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Significance of Pectin and Pectinase with Respect to Biological Application

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Abstract: ***Pectin, a naturally occurring polysaccharide, has become increasingly important in recent years. Because of its biodegradabilitynature, theadvantages of natural pectin are increasingly appreciated by scientists and consumers. The diethyl ester of polygalacturonic acid is pectin. Citrus peels and apple pomace are commercially extracted under slightly acidic circumstances for pectin. Pectinases are enzymes that break down pectin, which is a chain molecule with a rhamnogalacturonan backbone that is linked to other polymers and carbohydrates and pectinases are produced by bacteria, fungus, actinomycetes, and yeast, among other microorganisms.The pectinases are used in the pulp, textile, food, and water treatment sectors, as well as in the food, beverage industries for clarification and also pectin is used in the pharmaceutical business, as well as in the promotion and treatment of health. Matrix tablets, gel beads, and film-coated dosage forms have all been used as potential carriers for drug administration to the gastrointestinal tract. This paper focuses on the significance of pectin and pectinase, microorganisms producing pectinase, factors affecting its activity, enzymatic degradation and its various applications in different industries.***

Keywords: pectin, pectinase, sources, degradation, enzymatic factors

1. Introduction

Pectin and pectinase are important in industrial sectors nowadays because pectin is a galacturonic acid-rich structural acidic heteropolysaccharide with carboxyl groups esterified with methanol. In cereals, vegetables, and fruits, the acidic heteropolysaccharide is a prominent component. Pectic compounds are high-molecular-weight, biocompatible, non-toxic, anionic natural polysaccharides that are important components of the plant's middle lamella and primary cell wall (Chen, J *et al.*,2015;Sat apathy *et al.*, 2020). Pectinase enzyme breaks down pectin, which is often found in plant cell walls and is widely used in commercial preparations like saccharification, fruit juice clarification, coffee and tea fermentation, paper making etc. Pectinase has a complex enzymatic system that breaks down pectic compounds such as pectinic acids, propectins, pectins, and pectic acids (Kittur *et al.*, 2003). Pectin's main chain is made up of methyl esterified 1, 4-D-galacturonan and a demethylated form of pectin called pectic acid (pectate) or polygalacturonic acid. Pectinase works by breaking glycosidic bonds and converting polygalacturonic acid to monogalacturonic acid (Raju *et al.*, 2013). Pectinolytic enzymes are used in a variety of industries, although they are most commonly used in the food industry for procedures like fruit juice clarifying and production of wines and the extraction of vegetable oils (Joshi *et al.*, 2006). The benefit of pectinase also extends to maintaining gut microbiota homeostasis by increasing nutritional prebiotic properties in plant-based foods during processing. Enzymatic pectin formulations are also used in the pharmaceutical industry to generate low-methoxyl pectin, which is advantageous for diabetes patients (Wilkowska *et al.*, 2019).

Pectin:
Pectic substances are acid polysaccharides with a high molecular weight found throughout the plant kingdom. The pectin molecule can be altered biologically or chemically. For more than 50 years, the chemical structure of pectin has been the subject of numerous scientific reports, (De Vries *etal*., 1981) as pectin functions as a lubricating or cementing agent in the plant cell wall andits role during fruit ripening, its role in food processing, and its role as nutritional fiber, elucidation of this structure was and continues to be important (Takuo Sakai *et al.*,1993).Pectic substances found in the cell wall and middle lamella, such as pectin, protopectin, and pectic acids, contribute firmness and structure to plant tissues. D galacturonic acid units in pectic substances are linked together by a-1, 4- glycosidic linkages, and the carbonyl side groups are 60/90 percent esterified with methanol (Sathyanarayana*et al.*, 2003).

Structure of Pectin
Pectin is a polysaccharide that is essentially linear. It is polydisperse and polymolecular, like most other plant polysaccharides, and its composition varies depending on the source and isolation conditions. Parameters such as molecular weight and concentration of certain subunits will vary from molecule to molecule in each pectin sample (Sriamornsak *et al.*, 2003). Because pectin can alter during isolation from plants, storage, and processing of plant material, determining its structure is difficult (Novosel'skaya *et al*., 2000). Impurities might also accompany the primary components. Pectin is now assumed to be made up primarily of D-galacturonic acid (GalA) units linked together in chains by á-(1-4) glycosidic linkages (Mukhiddinov *et al*., 2000) alpha (1-4) glycosidic linkage connects them in chains. Some carboxyl groups in these uronic acids are present naturally as methyl esters, while to form carboxamide

groups others are commercially processed with ammonia. Pectin includes a chain-like shape of a few hundred to around 1000 saccharine units, corresponding to average molecular weights of 50,000 to 150,000 Daltons. There may be significant variances between samples and between molecules within a sample, and estimates may differ depending on measuring methods. Rhamnose (Rha) is a small component of the pectin backbone that causes a kink in the straight chain, while arabinose, galactose, and xylose are found in the side chains (Oakenful, 1991). The typical fragment is a chain of several hundred alpha-(1-4)-bonded GalA units with a diverse DE.

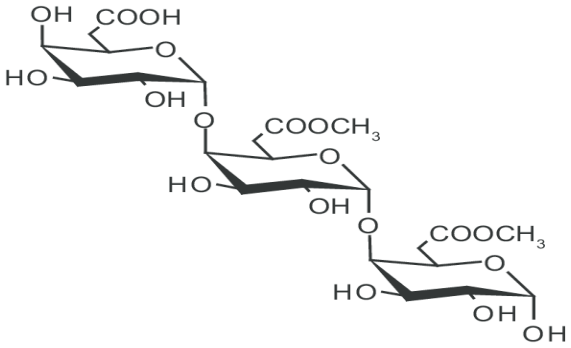

Figure 1: Structure of Pectin (Ali et al., 2015)

Sources of Pectin

Pectin is available naturally in many sources like in plants; it is present in high amounts in middle lamella of cell wall.Citrus fruits such as oranges, lemons, and grapefruits are high in pectin. Apples, guavas, plums, and gooseberries have a lot of pectin, whereas soft fruits like cherries, grapes, and strawberries have a lot less (Srivastava and Malviya, 2011). Apples contain 1–1.5 percent pectin, apricots contain 1%, cherries contain 0.4 percent, oranges contain 0.5–3.5 percent pectin, carrots contain 1.4 percent pectin, and citrus peels contain 30% pectin. Pectin is made mostly from dried citrus peel or apple pomace, both of which are by-products of juice manufacture. Sugar beet pomace is also used in a limited amount.Banana *(Musa acuminata L.*) Beet pulp (*Beta vulgaris*) Carrot {*Daucus carota*) Giant granadilla (*Passiflora quandrangularis L*) Guava (*Psidium guajava L.*) Lemon pulp (*Citrus limon*) Lychee (*Litchi chinesis S.*) Mango (*Mangifera indica L.*) Orange peel (*C. sinesis)* Papaya *(Carcia papaya)* Passion fruit rind Peaches *(Prunus persica*) Pineapple (*Ananas comosus L.*) Strawberries *(Fragaria ananassa)* Tamarind (*Tamarindus indica L.*) Tomato fruit (*Lycopersicon esculentum*) (Bonner, J. 1976; Renard*et al.*,1993;Oakenfulland D. G. 1991)It's tough to prepare apple pomace until it's been dried and stored for a while. Pomace is typically collected from a variety of drying plants throughout a large area (Romboutse*t al.,* 1986). As a commercial supply of pectin, sugar beet pectin has a number of drawbacks. Sugar beet is not employed as a raw material since its pectin gelling ability is inferior to that of apple and citrus pectin, despite its high pectin content, availability, and inexpensive cost. The large amount of acetyl groups and the short molecular size of pectin are primarily responsible for this (MICHEL *et al.,* 1985).

Significance of Pectin:

Pectin's have always been present in human food as a natural component and pectin is employed as a gelling agent, thickening agent, texturizer, emulsifier, and stabilizer in a variety of foods. Pectin has been employed as a fat or sugar substitute in low-calorie foods in recent years. Pectin's multifunctionality arises from the fact that its molecules have polar and nonpolar sections, allowing it to be incorporated into a variety of dietary systems (Hoefier, and A. C. 2012). Their gelling qualities are the fundamental reason for their use as a food hydrocolloid andthe texture required, pH, processing temperature, presence of ions, proteins, and the projected shelf life of the product are all elements to consider when choosing pectin for a certain meal (Voragen *et al.*,1986).

Some uses of pectin in various industries are as below:

1) Jams, Jellies and Preservatives

The most common foods that use a lot of pectin are jams and jellies. Jam is made by heating fruit for a short time to extract juice and pectin by converting protopectin to soluble pectin because sugar-free jams and jellies are becoming more popular, partially due to calorie-conscious consumers and partly to meet the demand for sugar-free products among diabetics. LM pectin is utilized in these goods, which results in pectin-calcium gels (Athira*et al.,* 2019).

2) Conserves

Conserves are sweetened only with fruit juice or fruit concentrate and do not contain any extra sweetener. As a result, their soluble solid content is lower than that of sweetener-containing goods. Consumers rate them as high-quality because they don't include any additional sweeteners and its soluble content is 55 to 62% (Therkelsenand G. H. 1993).

3) Baker's Jellies

Instant jellies, which are used in a variety of bakery goods, are made with pectin. Because HM pectin is thermally stable, it is used to make jellies that are cooked without being fluidized in the batter or dough. If the formula's fibre content is increased, fibre entanglements will further reinforce the gel structure, making it more stable (Hoefier, and A. C. 2012).

4) Confectionary Products

Flavored candies are made with HM pectin. Pectin with a neutral flavour (no fruit flavour) can be used to manufacture confectionary products to which an additional flavour can be added. Artificial cherries are made with pectin, which is a wholly synthetic medium that allows for precise control of setting conditions. In edible coatings, pectin is utilized to prevent lipid migration in confectionery items (Brake*et al.,* 1993).

5) Frozen Barriers

Pectin is added to frozen meals to prevent crystal formation and syrup loss during thawing, as well as to improve their shape. Ca2+ and pectin's have the greatest firming effect on frozen-then-thawed fruits. Fruit slices treated with calcium ions and pectin firm up more than the entire fruit. In frozen-then-thawed fruits, pectin, Ca2+, sugar, and vacuum all lower the drained weight (Van Buren, and J. P. 1983; Morris *et al.,* 1991). Fruits are coated with LM pectin's to improve their texture and quality before being used in ice cream.

Pectin improves the smoothness of frozen foods by reducing the size of ice crystals.

6) Beverages

Reducing the amount of sugar (sucrose, high fructose corn syrup, or a combination of the two) removes the beverage's mouthfeel or body, which is found in traditional soft drinks. A 0.05 to 0.10 percent HM pectin addition can help to restore mouthfeel. Pectin added to a dietetic fruit juice beverage with fruit pulp lowers "hard packing" (the deposition of fruit pulp into a hard clump that is difficult to disperse). Pectin is sometimes used as a clouding agent in beverages (El-Shamei, Z. and El-Zoghbi, M.1994).

7) Pharmaceutical Uses

In the pharmaceutical sector, pectin is used. Pectin has a positive effect on cholesterol levels in the blood and also works as a natural antidote against toxic cation poisoning. It has been proven to remove lead and mercury from the gastrointestinal system and respiratory organs (Kohn, R. 1982). Pectin shortens the coagulation time of blood when administered intravenously, making it effective for reducing hemorrhage or local bleeding. By modifying hepatic cholesterol homeostasis, pectin from prickly pear (*Opuntia spp.*) lowered plasma low-density lipoprotein (LDL) content without influencing cholesterol absorption in guinea pigs (Fernandez *et al.,* 1994). Pectin, as well as pectin mixtures with other colloids, has long been used to treat diarrheal illnesses, particularly in infants and children (Cerda *et al.,*1994).The use of a mixture of LM pectin, aluminium hydroxide, and magnesium oxide in the treatment of gastric and duodenal ulcers has been described (Ashford *et al.*,1994).

8) Other Uses

To generate sturdy, self-supporting films, pectin and starch blends can be employed. Color and taste substances in a pectin layer are released when liquids flow through the straw, and pectin's have been used to make biodegradable drinking straws (Endress, and H. U. 1991).

Degradation of Pectin with Pectinase Enzyme:

Properties of Pectinase:

Pectinases are enzymes that degrade pectic substances for nutritional purposes and are involved in plant pathogenesis. Pectic substances are widely distributed in fruits and vegetables (10/30 percent in turnips, orange peels, and tomato, pineapple, and lemon pulps), and thus serve as important natural substrates for pectinases (Gummadi, *et al.,* 2003).

Pectinases are a diverse group of related enzymes that hydrolyze pectin substances found primarily in plants. Pectinolytic enzymes are abundant in higher plants and microorganisms (Whitaker, J. R. 1990). They are vital to plants because they aid in cell wall extension and softening of certain plant tissues during maturation and storage (Jayani, R. S *et al.*,2005). Pectinases are classified into three groups based on their cleavage site: (1) hydrolases, which include polygalacturonase, PG (EC 3.2.1.15); (2) lyase/trans-eliminases, which include pectinlyase, PNL (EC 4.2.2.10) and pectate lyase, PL (EC 4.2.2.2); and (3) pectin esterase, PE(EC3.1.1.11) (Yadav, S *et al.*,2009). As a result, pectinase enzymes are frequently used in processes involving the degradation of plant materials, such as accelerating the extraction of fruit juice from fruit such as apples and sapota. Pectinases have also been used in the manufacture of wine (Nevadita Sharma *et al.,* 2013;Semenova, M *et al.,* 2006).The features of microbial pectinases are critical in the commercialization of industrial production and the application of these enzymes in a variety of applications. As a result, several studies have focused on pectinase stability, chemical modification, and catalytic efficacy.

Pectinase pH and Stability

The key restrictions on the rapid development of biotechnological processes are enzyme deactivation and stability. Stability studies can also reveal important details regarding the structure and function of enzymes. The selection and design of pectinases must take into account two crucial factors: improving stability and maintaining the desired degree of activity over time. Physical (pH and temperature), as well as chemical conditions influence the stability of pectinases (inhibitors or activators). *Rhizopusarrhizus* endo-optimum PG's pH was discovered to be in the acidic range of 3.8–6.5 (LIU*et al.,* 1978). Endo-PG from *Rhizopus stolonifer* proved stable in the pH range of 3.0–5.0, and it is highly selective for non-methoxylated PGA (Manachini *et al.,* 1987).

Enzymatic Degradation

Pectinases are a type of enzyme that catalysis the breakdown of pectic polymers in plant cells. The catalytic action of pectin-degrading enzymes is classified. Pectin de-esterification by pectin methyl-and acetyl-esterases produces pectate and alcohol. The enzyme's activity allows pectin-depolymerizing enzymes to reach the substrate. The enzyme works by hydrolyzing the methoxyl and acetyl groups of a galacturonate unit's ester moiety (Khan *et al.,* 2013).Polygalacturonases (PGs) hydrolyze -1, 4 glycosidic bonds in the homopolygalacturon backbone. They are members of the glycosyl hydrolase family 28, together with rhamnogalacturonases (Henrissat 1991). Based on their modes of action, there are four different categories of PGs. Endopolygalacturonase attacks the polysaccharide chains 1, 4-glycosidic connections at random, resulting in a multitude of galacturonic acid oligomers. They generate a rapid decline in substrate viscosity due to their endohydrolyzing activity. However, when the degree of methoxylation increases, the rate and degree of hydrolysis decrease significantly (Devi &Appu Rao 1996).Exopolygalacturonase I hydrolyze D-galacturonic acid from the nonreducing end of polygalacturonic acids, while exopolygalacturonase II releases di-galacturonate from the nonreducing end (Kenon & Waksman 1990).The eliminative cleavage of de-esterified pectin, which is a significant component of the primary cell walls of many higher plants, is catalyzed by pectin lyase (PLs) and pectin transeliminases (Carpita&Gibeaut 1993). Pectin polymers are immediately degraded by PLs via an elimination mechanism, resulting in the generation of 4, 5-unsaturated oligogalacturonides. *E. carotovora* and *Bacillus* cultures were the first to identify PL activity that required calcium ions (Starr & Moran 1962).

Factors Affecting Pectinase Activity:

1) Effect of pH

The pH of the medium is an essential factor (Shoichi*et al.*,1985)for production of microbial pectinase and growth of microorganisms,onmembrane permeability and biosynthesis and stability of enzymes(Murad *et al.*,1992; Murad *et al.*,2001) and the optimum pH from many moulds been reported to be acidic range. The ability of certain *Bacillus* species to produce pectinase. Their study attempted to optimize the impact of pH on pectinase production; the optimal pH range was 4 to 10. But pH 7 was where pectinase levels peaked. The maximum pectinase activities were found at pH 6.5, according to (rajakumar *et al.*,2012) among a wide range of pH values used for the optimization of this pectinase production. Using *Bacillus sphaericus*, the synthesis of pectinase was enhanced at various pH levels. The pH ranges were 4.4, 5, 5.6, 6.2, 6.8, and 7.4. The greatest activity from that pH range was found at 6.8 (Jayani *et al.*, 2010). Utilizing *Bacillus* species FW5 and *Erwinia* species FW2, the impact of pH on the synthesis of pectinase was also optimized. The pH scale was from 5 to 9 (Mehta *et al.*,2013). showed that among the several pH ranges, the *Bacillus* species FW5 and *Erwinia* species FW2 both produced the most pectinase at pH 7. Aspergillus sp. like *Aspergillus niger*, *Aspergillus aculeatus* etc., show high enzyme activity at pH 4 to 5 in acidic range (Suresh, B., &Viruthagiri, T.2010). So, it shows that maximum enzyme production occurs at pH range 4 to 7 for bacteria and fungi.

2) Effect of incubation period

The period of fermentation has a great effect on production of microbial product(Murad *et al.*,1992; Murad *et al.*,2001) maximum production from many moulds may vary from 1 to 6 days(Ghildyal *et al.*,1981). At the conclusion of 72 hours of fermentation under liquid state conditions, *Erwinia carotovora* MTCC1428 produced the greatest quantity of pectinase activity (Kothari *et al.*,2013). After 120 hours of fermentation time, utilizing a *Bacillus* species, the maximum pectinaseactivity was noted (Soares *et al.*,2001). It was determined that *Chryseobacteriumindologenes* strain was the other bacterial isolate, K6. At the conclusion of the 72-hour incubation period, this isolate produces the most extracellular pectinase (Roy et al.,2018). After 72 hours of fermentation durations, this species generated the greatest amount of pectinase (Kumar, A., & Sharma,R.2012). FW2 *Erwinia* species and FW5 *Bacillus* species were used to produce the most pectinase. By the end of the 96-hour fermentation period, those two distinct species had produced the most pectinase. (Haile, S., & Ayele, A. 2022; Mehta *et al.*, 2013*). Aspergillus* sp. showed maximum pectinase activity after 96 hours (Ali *et al.*, 2010; Suresh, B., & Viruthagiri, T.2010). It shows that the incubation time for maximum enzyme activity occurs after 96 hours.

3) Effect of Nitrogen and Carbon Sources

The impact of organic and inorganic nitrogen sources on pectinase synthesis was investigated. According to (Hours *et al.*, 1988) observation, lower quantities of (NH4) 2SO4 (0.16 percent) or KH2PO4 (0.1 percent) added to the growth medium as inorganic nitrogen sources had no effect on pectinase output. Pectinase synthesis is influenced by ammonium phosphate and ammonium sulphate (Galiotou-Panayotou *et al.*, 1993). A sufficient amount of carbon as an energy source is essential for optimum growth, which affects the organism's growth and metabolism. Pectic enzyme production is inhibited by high glucose concentrations in the medium, while enzyme production is stimulated by low glucose concentrations (Aguilar, G., & Huitron, C. 1987).To test their impact on pectinase production, carbon sources such glucose, maltose, lactose, starch, xylose, and sucrose as well as nitrogen sources like peptone, urea, yeast extract, KNO3, NH4Cl, and NaNO3 were added as separate components to the baseline media. The peptone in the media showed maximum activity than other nitrogen sources (Kalaichelvan, P. 2012). During fermentation utilizing cassava waste, *Bacillus* sp. MFW7 produced marginally more pectinase in response to the addition of carbon sources in the form of carbs. Lactose had the highest production rate. The synthesis of pectinase was severely inhibited when the bacteria was cultivated on glucose, maltose, or sucrose, but pectinase production was shown to be excellent when the bacterium was grown on lactose. Concurrent with our findings, *Bacillus sphaericus* was found to produce pectinase optimally from citrus pectin and xylose (Jayani *et al.*, 2010). It shows from the findings that maximum enzyme production observed with peptone, ammonium sulphate and ammonium phosphate as nitrogen source and lactose, xylose and glucose as carbon source showed maximum enzyme production.

4) Effect of Substrate Concentration

Different substrate concentrations increase pectinase activity till certain level then level drops off (Khan I. G and Barate D. L.2016). At 0.8 percent of pectin content, the most pectinase activity was found. According to Khan and Barate'sinvestigation, the maximum activity of pectinase was measured using various concentration ranges of pectin (0.1-1%) at intervals of 0.1 percent. Other investigations showed that the activity of pectinase rose up to the optimal concentration as the concentration of pectin increased, and then it dropped after the optimal concentration. The largest quantity of pectinase activities was reported at 0.5 percent of pectin concentration and reduced after this concentration among the different pectin concentration ranges of 0.1 percent, 0.2 percent, 0.5 percent, 1 percent, and 1.5 percent (Haile, S., &Ayele, A. 2022). It shows that on increasing substrate concentration in the medium with all the optimal conditions keeping constant the enzyme activity increases and maximum activity is obtained until the optimal concentration is reached (Khan*et al.*,2012).

5) Effect of Activators And Inhibitors

Different metal ions show activating and inhibiting property on pectinase activity like zinc, magnesium, calcium, iron, manganese and potassium. On the activity of pectinase, the impact of several metal ions was investigated. Zinc was found to increase activity while also having an inhibitory impact. For all the isolates, magnesium, calcium, barium, and manganese served as effective activators. While increasing the activity of enzymes, iron exhibited an inhibiting effect on organisms. While strongly inhibiting the enzyme, potassium improved enzyme activity (Khan I. G and Barate D. L.2016). It shows that activators like magnesium, calcium, barium and manganese increased pectinase activity and inhibitors as zinc and iron inhibited pectinase activity.

6) Effect of Temperature

In various temperature ranges, various bacterial species produced the most pectinase, according to various researchers. Temperatures ranging from 25 to 50 degrees Celsius were used to generate polygalacturonase in five-degree intervals. *Bacillus sphaericus* produced the most pectinase from this temperature at 30°C (Jayani *et al*., 2012). *Enterobacter tabaci* NR1466677 developed the most pectinase activity at 35°c the study was conducted between 20 and 45 °C and 35°C was shown to be the ideal temperature for synthesis of enzyme (Obafemi *et a*l.,2019). The maximal pectinase that *Erwinia* species FW2 can make at various temperatures studied at rangefrom 20 to 65 °C and the temperature where the most pectinase was produced was 37°C (Mehta et al.,2013). While maintaining all other variables constant, the trials were carried out at varied temperatures of 35°C, 40°C, and 45°C. The activity of the pectinase enzyme was observed to increase when temperature rose from 30°C to 40°C for Aspergillus sp. which gave maximum enzyme production at 40°C (Suresh, B., &Viruthagiri, T.2010). Thus, the bacteria and fungi produce maximum pectinase at the range of 30°C to 40°C.

7) Commercial Production of Pectinase:

In liquid broths, commercial pectinases are frequently made from fungal sources. For the synthesis of enzymes, *Aspergillus* and *Trichoderma* are commonly used. Pectinase In solid state cultures, production have been recorded using agricultural wastes such as cassava fibre waste (Budiatman *et al.,* 1987) and wheat straw. For substrates, bran (Ghildyal *et al.,* 1981), apple pomace (Hours *et al.,* 1988), and citrus wastes (Garzón *et al.,* 1992) have been used, and these substrates have been determined to be the best for the SSF method. Trejo Hernanadez and colleagues (Trejo-Hernández *et al.,* 1991) made a comparison between the yields and productivity of pectinase using both approaches. SSF appears to be more productive than SmF.

Methods for Production:

Commercially available microbial enzymes are made using either submerged fermentation (SmF) or solid substrate fermentation (SSF) processes. SmF enzyme production processes are typically carried out in stirred tank reactors under aerobic conditions using batch or feed batch systems. The employment of SmF techniques in enzyme manufacturing is impracticable in a majority of developing country contexts due to high capital and energy costs, as well as the infrastructural requirements for large-scale production. Submerged fermentation is the culture of microbes in a liquid broth. It requires a large amount of water and constant agitation and produces a large amount of waste. SSF entails microbial growth and product generation on or within the particles of a solid substrate under aerobic conditions, in the lack or near absence of free water, and does not require aseptic conditions (Solís-Pereira *et al.,* 1993).

a) Submerged Fermentation (SMF)

In submerged fermentation, the fermentation occurs in presence of liquid and the organisms that require high moisture content for growth, grows in this type of fermentors. This process depends on supply of nutrients or replacement of supplement for production. Submerged fermentations (SmF) offer more chances for process control and analysis, as well as a foundation for planned experiments aimed at increasing fermentation yield through the use of optimal medium (Schmidt *et al.,* 1995). Molasses and broths are examples of free-flowing liquid substrates that are used in SmF. The fermentation broth is where the bioactive substances are secreted. The substrates are utilized quite rapidly; hence need to be constantly supplemented with nutrients (Subramaniyam, R. and Vimala, R. 2012). The organism is grown in liquid media with other essential nutrients and for enzyme production the required number of substrates are added with other nutrients and enzyme activity is calculated. This fermentation method works best for microorganisms like bacteria that need high moisture content during fermentation to produce bioactive compounds. This method has the added benefit of making product purification simpler. The primary function of SmF is to remove secondary metabolites from solid materials that must be utilized as liquids (Uzuner, S., & Cekmecelioglu, D. 2015).

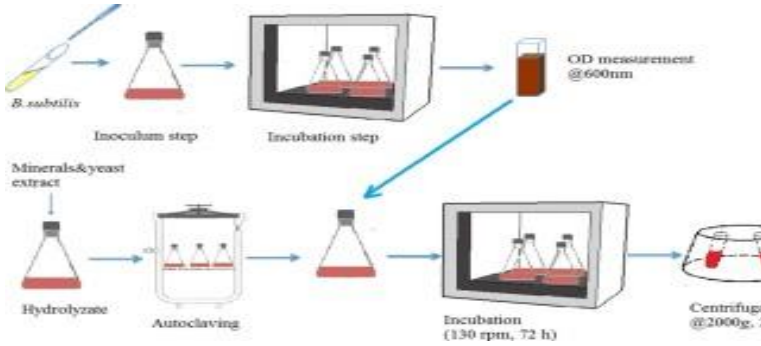

Figure 2: Submerged fermentation process (Uzuner, S., &Cekmecelioglu, D. 2015)

b) Solid State Fermentation (SSF)

The development of microbes on solid objects in the absence of free water is known as solid state fermentation (SSF). It has a lot of potential in terms of enzyme production (Aidoo*et al.,* 1982; Mitchell *et al.,* 2000). SSF makes use of solid substrates like paper pulp, bagasse, and bran. Utilizing these substrates has the primary benefit of making it simple to recycle waste materials rich in nutrients. The substrates used in this fermentation process are consumed very gradually and consistently, allowing the same substrate to utilize when fermentation takes a lengthy time (Subramaniyam, R. and Vimala, R. 2012).This method encourages the gradual release of nutrients. SSF is most appropriate for fungal and microorganism-based fermentation processes that demand low moisture contents. However, it is ineffective when employed with species like bacteria that demand high aw (water activity) in fermentation processes (Satyanarayana and Babu, 1996). The substrates used in solid state fermentation are agro waste that can be also used as carbon sources in the production of bioactive products. So, the advantage of this process is that the waste from agriculture industries can be unutilizedand recycled.

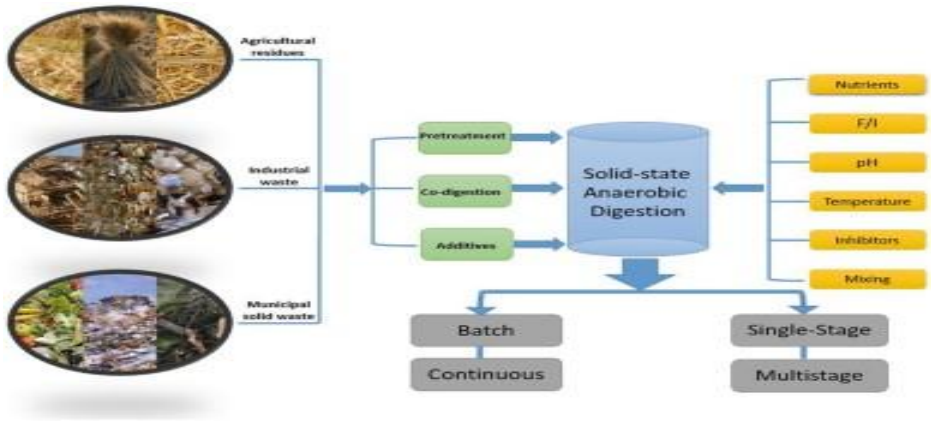

Figure 3: Solid State Fermentation (Steudler et al.,2019)

Applications of Pectinase in Various Industries

- **Juice Processing Industry**

Juice Clarification

Fruit juices are the most important product of a variety of fruits, and consumers enjoy them for their taste and nutritional value. The natural turbidity of each fruit's juice varies according to its physical and chemical properties. Due to the presence of insoluble materials like cell fragments from fruits pulp tissue or components, there is turbidity in juice, because of undissolved materials of fruit tissue. These insoluble materials, which gives taste, smell and colour characteristics, ranges in size from micro to larger pulp fragments. The use of enzymes can increase yield and aid in the clarification of a variety of juices, including apple, pear, orange, and peach. Enzymes break down pectin or cell walls, allowing for more juice extraction per ton of fruit (Daniele S. Ribeiro *et al.*, 2010).

Thus, pectinase enzyme is used to clarify the different types of juice like: -

a) **JUICE FROM APPLES:***Aspergillus* sp. pectinolytic enzymes have been widely used in the purification of apple juice (Amin F *et al.*, 2017). Exo-polygalacturonase produced by *Penicillium notatum* reduced the turbidity of apple juice by 73.78 percent, according to used polygalacturonase from *Penicillium* sp. and found that it reduced viscosity by 4.5 percent while increasing light transmission by 71.8 percent (Yuan *et al.*, 2011). It was reported that *Aspergillus*sp produced polygalacturonase that clarifies apple juice. *Aspergillusniger* pectinase reduced apple juice viscosity by 7.2 percent (Mukesh Kumar Patidar *et al.*, 2018).

b) **JUICE FROM ORANGES AND MOSAMBI:** Citrus fruits, such as oranges, lemons, and grapefruit, have the highest reported pectin concentrations in their tissues (Gallant*et al.*, 2014).Pectin accounts for roughly one-third of the insoluble material found within the juice cloud in commercially prepared citrus juices (Baker RA *et al.*, 1969). Because of the high levels of pectin methylesterase, which removes the methoxyl group from pectin, orange pectin is only partially methylated and by addition of pectinase enzyme orange juice is clarified (Mukesh Kumar Patidar *et al.*, 2018).

c) **JUICE FROM PEARS:** Pears are crushed and enzymes are added to the pulp in the maceration step at about 75 g ton and left for 30-60 minutes at room temperature. The pectinases PL, PME, PG, and arabanases improve juice extraction and yield. After pressing, the juice is depectinized for about 1 hour at 45-50°C with pectinases with high arabanase activities (D.R. Kashyap *et al.*, 2001).

d) **JUICE FROM GRAPES:** Grapes are difficult to crush and press due to their high pectin content. They are destemmed, crushed, and heated to 60°C or 80°C to release colour from the skins (in the case of black grapes) and destroy the endogenous polyphenoloxidase. Then, at a rate of about 50 g ton, enzymes such as Cytolase PCL5 (GistBrocades) or Ultrazyme (Novo Nordisk) are added to macerate the berries and increase yield. Different types of filters (rotary vacuum, earth) and/or centrifugation are used to separate the free running juice from the solids. The filtered juice is cooled to 0°C to prevent fermentation before being depectinized at 200 ppm for about 2 weeks (D.R. Kashyap *et al.*, 2001).

- **Wine Making**

In winemaking, pectic enzymes from fungi like *Aspergillus niger*, *Penicillium notatum*, or *Botrytis cinerea* are beneficial. Although berry, peach, apple, pears, and other fruit juices are used (Robertson G. L.1977;Pilnik, Walter & Voragen, A. G. 1993) grape wine is created in more volume. The enzyme treatment of the juice, either before or during fermentation, settles out numerous suspended particles and, in certain cases, undesired microorganisms. Finally, pectic enzymes are added to the mixture at the end of the fermentation process, increasing fermented wine filtration and clarity (D.R. Kashyap *et al.*, 2001).

- **Agricultural Substrate Saccharification**

Pectinases are also used in biorefineries to hydrolyze pectin found in pectin-rich agricultural and industrial wastes. These wastes are converted into simple sugars, which can then be converted into bioethanol or used as fermentable sugars (G. Garg *et al* 2016).

- **Processing of Textile Material**

Bio-scouring is an environmentally friendly method of removing non-cellulosic impurities from fiber using specific

enzymes. It makes the surface of the fiber more hydrophilic. Bioscouring also eliminates the high energy consumption and severe pollution issues associated with traditional alkaline scouring. Pectinases also protect fibers from damage (G. Garg *et al* 2016).Pectinase has been used in the textile industry to remove sizing agents from cotton instead of harsh chemicals, along with other enzymes such as amylase, lipase, cellulase, and hemicellulase. For the bioscouring of cotton to effectively achieve whiteness and absorbency, different combinations of enzymes have been used, such as cellulose with pectinase and cellulose with pectinase and protease. In the textile industry, the use of harsh chemicals has been reduced thanks to the use of enzymes like pectinases in conjunction with amylases, lipases, cellulases, and other hemicellulolytic enzymes to remove sizing agents.This has improved both the safety of working conditions for textile workers and the quality of the fabrics (Haile, S., &Ayele, A. 2022).

- **Retting and Degumming of Fiber Crops**

Pectinolytic enzymes are involved in the retting and degumming of coconut husk jute, axe, hemp, ramie, kena*(Hibiscus sativa*),A fermentation process in which certain bacteria (e.g., *Clostridium*, *Bacillus*) and fungi (e.g., *Aspergillus*, *Penicillium)* decompose the bark's pectin and result in the production of fiber is known as retting (D.R. Kashyap *et al.*, 2001).Gum-containing fibres must be removed before being used in the textile industry. The pectin that holds the fibres together should be broken down. Pectinases and xylanases can be used as an alternative to chemical degumming, which generates pollution. This method is both affordable and environmentally beneficial (Sharma and Satyanarayana 2012). For the retting and degumming process of fibres like ramie, flax, sunn hemp, and jute, alkaline pectinases are frequently utilized. Woody fabrics that are lengthy, strong, and stiff are macerated by pectinolytic enzymes released by soft rot bacteria, which weakens the fibers.

- **Fermentation of Coffee and Tea**

Pectinase treatment hastens tea fermentation while also destroying the foam-forming property of instant tea powders by destroying pectin. Pectinolytic microorganisms are used to remove the coat made up of mucilage from the coffee beans in coffee fermentation process. Pectinases are sometimes used to remove the pulpy bean layer that contains pectic subsces (VibhaBhardwaj et*al.*, 2017).The mucilage layer that surrounds and covers the Robusta bean is gelatinous and viscous in texture. With 84 percent moisture, 8.9 percent protein, 4 percent sugars, 2.8 percent pectin, and 0.9 percent ash, it tanhas these other ingredients as well. By mass, the mucilage makes up about 17% of the whole cherries. Depending on the variety, climate, estate management, and other factors, the Robusta coffee pulp's composition may change. The quality of the coffee bean is influenced by the conversion of mucilage to sugar. To remove the pectic substance-filled pulpy bean layer, pectinases are applied. Of coffee pulp, demonstrating waste recycling with value addition and being beneficial to the coffee business (Haile, S., &Ayele, A. 2022).

- **Paper and Pulp Industry**

Pectinase can depolymerize galacturonic acid polymers during papermaking, lowering the cationic demand of pectin solutions and peroxide bleaching filtrate (Vibha Bhardwaj et *al.*, 2017).The use of chlorine-containing bleaching agents results in the production of hazardous, mutagenic, and bioaccumulative organochlorine byproducts in the paper and pulp sector. These are the main culprits of the ecosystem's serious upheaval. Pectinase is used in this situation to reduce the ecosystem's toxicity from chlorinated chemicals. Galacturonic acid polymers are depolymerized by pectinases during the papermaking process, which lowers the cationic need of pectin solutions. The presence of pectin in the pulp, which cause the paper to turn yellow, has an impact on sheet formation in the paper and pulp business (samanta 2021).

- **Treatment of Pectic Wastewater**

Environmentally, wastewater from citrus processing industries containing pectic substances is treated in several steps, including physical dewatering, chemical coagulation, direct activated sludge treatment, and chemical hydrolysis, which results in the formation of methane. These have several drawbacks, including high treatment costs and longer treatment times, as well as environmental pollution from chemical use. Thus, using pectinases from bacteria, which selectively remove pectic substances from wastewater, is an alternative, cost-effective, and environmentally friendly method (VibhaBhardwaj et*al.*, 2017).

- **Extraction of Oil**

Oils derived from rape seed (Canola), coconut germ, sunflower seed, palm kernel, and olives are traditionally extracted using organic solvents. Hexane, a potential carcinogen, is the most commonly used solvent. Recently, a plant cell-wall-degrading enzyme preparation has begun to be used in the production of olive oil. The enzymes are added during the olive grinding process (Nevadita Sharma *et al.*, 2013).Because pectinases alter pectin's emulsifying qualities, which prohibit oils from being taken from citrus peel extracts, citrus oils, such as lemon oil, can be extracted (Nevadita Sharma *et al.*, 2013).

- **Detergent Industry**

Detergents with pectinases remove stains created by plant based things like jams, sauces and jellies making them easier to remove from fabrics during washing. Novozymes introduced a pectinase-based detergent, to remove pectin-based stains (Sarmiento*et al.*,2015). Detergent applications for pectinolytic enzymes are also interesting. These alkaline enzymes are now necessary for all automatic dishwashing machines and heavy-duty laundry detergents worldwide (Ito et al., 2005).

2. Conclusion

Pectinases are the enzymes that are used widely in many industries for the treatment of plant mass rich in pectin, which a long and complex molecule is occurring as structural polysaccharides in the middle lamella and cell walls of plants. Plants based thingsarerich in pectin and pectinases are used in many different industries like juice industries for clarification of juice, textile industries for retting and degumming of fibers, paper and pulp industry,for

treating pectic wastewater released from industries, in the fermentation of tea and coffee, extracting oil from plants, purifying plant viruses and making detergents for removing stains of things containing pectin -like jams, jellies and sauces from clothes andit has also been employed as a carrier for a wide variety of biologically active substances in pharmaceuticals and formulation of the drug, not only for sustained release applications butfor targeting medications to the colon for either local or systemic activity.

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