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PRODUCTION AND APPLICATIONS OF PECTINASES: A REVIEW

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ABSTRACT: Pectinases are the pectinolytic enzymes that constitute an important component of the plant cell wall and of wide use with their target as pectins. Pectinases cover three different enzymes: Pectin-esterases (PE) act upon ester bonds in pectin breaking them into simpler forms, Polygalacturonases (PG) target the bonds between the galacturonosyl residues, and Pectate lyases (PL)target the alpha 1,4 glycosidic bond. These enzymes can be biosynthesized with the help of various microbes, including bacteria and fungi, using biological waste as a source of nutrients, carbon, and energy. These are commercially important because of their extensive use in the food and textile industry, clarification of beverages, maceration of plant tissue, treatment of wastewater, pulp liquefaction, textile industry and plant fiber processing, coffee, tea and oil extraction, de-gumming plant fibers, and improvement of chromaticity and stability of red wines. This review mainly aims at the pectinase's, their types, mechanism of action, and the substrates on which they act. It also gives a broad view of the applications of these enzymes in the industrial sector.

INTRODUCTION: Pectinases are enzymes involved in the hydrolysis of pectin. Pectin, the constituent of primary cell wall of terrestrial plants, was first isolated and described by Henri Braconnot in 1825. It is a high molecular weight heteropolysaccharide rich in arabinose, galactose, and galacturonic acid with a rhamnogalacturonan backbone. It forms a jelly-like substance in the cell wall and exists as calcium and magnesium pectate. In addition to the plant cell wall, it is also present in fruits in very high concentrations.

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During ripening, pectinase breaks pectin into simple sugars, which results in the sweetening and loosening of fruits. There are different types of pectin like protopectin, pectinic acid, gelatinous acid, and pectic acid. Pectinases is a broad term that covers three different enzymes: Pectin-esterases (PE) act upon ester bonds in pectin breaking them into simpler forms, Polygalacturonases (PG) target the bonds between the galacturonosyl residues, and Pectate lyases (PL) target the alpha glycosidic bond. Pectinases are classified as exo and endo polygalacturonases depending on the position of bond cleavage.

Exo-polygalacturonases (EC 3.2.1.67) cleaves simple sugars from the non-reducing end while endo-polygala cturonases (EC 3.2.1.15) break inner glycosidic bonds [1]. **Fig. 1** gives a view of classification of pectinases [1, 4].

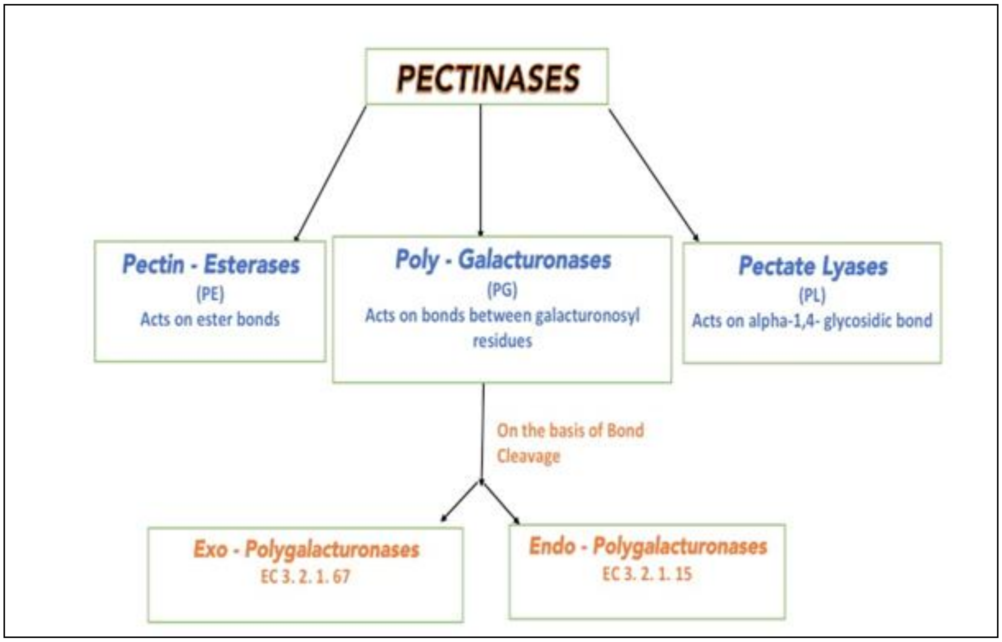

FIG. 1: CLASSIFICATION OF PECTINASES

Pectinase is also used in the pharmaceutical industry as well as many other biotechnological industries. Pectinase enzyme has adjustable catalytic activity, and it is non-toxic to humans. It can be easily produced by a large number of techniques at a cheap rate with good quality of yield. Microorganisms and plant material are the rich sources of pectinase production [2]. Microbial sources account for a total 25% of enzyme usage of pectinases for food processing worldwide [3].

Among fungi, many species of genus *Aspergillus* (sp. including awamori, niger, oryzae) and of genus Penicillium (sp. restrictum, expansum) are employed for high and efficient yield. Others like Mucor and Yarrowia are also used. The most common bacteria employed for the purpose involves *Bacillus*, *Lactobacillus*, *Aeromonas*, etc.

Yeasts like Saccharomyces, Candida, and Actinomycetes etcare also used for industrial production of pectinases. The microbes produce these enzymes through fermentation. Depending upon the state of substrate used, it can be either called submerged state fermentation (SmF) or solid-state fermentation (SSF).SSF uses various solid substrates as carbon and energy sources for the process of fermentation; however, the process uses a very little amount of moisture content/water. Various solid substrates employed for the process may include wheat, dried fruit and vegetable peels, soya beans, rice, corn, tubers, legumes, low-grade ores, *etc*. It provides high volumetric productivity and generates less effluent. In submerged fermentation, both the microorganisms and nutrients are submerged in water. The nutrient supply for growth is provided via liquid substrate, which may be a nutrient broth, alcohol or oils, etc. All the bioactive compounds are synthesized in the liquid medium itself and are regularly harvested from it. This technique is highly beneficial for the growth of those microorganisms which require high moisture content.

This review mainly describes the types and importance of pectinase, their types, mechanism of action and the substrates on which they act and applications of pectinases in the industrial sector. Substrate and pectinolytic microorganisms Most industries and laboratories exploit a large number of microorganisms for the production of enzymes in accordance with their objectives.

Bacteria, fungi, and actinomycetes have ability to produce a large amount of enzymes. 85% of pectinase are produced from microorganisms, and remaining 15% are from plants. *Aspergillus niger* is commonly used. Microbial pectinase accounts for 25% of global food and industrial enzyme sales and market [4]. All these microorganisms have been used for the industrial production of enzymes as per safety guidelines. **Table 1**. indicates the type of microorganisms used for the industrial production of pectinase and their importance.

TABLE 1: TYPE AND CONTRIBUTION OF DIFFERENT MICROORGANISMS IN INDUSTRIAL PRODUCTION OF PECTINASES

Microorganisms	Contribution
Bacteria	Produce 35% of Pectinase enzyme. (Easy reproduction and typically unicellular)
Fungus	Produce 35% of pectinase. (Fermentation is easily feasible)
Algae	Not used because photosynthesis are required
Protozoa	Not used because motile and mostly predators
Virus	Not used because the majority of them are infectious agents

Substrate plays a very important role in the production of enzymes because the raw material that contains good quality pectin will produce a large amount of pectinase enzyme. Pectic substances in fruits and vegetables are natural substrates for pectinases. Keeping a concern towards environment, many industries and laboratories are trying to use various types of waste material from fruits, and vegetable like peels of banana, apple, pomegranate, orange, lemon, pomace, potato, *etc*. as well as they involve some other kitchen waste like wheat bran, so they are able to produce a good amount of enzymes at reasonable prices. To improve the yield of microbial pectinase, researchers have constantly been working on different strategies; studies using different strains of the same microbe, varying conditions of fermentation, or modifying the nutritional parameters of the culture media have been reported.

In solid-state fermentation, wastes from the food and agriculture industry were widely used for *Aspergillus* pectin lytic enzyme production [5, 6]. These conditions yielded homogenous preparation of the enzyme and three different acidic pectate lyases were identified from MUG16 strain of *A. niger*. One study reported three times more activity of polygalactouronase (6.6U/mL) when bhimkol banana peel was used as a substrate for crude pectinase production from *A. niger* followed by its purification by ethanol. The highest clarification was achieved when raw banana juice was incubated for 60 min with a 2% concentration of partially purified pectinase [7].

Fungi serve to be one of the most common sources for isolation and production of pectinases. Sudeep *et al.* successfully produced and characterized pectinase enzymes isolated from various fungal strains and concluded *Aspergillus* species to be associated with the highest pectinolytic activity. The two most common agar systems generally employed for screening pectinase-producing fungi include Potato Dextrose Agar (PDA) and Pectinase Screening Agar Medium (PSAM) [8].

Aspergillus niger is used for producing low-cost pectinase enzymes for industrial purposes. Media and time are the most important factors for increasing production rate. Pectinase is a heterogeneous group of enzymes that hydrolyze pectin [9]. The pectic substance is mainly found in the middle lamella and primary plant cell wall. Pectinases can be obtained from various strains of bacteria and filamentous fungi. *A. niger* is used to obtain pectin from waste material with flask fermentation and bioreactor cultivation. Pectin is the most important factor that affects the amount of pectinases produced, and many other factors are also responsible, and they are measured with Pareto chart of standard effects. Bioreactor cultivation should be done with various control methods. This method of obtaining pectinases is highly useful for industries [10].

Different microbial strains from soil have been isolated based on pectin hydrolysis, identified microscopically, and screened for pectinase production by SSF. The highest pectinase production was shown by *Aspergillus niger* IBT-7. Xylose (1.5%) best supplement carbon source and yeast extract (1%) best nitrogen source, gave the highest pectinase production (39.1U/ml/min) [11]. Though plant and fungal pectinases are well known and employed, many bacterial species are also known to be extensively targeted as a source of pectinase enzyme extraction. Karabi Roy and companions have documented a novel bacterium Chryseo bacterium indologenes Strain SD as a vital source for the production of extracellular bacterial pectinases. The team has been successful in isolating this bacterium from soil samples as a novel pectinase-producing bacterial strain [12]. Polygala cturonase, pectin lyase, pectate lyase, and pectinesterase are the most complex pectinase enzyme. The solid-state fermentation method is more efficient for the production of the pectinase enzyme. For producing low-cost pectinase enzyme, filamentous fungi should be used over bacteria or any other source [13]. *Bacillus substilis* SAV-21 helps in cost-effective production of pectinase and pectin lyase using agro residues. Pectinase and pectin lyase are pectin degrading enzymes that were produced in solid-state fermentation by Bacillus substilus SAV-21 was identified by 16s rDNA sequencing. Maximum production of pectinase and pectinlyase were 3315u/gds and 10.5u/gds, respectively [14]. Immobilised *Geotridum candidum* AA15 can be used to reduce the high cost of pectinase production and can produce a greater yield of pectinase production (0.115 IU mL1 of pectinase for up to 6 production cycle in citrus pectin) as compared to free cell (0.046 IU mL1 of pectinase). Immobilization in combination with naturally available waste biomass can be used for pectinase production [15].

TABLE 2: PRODUCTION OF ALKALOPHILIC POLYGALA CTURONASES

Micro-organism	pH	Pgase activity*
Bacillus sp.	10	23076.0U/g
Bacillus sp.	8	8050.0U/g
Streptomyces sp.	8	4857.0U/g
Bacillus gibsonii	10.5	3600.0U/g
Bacillus sp	10	360.0U/mL
Streptomyces sp.	8	76.0U/mL
Bacillus sp.	10	6.0U/mL

Applications of Pectinase: Pectinase enzyme is a most significant enzyme in various industries. It is mainly obtained from microorganisms as they produce a very good yield. It is used in industries like the textile industry, paper industries and vegetables, and fruits processing industries, paper and pulp industries, wine industries, animal feed industries, tea and coffee industries *etc*. There are many more industries that are going to be dependent on pectinase enzyme production for various uses in the future. It is used in industries for avoiding chemicals that may or may not produce some harmful effects on the environment, humans, flora and fauna [16].

Processing of Animal Feed: Use of pectinases is very common in the production of ruminant feed as their enzymatic action releases nutrients resulting in their increased absorption and decreased amount of faeces [3, 5]. Improved fiber digestion was shown to be associated with increased digestible energy intake [3] and body weight gain, ultimately giving a better animal performance.

Inclusion of different types of enzymes like pectinases, cellulases, xylanases *etc*. in ruminants, diet has become a significant and economic tool for improving the digestive process in the ruminants [17]. In this study, pectinase was produced by *Aspergillus* terreus using beet pulp as a cheap substrate under the optimum fermentation conditions, and the method was very economical. The pectinase produced in the laboratory had many benefits over the commercial enzyme as it not only helped to overcome the feed gap in an economically viable and environmentally friendly way but also showed improvement of the feeding value of dairy animal's diets rich in pectin [18]. Nowadays, agricultural byproducts are the major source of pollution. Nonetheless, these are the rich source of pectin also. Supplementing agricultural byproducts with pectinases provide a highly nutritious feed for ruminants. This feed improved the production of milk and its fatty acids content. This method also helps in reduction of pollution [18].

Tea and Coffee Processing: Pectinase enzyme is used in each and every field. It is used in tea and coffee industries, bioleaching of pulp, treatment of wastewater, clarification of fruit juice, and extraction of oils. It is not toxic to the environment and humans [19].

Treatment of tea leaves with pectinase enzyme speeds up the fermentation of tea and reduces the foam-forming property of instant tea powders by breaking up the pectin present in the cell wall of tea leaves [3]. Application of pectinase, xylanase, and cellulase isolated from yeast Pichia sp NRRL Y-4810 and Zygosaccharomyces sp. NRRL B-2357 increased the black tea components over conventional treatment [3].

Pectinases are used in the coffee fermentation to remove the mucilaginous coat from the coffee beans, enhancing its quality. To make this process cost effective, waste mucilage was used as a source of microbial pectinases for the processing of coffee beans [20]. Pectinase from *Bacillus subtilis* strain Btk 27 have potential in the removal of mucilage from coffee bean and has a property of alkaline and thermophilic. The complete removal of mucilage from coffee beans within 20 h of incubation was observed. The process was efficient as fermentation time as well as loss of quality of coffee beans was significantly reduced as compared to traditional coffee processing [21].

Saccharification of Agricultural Substrates: Pectinase, hemicellulases, and cellulases present in the plant cell wall are involved in simplifying sugar. Simple sugars are used as fermentable sugars and converted into bioethanol. There are many industries that are dependent on the pectinase enzyme for their products and it leads to an increase in the demand for the enzymes. But day by day, increasing demand leads to choose some alternative sources for the production of enzymes like agro-industrial residues. *B. safensis* M35 and B. altitudinis J208 b strains of bacteria are used for the production of pectinases enzyme by submerged fermentation. It produces a large and good amount of pectinase enzyme for industrial purposes [22].

Bio Bleaching of Kraft Pulp: Paper and pulp industries have increased the use of various enzymes like pectinase, xylanases, ligninases and mannanase in the biobleaching process. Bio bleaching of mixed hard wood and bamboo kraft pulps involve pectinase enzymes with combination of other enzymes which are easily produced from microorganisms. Using the enzymes in the bio bleaching process lead to the less use of bleaching chemicals for attaining the brightness of pulp; this also enhances the physical quality of paper sheet. It also reduces the amount of organochlorine released in the effluent as compared to the conventional method. During sheet formation from pulp, dewatering occurs due to high cationic demand during peroxide bleaching that causes yellowness of paper. As pectinases depolymerize polygala cturonic acids, it decreases the cationic demand and improves the quality of the paper. Kappa numbers are also reduced by a combination of xylanase and pectinase produced from *Bacillus pumilus* and *Bacillus subtilis*, respectively 23 as 20% less chlorine is consumed [24]. Depolymerization of xylan and pectin present in the pulp fibre by xylanase and pectinase together reduces viscosity of the pulp and allows easy access of bleaching chemicals [25].

Recycling of Waste Paper: Recycling of waste paper involves a process in which paper is deinked, resulting in the release of many chemicals in the environment. The use of enzymes like pectinases, hemicellulases, and lignolytic enzymes produces less pollution, better quality of product with the desired deinked paper and saves a large amount of energy. It has less disposal issues than other methods. Enzymatic deinking alters bonds near the ink particle and removes the ink from the fibre surface. The released ink is then removed by washing or floatation. A combination of xylanase and pectinase has been used for deinking school waste paper. BOD and COD values in deinking are reduced by the use of enzymes, thus lower the cost of wastewater treatment in an environment-friendly manner [25].

Paper Making: Nowadays enzymes are used in the paper and pulp industries for manufacturing activities. Pectinase is used in papermaking [23]. Conventional paper industries produce a diluted waste containing fiber fragments and inorganic particles so there is need for proper water drainage. Retention aids like cationic polymers are added to pulp in modern papermaking and to speed the drainage of water. Polymers of galacturonic acid are depolymerized by pectinases and subsequently lower the cationic demand of pectin solutions and the filtrate from peroxide bleaching [23].

Waste Water Treatment: Water pollution is a great problem these days because a large number of effluents are discharged into water bodies. Conventional methods are costly and time-consuming. The best alternative source of sewage water treatment is the pretreatment of water with pectinase enzymes. Erwinia carotovora (FERM P-7576) secretes endo-pectate lyase, has been reported to be useful in the pretreatment of pectinaceous waste water. However, indirect pretreatment by the pectolytic enzyme has also been compared and reported to solubilize almost all the pectic substances contained in the wastewater

[26]. Microbial pectinases obtained from alkalophilic microorganism degraded pectins present in the effluents of citrus processing industries, which go in wastewater [27].

Wine Production: There are many enzymes that are helpful in the wine-making process; amongst these, pectinases have important roles in the winemaking industry. Pectinases facilitate filtration, help the extraction process, increase juice yield and accentuate the flavour and colour. Wines that are enzymatically treated showed reduced filtration time and were more stable as compared to control wines. Treatment of macerated fruits with pectinases before the addition of inoculum resulted in progressed characteristics of wine (colour and turbidity) as compared to the untreated wines. Pectinases stabilize wine colour by growing phenolic compound that is secondary metabolites. Enzymatically treated red wines are considered better than control wines. The maceration and viscosity are reduced to increase the growth of press juice. Boss stated a decrease in n-propanol concentration and an increase in 2-phenyl ethanol and isoamyl alcohol when the fermented grapes were pre-treated with pectin lytic enzyme. The yield of juice was increased after treating with pectinase. The activity of pectinase esterase in the winemaking process results in an increased level of methanol; however, its concentration should be regulated because of its poisonous nature, which is why in a commercial mixture, the level of pectin esterase should be of low concentration [23].

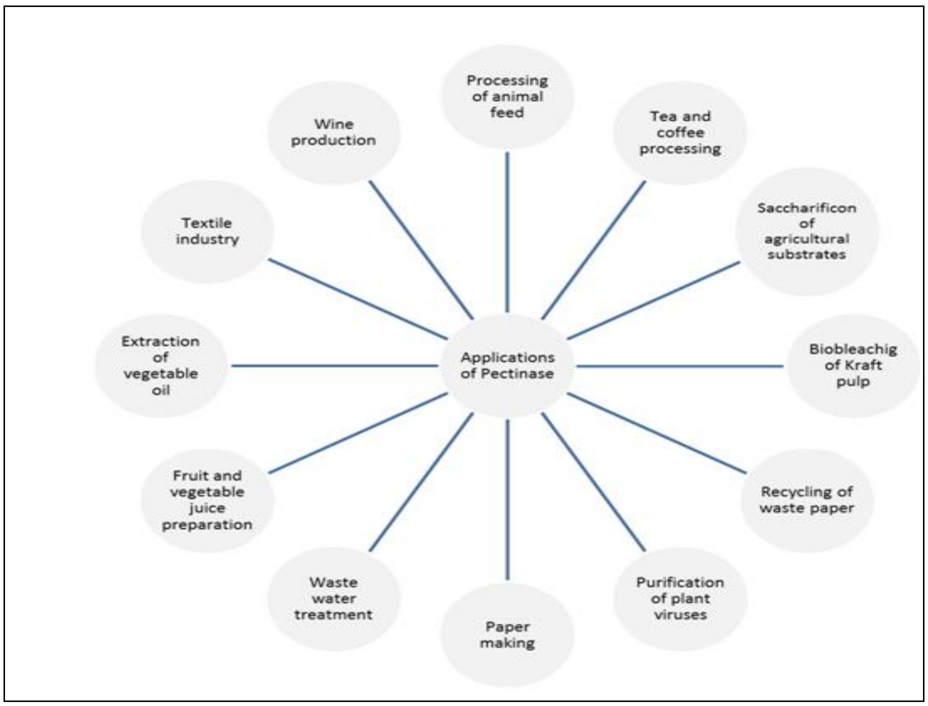

FIG. 2: INDUSTRIAL APPLICATIONS OF PECTINASES

Extraction of Vegetable Oil: Production of vegetable oils such as olive, palm, coconut, sunflower oil involves extraction with organic solvents such as hexane that is carcinogenic in nature. However, use of pectolytic enzymes allows the extraction of vegetable oils in an aqueous process by degrading the component of the cell wall. Oil yield and its organoleptic quality are also enhanced [26]. To make the process cost-effective, in this study author has used pectinases enzyme from *Aspergillus giganteus* NRRL10 with solid-state fermentation [27].

Fruit and Vegetable Juice Preparation: Generally, fruit and vegetable juices are prepared by enzymatic treatment of the pulp. This forms the biggest application of pectinases. Acidic pectinases have a major role in the fruit and vegetable juice industry; they are produced by fungi mainly from *Aspergillus* spp. due to its distinct role in food industries [23]. These enzymes are generally employed during fruit and vegetable juice extraction at the stage of pressing. The most important enzyme for the extraction of vegetable and fruit juice is the pectin lyase, with an optimum pH of 3-4 [27]. Pectins generally make the juices thick and cloud, which is not desired. In order to avoid this, they are generally treated with an enzymatic preparation, of which pectinases form a major fraction.

This treatment also leads to an increase in the bulkiness of the fluids [23]. This enzymatic process also increases the efficiency of pressing down the substrates for juice production. This helps enhance the quality, stability, and yield of extractive processes. Different pectic enzymes reduce the viscosity of the beverages to a different extent. These enzymes help fasten the process of clarification of juices. Pectinases are used for the destruction of the cell structures of the fruits and vegetables along with cellulases and hemi-cellulases and lead to the release of anthocyanins, which are naturally occurring pigment molecules. These enzymes also help remove the peels off the vegetables and in the maceration of vegetables to produce a paste of these fruits and vegetables.

Pectin lyase has a good haze removal activity. Pectin lyase was obtained from Acinetobacter calcoacetius bacteria with the help of a three-phase separation technique and immobilized *via* glutaraldehyde on CM cellulose (natural support material), magnetized with Fe_3O_4 nanoparticles. The result showed good reusability for pectin hydrolysis. Immobilized enzymes are effective in clarifying fruit juices. Immobilization of enzymes is better than using soluble enzymes and important in the food industry to contribute to a sustainable nutrition system [29]. Chryseo bacterium indologenes strain SD is isolated from forty isolates which are produced from vegetable dump waste soil using standard plate count. Hydrolysis of pectin is the key source for screening. Fruit juices are clarified with the help of it [30].

Pectinase has been used to reduce turbidity in fruit juice. Pectinase produced from Bacillus subtilis was isolated from Kilis soil and purified using cell-free supernatant, ethanol, and ammonium sulfate precipitation methods. The maximum specific activity was observed in enzyme preparation purified by ethanol precipitation, 217.44 U/mg [31]. A wild type of *A. Nigerpectinase* was isolated from a rotten orange texture (used as substrate) through solid-state fermentation evaluated on natural apple juice. The concentration of soluble sugar, the viscosity of the juice, clarity, and the yield of extracted juice was significantly improved by the enzymatic hydrolysis activity of pectinase [32]. 60% turbidity and 40% viscosity of the juice was reduced by enzymatic treatment. After the enzymatic treatment, the total phenolic compounds, total anthocyanins, and antioxidant activity were preserved [33]. Pectinase increases the yield of juices by enzymatic liquefaction of pulps, also helps in the formation of pulpy products by macerating the organized tissue into a suspension of intact cells [34].

Textile Industry: One of the major tasks involved in the textile industry is the separation of non-cellulosic impurities from the fiber. This process of scouring was earlier practiced using harsh chemicals, which further poses a problem of their disposal and hence pollution. However, bio-scouring involves the action of various enzymes, which reduces the hydrophobicity of the text tile material. Another important step involved in the textile industry is degumming of fiber crops which is well aided by pectinases [27].

Depectinisation is important to eliminate loosened waxes from cotton. Best results for scouring are produced when alkaline pectinases are used along with other enzymes like amylases, cellulases, hemicellulases, lipases, *etc*. Usage of enzymes in place of chemicals also fastens the processing of textiles and confers them the water retention ability and high tensile strength. Bio scouring of fabric with pectinase resulted in the enhancement of various physical properties of fabri. Pectinolytic enzymes obtained from Actinomycetes are known to produce a good correlation between pectate lyase activity and degumming effects [24]. The combination of chemical and enzymatic processes enhances the degumming of bast fibers and decreases the consumption of chemicals and energy [35].

Reducing Pollution: With the increased use of natural resources in food processing industries, the disposal of agricultural wastes is adding to the problem of environmental pollution. Wastewater effluent from these industries is a major challenge. The use of conventional physical and chemical methods of water treatment is not only time-consuming and expensive but also adds to water pollution by releasing a chemical into it. Pectinases find excellent alternative and environment-friendly application here as the agriculture waste is rich in pectin and a suitable substrate for these enzymes [36]. Wastewater pretreated with pectinases to decompose pectin is subjected to activated sludge treatment. Extracellular endo pectate lyase produced by alkalophilic Bacillus sp. (GIR 621) at pH 10.0 has proved to be effective in the decomposition of pectin residues from wastewater [37]. Many authors report such applications of Pectinases for the treatment of wastewater coming from different industries [38].

CONCLUSION: Pectins are a group of polysaccharides that are present in the plant cell wall. There are different types of pectin like protopectin (water-insoluble pectic substances occurring in plants), pectinic acid (water-insoluble transparent, gelatinous acid existing over the ripe fruits and vegetables), pectic acid (water-insoluble transparent, gelatinous acid existing over the ripe fruits and vegetables). Pectinases are used to degrade the pectin. Pectinases are classified into different classes: polygalactouronase, pectin esterases, pectin lyases.

Microorganisms that are used for the production of pectinases are filamentous fungi, bacteria, and some strains of actinomycetes. *Aspergillus niger*, *Bacillus subtilus*, *Bacillus cocci*, etc., are mostly used in the production of pectinases. But the studies revealed that *A. niger* is mostly used in the industry for the production of pectinases because it is more efficient than other microorganisms in the production of pectinases. Different substrates can be used for the production of pectinases like a banana peel, orange peel, apple peel, wheat barn, pomegranate, etc. Solid-state fermentation and Submerged fermentation are the methods used for the production of pectinases. However, the production of pectinases is very expensive, but using simple fermentation techniques and using the cheap carbon source, adequate temperature, optimum pH, adequate inoculum size leads to inexpensive and increased production of pectinases. However, if an enzyme becomes immobilized, it will lead to the reuasage of enzymes repeatedly, and it will be economical.

The production of pectinases is eco-friendly because in this, only agro and food wastes can be used. Pectinases are used in different industrial sectors like fruit juice extraction, coffee, and tea fermentation, textile industry, fruit juice industry, *etc*. As pectinase is used widely in the industry so whenever the degradation of pectin is needed, it is important to undertake research in the screening of microorganisms for pectinases and investigate the optimal conditions for the production of microbial pectinases.

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