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Industrial Applications of Microbial Protease: A Review

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Abstract

Protease enzymes are found in all of living organisms, which promote differentiation and growth of cell. They also have physiological functions in both of synthetic and degradation processes. Protease enzymes can be classified depending on different basis such as their pH optima, catalytic mechanism, active site, their evolutionary relationships and sequences of amino acids. In addition these enzymes can be classified depending on their site of action in the polypeptide chain to endopeptidases and exopeptidases. Alkaline protease is gained from many sources such as bacteria , insects and fungi, this type of protease is used in the industry of detergent and leather. The acid protease from microbial origin possess a potential role through industry of different feed and food. Acidic proteases also have been discovered in few bacteria. The neutral protease enzymes are also produced via the *Bacillus* genus and used in a variety of sectors. Since ancient times, microbial protease have been used in food fermentation process, now these enzymes are successfully used in various industries. Because of the increasing demands and uses, researchers are looking into numerous strategies to find, artificially manufacture or redesign enzymes having increased suitability through industrial procedures because they can withstand adverse circumstances. These enzymes not only have commercial and economic worth, but they also provide a safer solution for the environment. In addition to all of these uses scientists have successfully used proteases in the medical field on a large scale over time.

Keyword: Microbial proteases, Industrial applications, Protease in medical field, Protease classification

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التطبيقات الصناعية للبروتييز المايكروبي: مقال مراجعة

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الخلاصة

توجد إنزيمات البروتييز في جميع الكائنات الحية ، والتي تعزز تمايز الخلايا ونموها ولها أيضًا وظائف فسيولوجية مختلفة. تصنف إنزيمات البروتييز اعتمادًا على أسس مختلفة مثل درجة الحموضة المثالية وآلية التحفيز والموقع النشط وعلاقاتها التطورية وتسلسل الأحماض الأمينية .كما يمكن تصنيف هذه الإنزيمات اعتمادًا على موقع عملها في سلسلة متعدد الببتيد إلى endopeptidases و exopeptidases. يتم الحصول على البروتييز القلوي من مصادر متعددة مثل البكتيريا والحشرات والفطريات ، ويستخدم هذا النوع من البروتييز في صناعة المنظفات والجلود. يمتلك البروتييز الحمضي من أصل جرثومي دورًا مهما في صناعة الأعلاف والأغذية المختلفة. وبالنسبة لأنزيمات البروتييز المتعادلة تنتج من قبل جنس *Bacillus* وتستخدم في مجموعة متنوعة من القطاعات. منذ العصور القديمة ، تم استخدام البروتييز الميكروبي في عملية تخمير الطعام ، والآن يتم استخدام هذه الإنزيمات بنجاح في مختلف الصناعات. لا تتمتع هذه الإنزيمات بقيمة تجارية واقتصادية فحسب، ولكنها توفر أيضًا حلاً أكثر أمانًا للبيئة. وبالأضافة لكل هذه الاستخدامات نجح العلماء في استخدام البروتييز في المجال الطبي على نطاق واسع بمرور الوقت.

الكلمات المفتاحية: البروتييز المايكروبي، التطبيقات الصناعية للبروتييز، استخدام البروتييز في المجال الطبي، تصنيف البروتييز.

Introduction

Proteases is a class of enzymes that hydrolyze or break down proteins or peptides. Proteases attack and cleave the peptide bonds that connect residues of neighboring amino acid in a molecule of protein , given the short peptides and amino acids **[1]**. These enzymes do not only have degradative actions, but they also have the ability to synthesize. Proteases have been used to synthesize peptides for use in a variety of fields, including the industry of food, agriculture, medicine and others **[2]**.

These hydrolytic enzymes are found in all life things, including eukaryotes such as animals, plants, fungi, and protists, in addition to prokaryotic domains such as bacteria and archaea.

Furthermore, some viruses have been discovered that have the ability to encode their own proteases **[3]**. *Bacillus* sp. are the most active and dynamic extracellular alkaline protease producers in the industrial sector. Proteases are one of the three most important categories of industrial enzymes, and their global market is rapidly expanding. Proteases account about 20% of the 60 % of enzymes sold worldwide **[1]**.

Proteases are extremely important in the metabolism and physiology of all living beings. Apart from their vital role in protein and peptide digestion, these enzymes play an important role in regulating of a wide range of physiological activities by regulating various phases of protein activation–inactivation, signaling, protein synthesis, turnover of protein, and expression of gene **[4]**. Protease and inhibitors of protease make up more than 2% of all human genes. Proteases play a vital part not only in the body normal functioning and the up keep of homeostasis, but also in inflammation, infections, immunity, and development of disease **[5]**. There are different proteases uses in the medicine field **[4,6]**. Various cancer-related characteristics, such as uncontrolled growth, metastasis, immune evasion and angiogenesis have all been linked to abnormal protease activity. Proteases, on the other hand, have been discovered to play an important role in suppression of tumor. As a result, in the field of cancer management, proteases are used for predictive diagnostic and therapeutic purposes **[2,7]**

The industry of leather is reducing the use of laying and chemicals because of the utilization of proteolytic enzymes for performing various steps in leather processing **[10]**. The keratinous wastes which is produced via slaughter houses, poultries, etc. cause many problems such as water pollution, soil pollution, spreading of diseases and clogging of drains, as well as this type of wastes is hard to deal with **[11]**. Furthermore, the proteases have an important role in different industries, such as an additive to detergent and cleaning solution for contact lens. The industry of textile utilize the proteases for different purposes like silk degumming and wool biopolishing **[2,12,13]**.

Classification of protease

Protease include complex and large group of different enzymes, these enzymes are classified depending on their stability profile, temperature and pH optima, specificity to substrate, catalytic mechanism and active site. There are different ways used to classify protease enzymes, such as depending on the catalytic mechanisms these enzymes can be classified into metallo-proteases, cysteine proteases, aspartic proteases and serine proteases. Also protease can be classified into various families and clans basis on their evolutionary relationships and sequences of amino acids **[14]**. Protease enzymes can be classified as exopeptidases which destroy the N- or C- terminal bonds that belong to the peptide and endopeptidases which destroy the bonds found inside the peptide **[15]**. While depending on their activity at optimal pH, they are categorized into alkaline, neutral and acid protease **[16]**.

Microbial protease

Protease studied and discovered in early times, there are several proteases from plant origin like papain and protease, and from animal origin like trypsin and pepsin. These enzymes characterized and discovered in the early 1800s or 1900s **[4]**. Microbial proteases were the most studied enzymes since the beginning of the enzymology. These enzymes have drawn interest not only due to their crucial role for metabolic functions but also due to the fact that they are widely used in different industries **[1, 17]**. The proteases from bacterial and fungal origins have a big attention from the commercial view so many researchers focused on these enzymes. Two-third from total protease which is used in different industries are from microbial origin **[18]**. The protease from microbial origin have become a focus of interest because owing several advantages. These advantages include rapid rates of production, low cost in terms of time and space requirements, and it is also do not affect by climate change **[2, 19, 20]**. Proteases from microbial origin are divided into groups depend on their basic or acidic characteristics. Functional groups presence and peptide bond position are also used to classify them. Microbes produce abundant intracellular proteases that are important in turnover of protein, differentiation, regulation of hormone and pool of cellular protein. While the extracellular proteases consider important in hydrolysis of protein, as in photographic film processing, and enzymatic production based on preparation of detergent and solvent, thermal tolerance, specific substrate and zein hydrolysates production **[1, 21, 22]**. With respect to the commercial producing of proteases, the *Bacillus* genus possess very important place, while the proteases from fungal origin dominated via the genera *Penicillium*, *Aspergillus*, *Trichoderma* and others **[18]**. The microorganisms are found in diverse environments, consequently, it is simpler to look for microorganisms with enzymes that have advantageous properties. Because the shorter time of generation and simpler make-up of genetic material, the manipulation of genetic material is easier for microorganisms **[23]**.

As well as several microbes can grow on waste material or cheaper substrates, so the process become more economical. There are many projects accomplished on different proteases from microbes (Table 1) **[2]**.

Table:1 Various microbial proteases with their applications

Microorganism	Isolation source	Protease type	Practical application	Reference
Alcaligenes faecalis	Sediments of marine	Halophile organic solvents tolerant proteases	Deproteinization of the shell and shrimp residue	Maruthiah *et al*. [24]
Bacillus subtilis	Tannery location soil	Alkalic protease	Animal hide dehairing, detergent additive	Hussain *et al.* [25]
Halobacillus sp.	Hyper saline lakes	Solvents' stable thermo proteases	Detergent formulation and synthesis of peptide	Daoud *et al.* [26]
Aspergillus foetidus	Soils of Savannah	Acidic proteases	Food Industry	Souza *et al.* [27]
Exiguobacterium indicum, *B. subtilis*	Mixed soil poultry waste	Alkaline proteases	Dehairing of animal hide, eradication of gelatin from film of X-ray	Hakim *et al.* [28]
Bacillus halotolerans	Tunisia potatoes	Alkali proteases	Detergent additive	Dorra *et al.* [29]
Bacillus cereus	Mixed soil organic substance	Alkali proteases	Additive to detergent	Asha and Palaniswamy [30]
Bacillus tequilensis	Soils	Fibrinolytic proteases	Blood clot dissolution	Xin *et al*. [31]
Bacillus safensis	Oil location off-shore	Serine alkalic proteases	Additive to detergent	Rekik *et al.* [32]
Salipaludibacillus agaradhaerens	Soda lakes	Serine alkalic proteases	Additive to detergent	Ibrahim *et al*. [33]

Lactobacillus paracasei	Goat milk	Rennin like proteases	Milk clot	Putranto *et al*. [34]
Streptomyces sp.	Soils thermostable	Alkalic proteas	Degradation of keratine residue	Al-Dhabi *et al*. [35]
Bacillus atrophaeus	soil contaminate with hydrocarbon	Serine alkali proteases	Detergent additive	Rahem *et al*. [36]
Bacillus stearothermophilus	Mill sols of olive oil	Thermostable alkalic proteases	Additive to detergent	Karray *et al*. [37]

Alkaline protease

Alkaline protease is obtained from different sources such as bacteria , insects and fungi. Although most alkalophilic microbes produce alkaline protease, researchers are mostly interested in those that produce large amounts of the enzyme.Fermentation media must be optimized properly to achieve commercially successful production of enzyme. Various factors such as pH, carbon source , metal ions ,nitrogen source,inhibitors and temperature all have an impact on enzyme synthesis **[38]**. *Bacillus* genus have an important role in producing alkalic protease (EC.3.4.21-24.99), this type works under alkalic pH from 9 to 11. The producers of alkaline protease found in soil,water, and conditions which is highly alkaline.The industry of detergent consumes large amount of alkaline protease, which known as serine proteases and active at alkaline pH **[1,39]**. Another example of *Salinivibrio* sp. is strain AF2004 which secretes metallotype protease, this enzyme possess a wide level of pH between 5–10 and thermal tolerance. This strain is highly recommended because its halophilic and thermal characteristics **[40]**. There are several studies indicated that various mushrooms secrete alkaline protease **[1,41]**.

Acidic protease

Among the protease enzymes, the acid protease from microbial origin have extensively utilized in pharmaceuticals beverage and food, they possess potential role through industry of different feed and food like dairy industry (hydrolysis of whey and casein which is used in development of cheese flavor and milk protein) the industry of baking (flour treatment through the production of the baked goods, improvement of flavor, color and dough texture in cookies), the industry of wine, hydrolysis of soy protein and production of soy sauce **[13]**.

The acidic protease enzymes show its activity at the acidic range of pH, mostly at pH between 3.8 - 5.6 and optimum pH between 3–4 while the point of isoelectric between 3 - 4.5 and are member of the aspartic proteases family. Rennin- and pepsin-like enzymes are two sorts of the acidic proteases that are derived from microbes **[1,2]**. In contrast with alkaline protease enzymes, which are produced mostly via the bacteria, acid proteases are mostly produced by fungi, with the genera *Penicillium*, *Aspergillus*, *Mucor*, *Endothia* and others **[13]**. Molds and some yeasts, have been shown to synthesize novel acid proteases **[42]**. Acidic proteases also have been discovered in a few bacteria. Protease enzymes from *Lactobacillus plantarum* strain 1.13 and *Enterococcus faecium* strain 1.15, derived from the Bakasam (a kind of fermented meat), showed like rennin activity **[43,44]**.

Neutral proteases

The term "neutral proteases" refers to proteolytic enzymes that are active at neutral range of pH or mildly alkaline range or mildly acidic range and the maximum action located with pH 5-8. Similar to the alkaline protease enzymes the neutral protease enzymes are also produced via the *Bacillus* **[1,45]**. Many of neutral proteases are metalloproteases, which require the presence of the divalent positive charge ion in order to function **[2]**.

The neutral protease enzymes can be used in a variety of sectors, including baking and brewing **[1]**. Ao *et al*., **[46]** characterized a neutral protease of *Aspergillus oryzae* Y1 obtained from broad beans naturally fermented, finding it is a protein with 45 kD and the optimal pH is 7.0, while the optimal temperature is 55 °C. The majority of neutral protease enzymes belongs to *Bacillus* genus, these enzymes have poor thermotolerance range of pH 5 – 8. They produce less bitterness through the hydrolysis in food proteins, so these enzymes considered more useful in food industry **[1]**.

Microbial protease enzymes and their industrial applications

Protease is utilized widely in industry with numerous applications such as detergent, drinks, silver recovery, leather, baking, dairy and pharmaceuticals and many others.

Detergent manufacturing

Protease enzyme was already employed in the detergents since the late 1990s, and it is regarded as one of the biggest industries that uses protease. Protease enzymes are used in the industry of detergent, which comprise about twenty percent of worldwide enzyme sales and (30–40) % of worldwide enzyme income, and is expected to grow at a compound annual growth rate (CAGR) of roughly 15.5 % during 2020 – 2025 **[1]**. Protease breaks down foods like fish, beef, egg, milk as well as blood, stains, biological secretions and improves quality of the detergent with agitation time and less soaking.

The protease enzymes employed as additives to detergent which added in tiny concentrations due to their ionic capabilities, over a wide range of temperature and pH also possess a longer life span as well as better detergent performance **[19]**. Recently, Serene Alkaline Protease is being utilized in the waste management processes of numerous food industries and home tasks. The protein-based remains are a prominent contaminant through food bioprocessing industries like milk and meat processing **[47]**. Enzymatic substitutes used in various cleaning procedures save cleaning costs also save for environment and significantly extending the lifespan of equipment. The attractive aspect of enzymes that is used for cleansing processes is that they degrade naturally, also they don't harm the environment once they have achieved their goal. Unlike other remediation methods, there is no accumulating of pollutants or biomass which must be eliminated. However, the disadvantage of utilizing protease enzymes for bioremediation is their high cost **[48]**. *Bacillus pumilus* MP 27 was shown to possess an alkaline protease. Under a wide range of temperature and pH, the enzyme remains stable. In pH 11, it preserved 70% from its action, whereas at pH 9, it retained 50% from its action. Commercial detergents were very compatible with it. It was compatible with Triton X 100 in 80% of cases and Tide brand detergents in 100% of cases. At 4°C and 50°C , the destaining performance was excellent. As a result, the above alkaline protease can be added to commercial detergents as a stain remover in washes that use cold water **[50]**. *Bacillus licheniformis* NH1 produced alkali serine protease. The protease appeared active at pH range 7–12 and optimal pH range is 10–11. After 60 minutes at 40 °C incubation using 7mg/mL from new Dex, Dixan and Axion, the enzyme maintained 90% from initial activity **[51]**. These characteristics of proteases show they are appropriate additive in the industry of detergent **[52]**. However, caution is needed as a result of respiratory sensitivity from inhalation when handling concentrated detergents enzymes **[15]**.

The industry of leather

Protease enzymes are utilize for break down the non collagenous materials of the skin and non fibrillary proteins are removed. The use of proteases through leather industry improves the leather quality, making it stronger and softer with fewer spots. The utilize of enzymes through the production of leather helps increased the quality of the product while minimizing pollution. Due to its keratinolytic and elastolytic characteristics, the use of alkali protease is increasing in the expanding leather industries. The influencing features of alkaline protease are very helpful in the leather industries. It was discovered that protease is helpful throughout the hair removal, bating, and soaking stages of preparation for hair and skin **[2,53]**.

Chemicals like lime and sodium sulfide were used in leather preparation in the past. These chemicals are both expensive and dangerous. Because of the issues with wastewater disposal, the use of these compounds is not considered environmentally beneficial. Another strategy is the utilizing of enzymes that have increased the quality of leather and reduced pollution. Earlier, a wide range of enzymes were utilized through the industry of leather, but they were all unsuccessful. Protease enzymes produced from specific bacterial species have been shown to be effective in the eradication of hair **[54]**. Removing the hair by enzymatic method causes no damage to the surface, and leather with high-quality could be recycled requiring no significant chemical reduction. In the leather industry, microbial alkaline proteases are becoming very popular. The use of enzymes in the processing of leather could aid in the development of environmentally friendly processes. Given the high dangers associated with traditional processing of leather, there is a strong focus on creating safer and cleaner enzymatic techniques **[55,56]**. Microbial alkali protease speeds up the hair removal phase due to the swell of the hair roots then subsequent invade the follicle protein of hair via proteases. Batting removes undesirable inter-fibrillary proteins, resulting in leather that is softer, silkier, and more flexible. Alkaline proteases obtained from various microbiological sources have been found to be efficient bating agents. The fundamental goal of the bating stage is to promote separation of collagen via destroying keratin protein, providing surface that tanning agents can interact with **[15]**.

Management of waste

Proteases are enzymes that are used to break down several types of garbage, including hazardous waste, liquid and solid. Proteases also help with degradation of waste via reducing waste into small simple molecules which can be used for metabolic activities via other organisms, reducing the amount of biological oxygen needed in aquatic environments. Genex, is a hydrolytic enzymes preparation obtained from the *Streptomyces* sp. , *Bacillus amyloliquefaciens* and *Bacillus subtilis*, utilize for cleaning and depilation of hairs that found in clogged pipes and drains **[48]**. Another study mentioned the possibility for keratin decomposition derived from feathers to be used in the formulation of hair shampoos **[49]**.

Recently, alkalic protease enzyme will be utilize through the waste management for several food industries and household activity. Protein wastes are a prominent contaminant in industries of food bioprocessing like processing of meat and milk. Different cleaning processes utilizing enzymatic solutions reduce cleaning costs and any environmental risks while also extending the life of equipment. A commercially available product that includes proteolytic enzymes from *Streptomyces sp*., *B. amyloliquefaciens* and *B. subtilis*, in addition to disulfide reducing factor, thioglycolate , helps in the clogged pipes clearing **[2,48]**.

Dairy industry

For the manufacturing of, yogurt, cheese, kefir, and other fermented dairy products, microbial cultures with a broad enzyme spectrum, particularly possessing proteolytic activity, frequently are utilized in dairy sector. Proteolytic enzymes are known to be employed in the production of cheese to coagulate milk proteins in addition to hydrolyzing proteins. Using proteolytic enzymes, several protein hydrolysates are produced from milk. Dairy products that are simple to digest are made for children and the ill. Exogenous proteolytic enzymes are crucial for the formation of cheese **[1,57]**. Chymosin may be substituted by some microbial proteases produced by lactic acid bacteria (LAB) when making cheese **[58]**. Because *Enterococcus feacalis* can produce active protease, there is a chance that it could be used to contribute in the digestion and whey protein generation of bioactive peptides **[57]**. As an excellent candidate for the production of dairy products that are hypoallergenic, LAB *(E. faecalis* VB43) also produces enzymes that can hydrolyze allergenic proteins in milk **[58,60]**.

Meat industry

Partial breakdown of muscle proteins, disulfide bond breakage, when reacting to carboxyl, hydroxyl, sulfhydryl and amino groups all take place under the impact of proteases in meat. Sarcoplasmic proteins undergo substantial hydrolysis during this process, while myofibrillar and connective tissue proteins may also undergo partial hydrolysis based on the specificity of the enzymes. More meat is made available to proteolytic digestion enzymes as the meat becomes more tender, its level of hydration rises, and its protein digestibility rises. These procedures increase the amount of free and solubilized amino acids as well as smaller peptides, that enhances meat flavor. The maturation period of fragility is accelerated and also improves. Proteolytic enzymes help to improve flavor and act as a defense against a variety of harmful microorganisms. They have an impact on the ideal meat texture, flavor, and color, as well as a superior sausage cut **[61]**. Proteases added to meat may prevent lipid oxidation **[60,62]**.

Bakery industry

Proteolytic enzymes are utilized to make bread and pastries. Sour wheat dough's rheology and the bread's texture are impacted by the LAB enzymes' contribution to the breakdown of the gluten protein **[63]**.

In the baking sector, *Lactobacillus sanfranciscensis* is a fascinating species, particularly for creation of cakes made with wheat. This is due to the strain's distinctive characteristics, which include its high saccharide utilization efficiency, production of antimicrobial compounds, high proteolytic ability and acidifying activity **[60,64]**.

Brewing industry

Proteinases are being used in the brewing industry to break down proteins and keep beer from clouding up after cooling. It is important to note that native proteases are active during the malting of grains, often barley. These enzymes help the brew become clearer and have a higher nutritional importance for being a substrate for the growth of yeast also a finished food component. The homogenous protein distribution is undesirable since the flavor of the foamy beer is lost and it is rendered "empty". However, improper distribution of protein results in filtration being more challenging and contributes to beer becoming hazy when in storage **[60,65]**.

Food Additives

When *Xanthomonas campestris* produces xanthan gum, proteases can be utilized to remove the cell mass **[58]**. Food concentrates, soy sauces and protein hydrolysates, are all made with the help of proteolytic enzymes **[58][66]**. Numerous lactic acid bacteria have demonstrated probiotic-synthetic activity, benefiting customers' health in the process. Live bacteria known as probiotics are found in food in small amounts and, when taken, help the host's health by enhancing the flora in their intestines. One effective way to control the proteolytic activity of a certain food product may be the use of these bacteria as food additives **[60,67]**.

Feed Industry

For the natural disposal of fibrous animal protein (keratin) from nails, hair, horns and feathers, keratinolytic peptidase may be utilized. The goods acquired in this manner could be added to animal feed **[68]**. Using proteolytic enzymes, eggs can be converted into incredibly nutritious animal feed **[69]**. In the amino acid studies, proteolytic enzymes shown non-specificity assessed in multiple research resulting in enhanced animal feed digestibility **[68]**. In conclusion, the need for high income in animal production has led to a widespread development of different preparations for animal nutrition. Because of their strong probiotic activity and desirable proteolytic capabilities, lactic acid bacteria(LAB) are likely to be used more frequently in agricultural, industrial and pharmaceutical applications **[60]**.

Medical field

In the medical sector, proteases exhibit promises therapeutic qualities in the form of non-woven tissues, gauze, and ointment formulation. Alkali proteases are administered orally to help detect particular lytic enzyme deficiency diseases **[70]**. The usage of the fibrinolytic enzyme indicated that it will be used in thrombolytic therapy and as an anticancer medication in the future **[71]**. Bandages that are stabilized with a preparation of elastoterase enzyme is used to treat a variety of illnesses, including wounds, burns, furuncles, and carbuncles.

Proteases have unique therapeutic capabilities that are useful in creating a variety of medications against deadly diseases, such as those that fight cancer, bacteria, inflammatory infections, and dissolve blood clots, among many other uses **[41]**. Collagenase and *Escherichia coli* asparaginase enzyme both significantly contribute to the asparagine elimination from blood in different types of lymphocytic leukemia, wounds and burns **[72]**.

Contact lens cleansing

Proteases play a significant role in biomedical goods such as contact-lens enzyme cleaner. It is commonly recognized that proteins, lipids, and mucin are the important ingredients of tears found in the sediments on contact lenses. Our natural tears has a protein which interacts with contact lenses to generate protein sediments, which are natural residues that cannot be avoided. Pancreatin, trypsin, and chemotrypsin are examples of animal and plant proteases that have typically been used to make contact lens cleaning solutions, although most of them leave a bad smell in the cleansing solution or eventually develop one **[73]**.

Numerous microorganisms proteases of *Streptomyces* sp., *Aspergillus* sp. and *Bacillus* sp. were mentioned for washing tear films and foreign objects of the contact lenses. To resolve these disadvantages and to make the cleaning component safe and without foul smell, nor generating allergic reactions or irritation in the eyes, bacterial proteases are becoming more important **[74]**.

Conclusions

The protease enzymes are a wide family of enzymes which catalyzes a broad range of biochemical reactions. The value for these enzymes is generated from their importance in many industries and the medical sector. With the use of low-cost raw materials and the incorporation of genetic modification, the utilization and manufacturing are rising globally. Currently there is an exigent need for the utilization of such technologies that promise cleaner production as a substitute for the usage of potentially dangerous compounds.

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