential in finding solution of many environmental problems. They play a dominant role in transforming pollutants that harmful to the environment. Microorganisms reveal an extremely extensive metabolic diversity and therefore they are able to degrade a wide variety of chemical compounds. However many chemical compounds are poisonous and inhibit the growth and activity of microorganisms [2].

Microorganisms are the most common sources of commercial enzymes due to their physiological and biochemical properties, facile culture conditions and ease of cell manipulation. Among microbial enzymes, proteases are the most important for the industry, and constitute approximately 60% of the total industrial enzyme market. These enzymes are used for food processing, pharmaceuticals, leather processing, and silver retrieval in the x-ray film industry, industrial waste treatment and as detergent additives [3]. These microbes release many useful enzymes in the soil and among them proteases are the most important and significant group that split the polypeptides into small peptides and then peptides into free amino acids. Among the microbial protease, fungal proteases have attracted the attention of the scientists because fungi can easily grow on low cost substrates and release highest amount of enzymes. In this review, the importance of proteases in control of environment pollution, their classification and production, causes of environment pollution, mode of action, factors which affect the activity of protease, application of protease and future of proteases will be discussed.

## CLASSIFICATION OF PROTEASES

Proteases are the significant group of the hydrolytic enzymes. They are also called proteolytic enzymes. Proteases can be classified in several categories on the following basis.

**On the Basis of Site of Action**

The proteolytic enzymes are subdivided into two major groups i.e. exopeptidases and endopeptidases, depending on their site of action. The exopeptidases act only near the ends of polypeptide chains. Based on their site of action at the N or C terminus, they are classified as aminopeptidases and carboxypeptidases. Endopeptidases cleave peptide bonds far-away from the terminal of the substrate [4, 5].

**On the Basis of Origin**

Based on their origin proteases can be classified in several categories like- microbial (bacterial, fungal and viral) proteases, plant proteases, animal and human proteases enzymes [5].

**On the Basis of pH**

pH is the most important factor for all enzymes to carry out their work efficiently. There are three categories of proteases based on pH optima.

***Acidic Proteases*****:** Acidic proteases work well on acidic condition (pH 2.0–6.0). They are commonly produced by *Aspergillus niger* [6, 4, 5].

***Neutral Proteases*****:** Such proteases work efficiently on neutral pH (6.0 to 8.0). Neutral proteases are produced by *Aspergillus carneus, Tricholoma columbetta, Fusarium culmorum,* etc. [6, 4, 5].

***Alkaline Proteases*****:** These proteases are needed alkaline condition (pH 8.0 to 13) to work proficiently [4, 5]. Alkaline proteases are produced by *Trichoderma harzianum, Aspergillus clavatus, A. fumigatus, Penicillium chrysogenum, Conidiobolus coronatus, Fusarium culmorum,* and *Cephalosporium* species [6].

**On the Basis of Functional Group Present on Active Site**

The endopeptidases are divided into four subgroups depending on their catalytic mechanism.

***Serine Proteases*****:** Serine proteases are the most extensively distributed group of proteolytic enzymes. The enzymes have a reactive serine residue in the active site [7]. These are generally active at neutral and alkaline pH, with an optimum pH between 7 and 11 [8].

***Cysteine Proteases*****:** Cysteine proteases are also well-known as Thiol proteases. Cysteine proteases contain a Cys–His–Asn triad at the active site. A histidine residue, presents in the active site act as proton donor and enhance the nucleophilicity of the cysteine residue [9].

***Aspartic Proteases*****:** These proteases are generally acidic proteases and show maximum activity at low pH (3.0–4.0) [5]. Aspartic acid proteases depend on aspartic acid residues for their catalytic activity.

***Metalloproteases*****:** Metalloproteases are characterized by the requirement for a divalent metal ion for their activity. These proteases are most diverse group of catalytic enzymes [10].

## PRODUCTION OF PROTEASES

Proteases production, especially in fungal protease, is very effortless due to high diversity, broad substrate specificity, and stability under extreme conditions; it offers an advantage of separation of mycelium by simple filtration. Fungal proteases can conveniently be produced in solid-state fermentation process [11, 4]. Proteases production carried out by solid state fermentation (SSF) and submerged fermentation (SMF) method. Both methods have advantages as well as some disadvantages. SSF is cost effective method over SMF. It requires less energy and gives more stability to the product because of a reduced amount of dilution in medium. Submerged fermentation posses many advantages like easy to recover product, mycelia and spores from the medium. It has a major disadvantage that the products or enzymes are less stable than of solid state fermentation because of much diluted medium. Filamentous fungi grow well in solid state fermentation because it provide solid surface for the growing mycelia. Less water content reduces the bacterial contamination problem [10]. Difficulties associated with the measurement of parameters in solid state fermentation such as microbial biomass, substrate consumption, concentration of product formed as well as the measurement of the physical properties of the system, for example, measurement of growth of the organism in solid culture is much more difficult than in liquid culture [12].

Proteases production is inducible and affected by the nature of the medium used in fermentation. Therefore, selection of appropriate medium has great importance [13, 14]. The ratio of C and N in medium greatly influenced the growth of fungi. Alongside some other factors such as pH, temperature, aeration, agitation, size of inoculum, age of inoculum and incubation time are also responsible for the affecting fungal growth [10]. In the industrial production of proteases, technical media usually employed that contained very high concentrations of carbohydrates, protein and other media components [8]. Proteases can be produced either intracellularly or extracellularly. Mostly microbial proteases produced as extracellularly. Intracellular proteases can be released by cell disruption method. Cell disruption can be achieved by both mechanical and non-mechanical methods. In mechanical method cell suspension are sheared at high rate. In non- mechanical method cell disruption can be accomplished by osmotic shock, autolysis, rupture with ice crystals (freezing/thawing) or heat shock. At the small-scale the microbial disruption can be achieved using various chemicals and enzymes. Following cell disruption, soluble proteins are usually separated from cell debris by centrifugation [15].

## CAUSES OF ENVIRONMENT POLLUTION

Now-a-days increasing population brings a lot of problems with them. People are totally depends on nature for their basic and all luxuriest requirements. They get all things from the nature but in return they are polluting them. That’s why our environment getting extremely polluted day-by-day.There are so many types of proteinaceous waste products are produced from different industries. Annually, great amounts of proteinaceous wastes, which could be measured in many billions of tons, are produced worldwide as residues from industrial processing. These waste products pollute the environment and work as a proteinaceous pollutants of environment. Proteinaceous waste products regularly generated from meat industries, leather industries, slaughter houses, oil refineries, dairy processing industries, egg and poultry industries and food industries [16, 17].

## MODE OF ACTION OF PROTEASES

Proteases have distinct properties to digest protein chains into shorter fragments by splitting the peptide bonds that link amino acid residues [16]. Some attack on the terminal amino acids of the protein chain called exopeptidases; others break internal peptide bonds of a protein called endopeptidases [4].The protease cleaves proteins by a hydrolysis reaction, an addition of a water molecule. Proteases usually activate a nucleophile, which is then attack the carbon of the peptide bond. The electrons in the carbon-oxygen double bond move onto the oxygen as the nucleophile attaches itself. This tetrahedral intermediate contains high energy and the proteases generally stabilize this intermediate. Then the intermediate decomposes into two parts and releasing the two peptide fragments [18]. On the basis of catalytic mechanism proteases are classified into four main classes i.e. serine proteases, cysteine proteases, aspartic proteases, and metalloproteases. These All four classes of proteases can use a different nucleophile or a different mode to activate the nucleophile. Serine and cysteine proteases use a catalytic triad to activate the side chain of either a serine or cysteine. Aspartic proteases use an aspartic acid residue to activate a water molecule and another aspartic acid residue to align the peptide for attack; while metalloproteases utilizes a metal ion to turn on a water molecule [18].

## FACTORS AFFECTING THE ACTIVITY OF PROTEASES

There are some key factors which influence the activity of proteases.

### Effect of Temperature

Temperature plays a crucial role in proteases activity. This is the major factor which affects the activity of proteases too.Enzyme activity increases as temperature increases, and in turn increases the rate of the reaction. A decrease in temperature caused a decrease in protease activity [19].

**Effect of pH**

This is another chief factor which strongly affects extracellular protease activities. Different types of proteases show optimum activity at different pH. At a culture pH of 4, acidic protease shows more activity than at pH 5 [20]. Alkaline proteases show more activity at pH 9.0–11 [4]. As well neutral proteases are more active between pH 7.0 and 7.5.

**Effect of C/N Ratio**

Protease activity increased with increasing concentrations of ammonium as nitrogen source [20] and also proteases activity increased if carbon sources provided with $Ca^{2+}$ [21].

## APPLICATION OF PROTEASES

Proteases are the group of novel enzymes and execute a large array of functions. Proteases have several commercial applications employed in food industries, leather, meat processing, cheese making, silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds and also in proteinaceous waste treatment. Some significant applications are mentioned below.

**Food Industries**

Proteases are very important to food industries because of their ability to act as catalysts and transforming raw materials into food products with improved quality. It is generally used in dairy industries to manufacture cheese. It is also used in baking and meat tenderization [4]. In baking process wheat flour is a significant ingredient. It contains an insoluble protein called gluten. Proteases modify wheat gluten to a lesser extent and give a better quality to dough, which is used to manufacture different types of bakery products [5].

**Textile Industries**

The application of proteases in textile industry is one of the most rapidly rising field in industrial enzymology. In traditional method cotton bleached to decolorize their natural pigments and give pure white appearance to the fibers. The hydrogen peroxide is common bleaching agent which is used in textile industries. This chemical agent affects the fibers quality with damaging them. Proteases are an alternative to this conventional method. Proteases bleach the fabrics without damaging them [22]. Proteases easily remove the oil, blood or other types of proteinaceous spots from the clothes. Therefore it is used as a detergent in laundry. In conventional method huge quantity of waste water generated after washing for removing the chemical agents but enzymatic method save the water [5].

**Leather Industries**

In leather industries chemicals are used to remove hair and other subcutaneous layer. These chemicals caused severe environmental pollution. Several studies have suggested that the enzymatic processing of leather not only solves environmental issues but also yield better quality leather [5]. Proteases have solved this problem because of its proteolytic activity it can easily degrade the hair made up by keratin protein. Now it’s simple and environmental friendly to remove hair and increase the quality of leather by mean of proteases [14].

**Medical and Pharmaceutical Industries**

Proteases play a vital role in medical field. Day-by-day proteases are increasingly used in treatment of various diseases like cancer, inflammation, cardiovascular disorders, necrotic wounds etc. [5]. Proteases have been studied as a substitute for the mechanical debridement (removal of dead skin) of burns [23]. It prevents blood coagulation and dissolves thrombus. Proteases have antimicrobial properties. Furthermore, proteases have ability to degrade keratinized skin which is used in preparation of vaccine for dermatophytosis therapy. It is also applicable in remove scar, regenerate epithelia, and faster healing processes [24].

**Waste Treatment**

Fungal proteases play a crucial role in waste treatment. They can degrade a variety of proteinic waste from the environment. Waste from poultry and slaughter houses like feathers and dead animal waste can be easily degraded by fungal proteases [4]. Proteases show best efficiency in silver recovery from X-ray films. X-ray films contain silver in gelatin layer. Burning of films to extract silver causing environmental pollution problem. Proteases hydrolyze the gelatin and recover silver without any environmental pollution issue [4, 5].

## FUTURE PROSPECTS

Since many decades proteases are used in different types of industries because of their specific way of action and ability to work on wide range of pH and temperature. Using chemicals for the cleaning of environmental pollution causes many toxic effects on nature but proteases are eco-friendly which never acts against the nature. Hence they attracted the attention towards their use. The use of proteases is going to replace the chemicals used for mitigation of environmental pollution [17]. These chemicals are polluting the environment too much. Now it's time to control pollution, so that our environment could be clean and our forthcoming generation can breathe in clean air. As the demand is for the cleaner and greener technology to preserve our mother earth for our descendant, the use of enzymes that can replace harmful chemicals are now very necessary. Enzymatic pollution mitigation is seen as one of the promising and sustainable alternatives to conventional method.

Use of protease will help in more complete digestion of animal feed leading to less animal waste. Waste treatment by proteases will be cost effective rather than chemical methods and it would be more feasible. Proteases will help in many industries to control the pollution. In silver recovery industries, generally silver recover by burning the x-ray films directly, which cause air pollution. With the help of proteases silver can be recovered in eco-friendly way from the film. By means of proteases it will be possible to manage slaughter and poultry waste easily [10]. In oil industries and refineries oil spilling is major problem which causes severe soil pollution and damage the soil quality. These types of soil pollution will be easily removed by use of proteases. That's why proteases are the leading enzymes with immense commercial potential and become more popular day-by-day and definitely it will change the world.

## CONCLUSION

Present review is primarily put emphasis on use of proteases in waste treatment and other related aspects. Now-a-days enzymatic technology finds a new way to reduce the use of chemicals and help to eliminate pollution from the earth. Among microbial enzymes, proteases are the most important for the industry, and constitute approximately 60% of the total industrial enzyme market. Proteases play a crucial role in textile, pharmaceutical, leather, food industries and in transforming pollutants that harmful to the environment. Fungal proteases have great potential to perform reaction in eco-friendly manner [25]. Therefore their applications in novel processes are likely to increase in the near future. Proteases are emerging in a big way in the field of waste management.

## REFERENCES

1. Rao MA et al. *Journal of Soil Science and Plant Nutrition.* 2010; 10(3): 333–353p.
2. Wainwright M. *An Introduction to Environmental Biotechnology*, Kluwer Academic Publisher 1999; New York.
3. Rodarte MP et al. *Acta Scientiarum. Agronomy (Online)* 2011; 33(3): 1–11p.
4. Veloorvalappil NJ et al. *Advances in Enzyme Research* 2013; 1(3): 39–51p.
5. Sawant R, Nagendran S. *World Journal of Pharmacy and Pharmaceutical Sciences.* 2014; 3(6): 568–579p.
6. Mikhailova R.V. Мікробіоëоãія і біотеõноëоãія 2011. 1(3). 47-61p.
7. Garcia-Carreno FL. et al. *Biochemical Education.* 1997; 25(3): 161–167p.
8. Ellaiah P et al. *Journal of Scientific and Industrial Research.* 2002; 61: 690–704p.
9. Verma S et al. *Front. Pharmacol.* 2016; 7: 107p. doi: 10.3389/fphar.2016.00107
10. De Souza PM. et al. *Brazilian Journal of Microbiology.* 2015; 46(2): 337–346p.
11. Vishwanatha KS. *Journal of Industrial Microbiology and Biotechnology.* 2010; 37(2): 129–138p.
12. Hesseltine CW. *Biotechnology and Bioengineering.*1972; 14(4): 517–532p.
13. Chinnasamy M et al. *Jordan Journal of Biological Sciences.* 2011; 4(3): 137–148p.
14. Srilakshmi J et al. *Journal of Pharmaceutical, Chemical and Biological Sciences.* 2014; 2(4): 218–234p.
15. Waites MJ et al. *Industrial Microbiology: An Introduction,* 1st Edn. Blackwell 2001, London, 109–123p.
16. Sharma PM. *Nature Environment and Pollution Technology.* 2010; 9(4): 707–711p.

17. Karigar CS, Rao SS. *Enzyme Research.* 2011; 1–9p.
18. Wikibooks. Structural Biochemistry/Enzyme Catalytic Mechanism/Proteases. https://en.wikibooks.org/wiki/Structural_Biochemistry/Enzyme_Catalytic_Mechanism/Proteases. Accessed 12 July 2012.
19. Klug C et al. *Berl Munch Tierarztl Wochenschr.*1998; 111(1): 9–12p.
20. Braaksma M et al. *Microbiology.* 2009;155: 3430–3439p.
21. Shivakumar S. *Archives of Applied Science Research.* 2012; 4(1): 188–199p.
22. Mojsov K. II International Congress. Engineering, Ecology and Materials in the Processing Industry. Goce Delčev University of Štip, Jahorina, Bosnia and Herzegovina, 9–11 March 2011.
23. Vellard M. *Current Opinion in Biotechnology.* 2003; 14: 444–450p.
24. Singh R et al. *Journal of Pharmaceutical, Chemical and Biological Sciences.* 2016; 4(3): 365–374p.
25. Binod P et al. *Journal of Scientific & Industrial Research.* 2013; 72: 271–286p.


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