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PRODUCTION OF FUNGAL CELLULASE ENZYMES AND THEIR APPLICATIONS

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ABSTRACT

Cellulose is the most common organic polymer. The enzyme cellulase may break it into smaller sugar components, such as glucose subunits, which can be utilized to make ethanol, organic acids, and other compounds. Cellulases are a set of enzymes that have applications in various industries, including food, textiles, pharmaceutical, detergent, biofuels, and paper, making them the third-largest industrial enzyme utilized worldwide with rising demand. Cellulolytic fungi were first screened by exposing them to Carboxyl Methyl Cellulose agar (CMC), a selective media for cellulolytic bacteria. Further testing can be done on Czapek-Dox agar medium supplemented with 1% CMC. The cellulase production was significantly improved by optimizing many parameters such as pH, temperature, and substrate concentration.

Keywords*: cellulolytic fungi, cellulase, fermentation, bioethanol*

1. INTRODUCTION

Enzymes are biological catalysts that are highly specialized, energetic protein molecules in every cell and are required for life to exist. Over 2000 enzymes have been identified, each involved in a single chemical reaction. They have incredible catalytic power, often outperforming synthetic or inorganic catalysts (Sharada et al., 2014).

Cellulases are enzymes catalyzing the hydrolysis of glycosidic linkages in cellulose and come in various forms. Exoglucanases (Exo-1,4-glucanases, EC 3.2.1.91), endoglucanases (endo-1,4-glucanases, EC 3.2.1.4), and -glucosidases (-D-glucoside glucohydrolase, EC 3.2.1.21) are involved in the enzymatic breakdown of cellulose (Legodi et al., 2019). Due to their complicated nature and vast commercial applicability, microbial cellulases have become the most common biocatalyst. Various organisms produce cellulases, including bacteria, fungus, protozoa, and animal species. In the extracellular matrix, where the enzymatic activity occurs, cellulases perform a catalytic function. Cellulases are in high demand in biofuel, where they are used to make bioethanol from cellulosic biomass (Sharada et al., 2014).

1.1 Cellulose:

Like any other abundant natural substance in the biosphere, cellulose abounds throughout nature. It is the most abundant renewable bioresource produced in the biosphere, at 100 billion dry tonnes each year, and the major product of photosynthesis in terrestrial ecosystems. The primary component of plant biomass is homopolymeric cellulose, anhydro-D-glucose linked by -1, 4 bonds. As a result, forest trash contains a significant amount of cellulose that is either unutilized or underused (Sharma and Sharma, 2017). Cellulase is an insoluble molecule made up of 2000–14000 residues that share several characteristics.

Anselme Payen, a French agricultural chemist, is credited with the systematic study of cellulose chemistry, which he discovered in 1837–1842 when he discovered that all young plants contain a fibrous substance with a uniform chemical component, which the French Academy named cellulose. Braconnot, on the other hand, found the first modified cellulose in 1839. (Klemm et al., 2005).

Cotton fibre has a cellulose level of 90%, wood has a cellulose content of 40-50%, and dried hemp has a cellulose content of around 57%. Cellulose has a variety of applications, including anti-cake, emulsifier, stabilizer, dispersion agent, thickening, and gelling agent. However, these are all secondary to its primary function of retaining water. Cellulose is a linear polymer of -D-glucose units linked by 1,4 linkages, with a polymerization degree ranging from 2,000 to 25,000. Cellulose chains create multiple intramolecular and intermolecular hydrogen bonds, resulting in hard, insoluble, crystalline microfibrils. Natural cellulose molecules have amorphous and highly organized crystalline areas and are structurally diverse. The degree of crystallinity varies depending on the cellulose supply, and highly crystalline sections resist enzymatic hydrolysis better (Jayasekara and Ratnayake, 2019).

1.2 Cellulases:

Cellulases are enzymes that catalyze cellulolysis, or the destruction of cellulose and similar polysaccharides, and are produced mainly through fungus, bacteria, and protozoans. Cellulases are enzymes that break down cellulose into monosaccharides like -glucose and oligosaccharides. The hydrolysis of the 1, 4--D-glycosidic bonds in cellulose, hemicellulose, lignin, and cereal -D-glucans is the particular process involved. Based on their mechanism of catalytic action, the following components of the cellulase system were categorized (Yoon *et al*., 2014).

Cellulases are a class of hydrolytic enzymes that can depolymerize cellulose and break it down into smaller molecules. Fungi mainly produce cellulases. However, some bacterial strains have been shown to make them as well. (Priyanka *et al.* 2017).

1.2.1 Endoglucanases or Endo-1, 4-β-D-Glucan Glucanohydrolases (EC 3.2.1.4)

Endo-1, 4-glucanase (EG), also known as endoglucanase, randomly cleaves intermolecular -1, 4-glucosidic bonds inside the cellulose chain. Viscosity reductions in carboxymethyl cellulose (CMC) solution are often used to test endoglucanases. Endoglucanases and exoglucanases have different mechanisms of action in that endoglucanases reduce the specific viscosity of CMC with little hydrolysis due to intramolecular cleavages. Still, exoglucanases hydrolyze lengthy chains from the ends gradually (Priyanka *et al.* 2017).

1.2.2 Exoglucanases or 1, 4-β-D-Glacan Cellobiohydrolases (EC 3.2.1.91)

Exoglucanases, also known as Exo-1,4-glucanases (exo-1, 4-D-glucan cellobiohydrolases, CBH), cleave the accessible ends of cellulose modules to release glucose and cellobiose. Cellobiohydrolases I and II from *Triochoderma reesei* act on the cellulose chain's reducing and nonreducing ends. Unlike endoglucanases and glucosidases, exoglucanases have no particular substrates in cellulase mixtures. However, the rate-limiting phase in the cellulose hydrolysis process is the enzymatic depolymerization step conducted by endoglucanases and exoglucanases (Priyanka *et al.,* 2017).

1.2.3 Exoglucanases or 1, 4-β-D-Oligoglucan Cellobiohydrolases (EC 3.2.1.74)

It removes cellobiose from celloligosachharide or Pnitrophenyl—D cellobioside, although it is inactive against amorphous cellulose and CMC (Priyanka *et al.* 2017).

1.2.4 β – Glucosidases or β-D-Glucoside Glucohydrolases (EC 3.2.1.21)

β -D-glucosidases hydrolyze soluble cellobiose and other cellodextrins in the aqueous phase to create glucose with a degree of polymerization (DP) of up to six. As the degree of polymerization of the substrate increases, the hydrolysis rate reduces dramatically. (Priyanka *et al.* 2017).

1.3 Biotechnological importance of lignocellulosic biomass

Potential candidates include lignocellulosic biomass resources, which can be converted into high-value bioproducts such as bio-ethanol and bio-fuels (Asgher *et al*., 2013). The conversion of lignocellulosic biomass into fuel ethanol is described in detail, step by step (Iqbal *et al*., 2013). Because they contain carbohydrates that must first be transformed into simple sugars and then fermented into ethanol, lignocellulosic or agro-industrial biomass could be a low-cost approach for producing bioethanol (Anwar *et al*., 2014).

1.4 Source of Cellulases:

Trichoderma, Humicola, Penicillium, and *Aspergillus* are the most commonly investigated cellulolytic fungus (Gupta *et al*., 2015). Basidiomycetes, such as Chrysosporium sp., are also widely dispersed and well-known for their ability to degrade cellulose (Thomas *et al.*, 2013).

Due to lower penetrating ability and inability to use a wide range of substrates for cellulase production, bacterial cellulase was also researched, though in smaller numbers than fungal cellulase. The well-known cellulase-producing bacterial species are *Bacillus sp., Cellulomonas*, and *Clostridium* (Thomas *et al*., 2014).

Compared to anaerobic bacteria, aerobic bacteria can perform better cellulase production of around 90%–95% of bacterial cellulose degradation. *Pseudomonas fluorescens, Bacillus subtiIIs, Escherichia coli, and Serratia marcescens.*

2. CASE STUDY

Legodi *et al.,* 2019 found that all *Trichoderma* and *Aspergillus strains* produced significant levels of all three enzymes (exoglucanase, endoglucanase, and -glucosidase) required for complete hydrolysis of cellulosic material in their study "Isolation of Cellulose Degrading Fungi from Decaying Banana Pseudostem and Strelitzia alb *Trichoderma* strains had more significant amounts of cellulase and endoglucanases, while *A. fumigatus* strains had higher levels of -glucosidase. The interaction of starting pH and incubation temperature on the microorganisms seems to impact the production of all cellulases components significantly. The efficiency of these fungal strains' cellulases in hydrolyzing agricultural lignocellulose wastes, such as banana pseudostem, for manufacturing bioethanol will be tested.

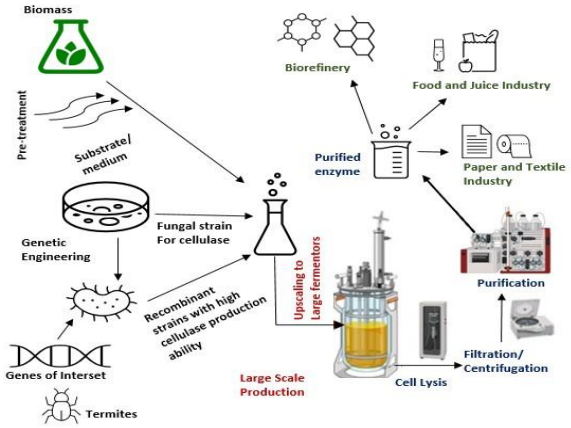

Fig. 1- (a) Production of Cellulase;

In the study "Study of Cellulase by Isolated Fungal Culture from Natural Resources and Application in Bio-ethanol Production," Patel and Desai, 2019 found that optimizing nutritional and environmental parameters for improving cellulase production by cellulase-producing isolated fungi improved cellulase production. The results demonstrated that the cellulase-producing fungi CPF3 and CPF-8 can grow in optimal conditions and be identified morphologically and partially purified. Both Solid State Fermentation (SSF) and Submerged Fermentation (SF) growth conditions produced an enzyme (SMF). Solid state fermentation, a cost-effective method, produced the most enzymes under these conditions. This is a viable approach for producing cellulase at a low cost using lignocellulose leftovers such as sawdust as a substrate. To make ethanol, the essentially purified enzyme transforms cellulose into a fermented reducing sugar, which is then fermented by yeast.

The potential of employing green tea waste as a substrate for cellulase synthesis under SSF by isolated polypore fungus was recognized and examined by Nguyen *et al.,* 2019 in their study "Production of high cellulase yields from polypore fungi in solid-state fermentation using green tea trash." This study enhanced cellulase activity by 1.6 times by employing *Microspores sp*. strain KA038 and a one-factor-at-a-time optimization approach.

Khattab *et al*., 2019 found that *Penicillium chrysogenum* has potential cellulase production characteristics to use agricultural wastes as a substrate for producing cellulase enzyme, which could be an effective additive to improve ruminant diet digestion and utilization in their study "Production Optimization of Fungal Cellulase and its Impact on Ruminal Degradability and Fermentation of Diet."

They found that a native Egyptian fungal strain (A. terreus) can be cultured using agricultural wastes as carbon sources to produce highly active cellulolytic enzymes in their study "Optimization and Characterization of Extracellular Cellulase Produced by Native Egyptian Fungal Strain."

Darabzadeh *et al.,* 2019 investigated "Optimization of cellulase production during solid-state fermentation by a new mutant strain of Trichoderma reesei" and found that "Cellulase production under SSF by a new mutant of T. reesei". After a "Plackett and Burman design" was used to screen seven factors, the most effective variables identified: moisture content, K2HPO4 concentration, incubation temperature, and incubation time. Based on the results, the cellulase activity was assigned the maximum value in the optimum condition, resulting in optimization factors being modified by 74% for moisture content, 2 g/L for K2HPO4 concentration, and 30°C for incubation temperature, and four days for the incubation period.

Soeka *et al*., 2019 discovered that Bacillus subtilis strain A8 possesses cellulolytic potential after studying "Production and characterization of cellulase from the newly isolated Bacillus subtilis A8 on rice bran and corncob." The cellulase enzyme activity of *B. subtilis* A8 on rice bran media has an optimum incubation time of 3 days at pH 6.0 and temperature of 60°C with a protein content of 0.13 g/mL. It is activated by Na+, Co+2, Cu+2, Mg+2, and Zn+2 ions, according to quantitative results of analyses. The cellulase enzyme activity of B. subtilis A8 on corncob media has a maximum incubation time of 3 days at pH 7.0, and 50°C with a protein concentration of 0.04 g/mL and is activated by Na+ and Co+2, whereas Cu+2, Mg+2, and Zn+2 are inhibitors.

"Fungal Cellulases Production for Biodegradation of Agriculture Waste" was researched by Srivastava *et al*., 2018. The generation of fungal cellulases is cost-effective for the SSF breakdown of certain types of wastes. Various fungi have been identified as potential cellulase producers and are being studied as alternatives to other recognized microbes. To reduce the cost of cellulase and increase its quality, efforts should be made to design more suitable and optimized combination processes.

Kulkarni *et al.,* 2018 investigated "Optimization of cellulase production by Aspergillus species under solid state fermentation" and found that nine cellulose-degrading fungi isolated from various materials tested for cellulolytic capacity in cellulose broth. Bl1 had the highest enzyme activity in the liquid medium of all the fungal isolates. Banana leaves were used as the Bl1 isolate's optimal medium, which included tryptone as an N source, a mineral salt solution pH of 5, and a moisture content of 67 percent. The optimal inoculum size and temperature found to be 7.35106 spores/ml and 37°C, respectively. Distilled water (pH 7) was the best solvent for cellulase extraction. The choice of CMC as the best substrate resulted in the highest activity level.

The study "Isolation, Production, and Optimization of Cellulase from a Combination of *Aspergillus niger* and *Trichoderma viride* Isolated from Decaying Woods" Mai AB *et al*., 2018 found that *Aspergillus niger* and *Trichoderma viride* produced cellulase effectively and could use for industrial cellulase production. At every stage of the optimization process, the combined effect of all the growth factors showed a tendency to increase cellulase activity. "Isolation of cellulose-degrading fungus from soil and optimization for cellulose degradation," according to (Priyanka P *et al.,* 2017).

The combined effect of all the growth factors noted a tendency to increase cellulase activity at every phase of the optimization process, confirming that the process parameters were optimized. The identified fungal strain could help produce cellulase from lignocellulose residues. Further research would improve cellulolytic capacity and extract cellulase enzymes for commercial use.

The study "Applications of fungal cellulases in befoul production: Advances and limits" (Srivastava et al., 2018) found that continuous and significant studies have increased cellulase enzyme production and efficiency for low-cost biofuel production. Low cell yield and submerged enzyme synthesis processes are also costly for biomass to biofuel conversion procedures.

Yadav *et al.,* 2016 investigated "Isolation, Screening, and Optimization of process parameters for increased cellulase production by solid-state fermentation" and found that fungal isolate P1 had a higher cellulase fermentation yielding point. The optimum temperature and pH for cellulase production were found to be 30°C and 6 pH. When rice straw was utilized as a solid substrate, lactose and peptone were determined to be the optimal carbon and nitrogen sources for cellulase synthesis by fungal isolate P1. P1 fungal isolate is a promising culture for cellulase generation in solid-state fermentation in the current study.

Lokhande *et al*., 2016 optimize the nutritional and ambient factors for enhancing cellulase production by the cellulolytic bacteria. The *Aspergillus niger* species demonstrated the ability to convert cellulose into reducing sugars, which might employ in various applications, including animal feed and as a feedstock for synthesizing valuable organic chemicals. The use of microbes for enzyme manufacturing is a promising strategy for large-scale production, as well as a potential dietary supplement or pharmaceutical business.

"Isolation and Identification of *Aspergillus protuberus* from Mahanandi Forest Sample and Investigation of Its Cellulase Production" (Yadav *et al.,* 2016) investigated cellulase production from *A. protuberus* under SmF conditions. For the formation of FPase and CMCase, glucose was found to be the best inducer, lactose found to be the best carbon source, whereas -glucosidase and protein content CMC and fructose found to be the best inducers and carbon sources, respectively.

The study "Purification and Characterization of Carboxymethyl Cellulase (CMCase) from *Penicillium ochrochloron* Isolated from Forest Soil of Neyyar Wild Life Sanctuary, India" found that when CMC was used as a carbon source in the culture medium, at 96 hours of incubation, at 27°C, and an initial pH of 5.5, the enzyme was purified and characterized. The enzyme was refined to homogeneity, and its molecular weight was determined to be 58 kDa. At a temperature of 60°C and a pH of 4.5, enzymatic activity is at its peak. Because the strain secretes a lot of CMCase, it's a suitable contender for industrial uses. (Yadav *et al.,* 2016)

Yadav *et al.,* 2016 investigated "Optimization of Cellulase Producing Fungi Isolated from Water" and discovered that *Trichoderma reesei* and *Penicillium chrysogenum* fungi generated cellulase successfully and may use for industrial cellulase production. Humans can benefit significantly from isolates with high cellulase activity in manufacturing pharmaceuticals and various other items.

The study "Optimization of culture conditions for Cellulase enzyme production from fungus" (Sethi *et al.,* 2014) found that cost-effective technologies are required to manufacture cellulases efficiently by employing Agobased waste as substrate. In microorganisms, enzyme production is tightly regulated, and these constraints can be relaxed to increase productivity. Cellulase yields appear to be influenced by a complex interaction involving variables such as inoculum size, pH value, temperature, inducer presence, medium additives, aeration, and growth period.

The need to use renewable resources to meet future fuel demand has heightened interest in cellulose, the world's most plentiful and renewable material. Our current research looked at the superiority of *Aspergillus niger* over the other fungal cultures tested in terms of producing extracellular cellulases. The data show that the technique for producing cellulose from coconut cake trash successfully produced a significant amount of this enzyme in laboratory settings. Furthermore, while the evolutionary operation factorial-design process may be useful in maximizing enzyme yield, all parameters are optimized one at a time. Cellulase enzymes' high activity and stability will be useful in various industrial and biotechnological applications.

3. APPLICATION

3.1 Paper and Pulp Industry:

The world's largest industries are paper and pulp. In 2013, the world's total paper production was expected to be 403 million tonnes. The pulp and paper industry is complex, with many kinds of mills, products, and processes. Effluents, therefore, vary significantly in quality depending on the process from which they originate. Due to this complexity, the requirements of water and effluent treatment at the different points of the manufacturing

process vary significantly. Membrane technologies are appropriate for many water recycling duties within the pulp and paper industry. Special mechanical forces grind and refine agro-industrial wood, resulting in pulp with a high degree of sufficient stiffness and bulk quantity. Biomechanical grinding and refining with cellulases and hemicellulases are more cost-effective, saving up to 20-40 per cent of energy while increasing the strength of paper sheets. The pulp and paper sector, which includes office and catalogue paper, glossy paper, tissue, and paper-based packaging, uses approximately 40% of all industrial wood traded globally, according to the World Wildlife Fund (WWF) (Jayasekara and Ratnayake, 2019). Cellulases, either alone or in combination with xylanases, help deink a variety of paper wastes (Imran *et al*., 2019).

Paper and pulp are natural resources that can replenished. As a result, two popular concepts in this market are recycling and reuse, usually accomplished through microbial cellulases. Cellulases are used in a variety of ways in this business. From the 1980s to the present, the potential applications have expanded into various fields. Deinking, pulping, bioremediation of industrial wastes, bleaching, and fibre improvement are only a few examples (Jayasekara and Ratnayake, 2019).

3.2 Textile Industry:

The most often utilized enzymes in the textile industry are cellulases. Biostoning of denim garments (jeans) and biopolishing of cotton and other cellulosic fabrics are both done with cellulases. Traditional stone washing of denim clothes involves amylase-mediated starch coating removal (desizing) and pumice stone treatment (abrasion) in powerful washing machines. Cellulases are used in the stoning of denim clothes to provide softness and a faded appearance, replacing the traditional use of pumice stones in the industry (Ray and Behera, 2017).

In the textile industry, fungal cellulases from *Trichoderma reesei* are the most commonly used enzyme. Other enzymes for decolourization and degradation of textile dyes include *actinomycetes* from the genera *Streptomycin* and Thermo Bifida, as well as bacteria from the genera *Pseudomonas* and *Sphingomonas* (Jayasekara and Ratnayake, 2019).

3.3 Waste management:

Waste management can benefit from the usage of cellulase. Cellulases, for example, are used to convert cellulosic municipal solid wastes into valuable chemicals and energy. Cellulases have the advantage of reducing the environmental impact of cellulose waste and pushing the conversion of pollutants to alternate sources of energy and chemicals, so reducing our growing reliance on fossil fuels (Jayasekara and Ratnayake, 2019).

3.4 Animal Feed Industry:

Cellulases are used in animal feed production to improve the digestibility of cereal-based foods and increase nutritional contents for better forage quality. *Trichoderma cellulase* has been used effectively as a feed supplement to improve feed conversion ratios and digestibility of cereal-based foods (Jayasekara and Ratnayake, 2019). Crude fibre measures the amount of fibrous, poorly digested material in the feed in terms of feeding value. Cellulases are utilized to increase the nutritional quality of forages in this setting.

Microbial fermentation has also been utilized to remove antinutritional components in animal feed, such as nonstarch polysaccharides such galactosides, glycerin, and -conglycinin.

Opazo (2012) cultivated mesophilic, aerobic, and cellulolytic bacteria (from the genera *Streptomyces*, *Cohnella*, and *Cellulosimicrobium*) and tested their capacity to decrease soybean meal galactosides and nonstarch polysaccharides using SSF.

According to Ray and Behera (2017), SSF reduced total nonstarch polysaccharides by roughly 24%, stachyose by 83 percent, and raffinose by 69 percent while increasing protein content.

3.5 Laundry and Detergent Industry:

Cellulases, particularly the EGIII and CBH I forms, are extensively employed in detergents for cleaning textiles, a relatively new development in the detergent industry. EG III variations, particularly those from T. reesei, have been reported to be appropriate for usage in detergents by several groups of researchers (Ray and Behera, 2017). Cellulases from fungi such as Trichoderma sp. (T. longibrachiatum, T. reesei, T. viride, and T. harzianum), Aspergillus niger, Humicola (H. insolens and H. griseathermoidea), and Bacillus sp. have been extensively explored for use in detergents thus far. The most suited additions to ordinary detergents are alkaline cellulases. It's due to their ability to remove dirt and soil particles from the fabric's interfibrillar regions.

Cellulases break down the rough projections of cellulose fibres or cellulose aggregates on the fabric. Due to this, the fabric gains a higher sheen and smoothness (Jayasekara and Ratnayake, 2019).

The manufacture of cellulase by thermophilic Bacillus sp. SMIA-2 uses sugarcane bagasse and steep corn liquor as substrates and the detergent industry's potential. After 120 h and 168 h of culturing time, maximum avicelase (0.83 U/mL) and CMCase (0.29 U/mL) activity were achieved, respectively. Cellulase compatibility differed by laundry detergent, being more stable in the presence of Ultra Biz and less stable in the presence of Ariel. The enzyme was also stable in the presence of sodium dodecyl sulfate and RENEX-95 but was inhibited by TritonX-100 and H2O2 (Ray and Behera, 2017)

3.6 Biomass Hydrolysis and Biofuel Production:

One of the most recently researched applications of cellulases in the bioconversion of lignocellulosic wastes is biofuel production. Although many cellulosic wastes are accessible, the cost-effectiveness of the biodegradation process is a crucial drawback. Cellulases transform lignocellulosic material into fermentable sugars such as glucose and maltose, which are then utilized as substrates to make bioethanol and other products. Although certain microbes can directly convert biomass to various alcohols, they are not exploited as a commercially viable source. To convert lignocellulosic material into bioethanol, this technology uses a multistep process. The proportion of hemicellulose and lignin is improved for further processing during the pretreatment process. The residues are next hydrolyzed at 50°C to yield fermentable sugars, and then microorganisms are used to convert cellulosic wastes to alcohol in the last stage (Imran *et al.,* 2016).

Reed has the potential to be used as a biobutanol and cellulase feedstock. Using Clostridium acetobutylicum under SSF successfully produced cellulase and biobutanol from reed. Without commercial cellulase, Organsolv pretreatment can efficiently produce reed hydrolysate that can turn to biobutanol. Furthermore, C. acetobutylicum fermented the hydrolysate medium and produced 14.24 g/L biobutanol and acetone/butanol/ethanol (ABE) with a yield of 0.33 g/g (Ray and Behera, 2017).

3.7 Wine and Beverage Industry:

In combination with glucanase, cellulase enzymes can increase the quality and yields of fermented products like wine and drinks. For example, cellulase, pectinases, glucanases, and hemicellulases are employed in wine production to increase colour extraction, skin maceration, must clarity, filtering, and wine quality and stability. Hydrolyzing glycosylated precursors into aglycones and glucose-glucosidases can improve the scent of wines (Li *et al.,* 2012).

3.8 Other Application:

Cellulases have also been utilized in agriculture to hydrolyze the cell walls of plant pathogens, thereby preventing plant infection and illness. Many cellulolytic fungi, such *as Trichoderma sp., Geocladium sp., Chaetomium sp., and Penicillium sp.,* are recognized to play an essential role in agriculture by improving seed germination, plant development and flowering, root system improvement, and crop yields Cellulases have also been utilized to improve the quality of soil. In addition, cellulases are employed in food processing to increase the extraction of fruit and vegetable juices (Sharada *et al*., 2014).

Cellulases, in combination with macerating enzymes, have been proven to boost olive oil extraction under cold processing conditions, as well as its antioxidant and vitamin E content (Sharma *et al.,* 2015). Furthermore, because humans have difficulty digesting cellulose fibre, using a digestive enzyme product containing cellulases, such as Digestin, can aid with digestive issues, including malabsorption. Finally, there has been discussion on using cellulase enzymes in chemical analysis, such as diagnostic and food analysis (Li *et al.,* 2012).

4. CONCLUSION

This study aimed to improve cellulase production by cellulase-producing isolated fungus by optimizing nutritional and environmental conditions. Because fungi are the leading players in the lingo cellulose breakdown process, this investigation revealed that the cellulase-producing fungi might grow under optimal conditions and can be identified by morphological identification and partial purification. Both culture conditions, such as Solid State Fermentation, were used to manufacture enzymes in this study (SSF). Solid state fermentation, a cost-effective method, produced the most enzymes under these conditions. This is a viable approach for producing cellulase at a low cost using lingo cellulosic left over's such as sawdust as a substrate. Saw dust has been confirmed as a valuable substrate for energy production, particularly in the biofuel industry. To make ethanol, the essentially purified enzyme transforms cellulose into a fermented reducing sugar, which is then fermented by yeast. Future parts of this research include reducing the cost of enzyme synthesis by employing waste lingo cellulosic materials, which is easily practical from an industrial standpoint. Biofuel has several advantages, including a reduction in greenhouse gas emissions and pollutants.

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