A REVIEW: Cellulose and Cellulase drive sustainable biomass conversion.

[1]Charmi Mahla and [2] Trupti Pandya

M.Sc. Student
Microbiology department, Bhagwan Mahavir college of Basic and Applied Sciences
Bhagwan Mahavir University, Gujrat, Surat, India.

Abstract- **Microbial enzymes have been used for centuries, but commercial use of enzymes has only recently become popular. Particularly, cellulases have found use in a variety of economic fields, including brewing, agriculture, laundry, pulp and paper manufacturing, and the textile industry. Cellulolytic enzymes potential for bio-conversion processes has been widely studied and explored, and they play an important role in a variety of industrial applications. A vital enzyme for cellulose-rich waste feedstock-based bio-refineries, cellulase is becoming more and more in demand across a range of industries, including paper and pulp production, juice clarifying, etc. In addition, there has been continuous progress in developing new strategies to improve its production, such as the use of waste feedstock as a substrate for the production of individual or enzyme cocktails, process parameter control, and genetic manipulations for enzyme production with increased yield, efficiency, and specificity. However, their role in the food industry has largely gone unnoticed. The use of immobilized cellulases has expanded their application in fruit and vegetable processing because it increases catalytic power while decreasing process costs. The current review provides a summary of current views on microbial cellulases as a potentially useful tool to build a sustainable idea for industrial applications.**

Keywords: cellulose, bacterial isolates, cellulase, and the food sector

INTRODUCTION

Cellulose is a biopolymer, the world's most plentiful and renewable carbon source. It is the most important component of plant cell walls, giving structural support and rigidity. Cellulose is a polysaccharide composed of hundreds of glucose molecules connected by -1,4-glycosidic linkages. However, because of its highly crystalline structure, cellulose is difficult to break down into its constituent glucose units, which is a significant barrier to converting it into valuable products. Cellulase enzymes come into action here.
Cellulase enzymes are a type of enzyme that breaks down cellulose into glucose molecules. These enzymes are produced by a range of microorganisms, including bacteria and fungi, as well as by some mammals, including termites. Endoglucanases, exoglucanases, and -glucosidases are the three primary types of cellulase enzymes. Endoglucanases break the cellulose chain's internal -1,4-glycosidic linkages, whereas exoglucanases split the cellulose chain's ends. The cellobiose molecules produced by endo- and exoglucanases are then hydrolyzed into glucose by -glucosidases.Because of their potential for sustainable biomass conversion, cellulose, and cellulase enzymes have received a lot of interest in recent years. The capacity to degrade cellulose into its constituent glucose units opens up new avenues for the manufacture of valuable products such as biofuels, bioplastics, and biochemicals. Furthermore, using cellulose as a feedstock for these products has the potential to reduce our reliance on fossil fuels while also lowering greenhouse gas emissions.

Cellulose

Cellulose is an organic substance that serves as the primary structural component of plant cell walls. It is a linear polymer composed of repeated -D-glucose units connected by -1,4-glycosidic linkages. Cellulose is one of the most prevalent biopolymers on Earth, with photosynthesis in plants, algae, and some bacteria producing an estimated 1011 tons per year. Cellulose offers an exceptional combination of qualities, including high tensile strength, low density, and biodegradability, making it an appealing material for a wide range of applications. Cellulose, for example, is used to make paper, textiles, food additives, and biofuels, and it has been studied as a potential biomaterial for tissue engineering and medication delivery.[1]

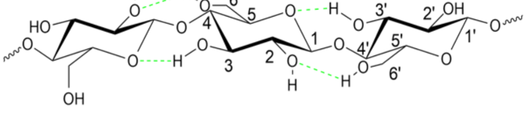

Fig.1 Chemical structure of cellulose

The high expense of turning cellulose into usable sugars, however, has prevented the use of cellulose as a feedstock for biofuels. Researchers are investigating several strategies, including the use of genetically engineered microbes and enzymes, to increase the effectiveness and lower the cost of this process.[2] Cellulose has been investigated for use in biomedical applications in addition to its potential usage in composites and biofuels. For instance, cellulose-based hydrogels have been created for tissue engineering and medication delivery. The antibacterial capabilities of cellulose nanocrystals and their possible application in wound dressings have also been studied.[3]

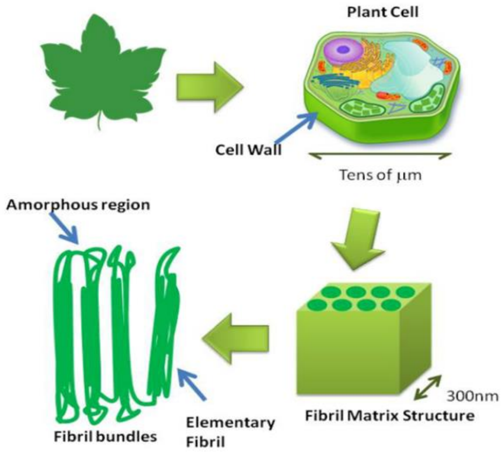

Fig-2- Cellulose in plant

Anselme Payen, "Mémoire sur la composition du tissu propre des plantes et du ligneux", Comptes Rendus, 1838, 7, 1052-1056.

For more than a century, scientists have been studying the structure and properties of cellulose using a range of techniques such as X-ray diffraction, solid-state nuclear magnetic resonance spectroscopy, and molecular dynamics simulations. In recent years, there has been an increase in interest in creating novel ways for producing and modifying cellulose, as well as investigating its potential applications in disciplines such as nanotechnology and sustainable materials.[4]

Cellulose source

Cellulose is found throughout the plant kingdom in its natural state.
In wood pulp or cotton fibers (cotton fibers are a virtually pure cellulose biological source).
Cotton fibers contain approximately 98% cellulose, whereas wood contains 40-50% cellulose. Table---1 [5]

Table-1: a summary of natural sources of cellulose

Source	Cellulose content (%)
Rice straw	40-45
Ramie	70-75
Kapok	70-75
Jute	60-65
Hemp	75-80
Flax	70-75
Cotton	90-99
Bamboo	40-55
Baggbasses	35-45

Other plant materials that can be used to make cellulose include maize cobs or stalks, soybean hulls, bagasse (sugar cane stalks), wheat straw, sugar beetroot pulp, bamboo, oat hulls, rice hulls, and fibers like jute, flax, and ramie.[6] Cellulose is found throughout the plant kingdom in its natural state.In wood pulp or cotton fibers (cotton fibers are a virtually pure c ellulose biological source). Cotton fibers contain approximately 98% cellulose, whereas wood contains 40-50% cellulose.

Rice husk, an agricultural waste product produced after rice milling, is one potential source of cellulose. The extraction of cellulose from rice husk has gained popularity in recent years because it provides a sustainable and low-cost source of cellulose. The viability of cellulose extraction from rice husk using a combination of chemical and mechanical techniques was examined in a study by Taflick et al. (2020). According to the study, rice husk contains about 34% cellulose, which may be removed by utilizing an alkaline treatment, bleaching, and mechanical processing. A high degree of purity and a low level of crystallinity was achieved, which is ideal for many industrial applications. The study also found that extracting cellulose from rice husk was more energy-efficient and cost-effective than standard cellulose sources such as wood pulp. This is because rice husk is a plentiful waste product that does not necessitate considerable resources to gather and handle. The researchers showed that high-quality cellulose can be extracted from rice husks using a mix of chemical and mechanical techniques and that this process is more energy-efficient and economical than other sources of cellulose. This study has enormous ramifications for the creation of cellulose supplies that are affordable and sustainable, which might have a big impact on a variety of businesses.[7]

Cellulose extraction methods

The acid hydrolysis technique, which includes the use of strong acids to break down cellulose into its constituent glucose monomers, is a common cellulose extraction method. This process was widely employed in the past, but it is connected with several environmental concerns, including the production of vast amounts of waste acids and the release of hazardous byproducts such as sulfur dioxide and nitrogen oxides.

The alkaline extraction procedure, which uses alkaline solutions to break down the lignin and hemicellulose components of plant materials, leaving behind the cellulose fibers, is another cellulose extraction method. This approach is less harmful to the environment than acid hydrolysis, but it requires higher temperatures and longer processing durations.

Recently, there has been an increase in interest in creating environmentally friendly and sustainable cellulose extraction processes that make use of non-toxic solvents including deep eutectic solvents and ionic liquids. The ability of these solvents to dissolve cellulose with the least amount of negative environmental impact has been demonstrated. The type of plant material being utilized, the desired purity of the extracted cellulose, and the extraction process's impact on the environment are all factors that influence the choice of cellulose extraction method.[8]

Cellulose applications

Due to its plentiful availability and distinctive characteristics, cellulose, a biopolymer that occurs naturally, has drawn considerable interest. Cellulose can be harvested using a variety of techniques from a variety of plant-based sources, including wood, cotton, and other agricultural wastes. Cellulose has developed a wide range of uses over time, including in the textile, paper, packaging, pharmaceutical, and food industries. We will go through some of the important uses for cellulose and its derivatives in this overview.

1) Paper and packaging: Cellulose is a key ingredient in paper and has been used in the paper industry for thousands of years. Due to their durability, biodegradability, and recyclability, cellulose-based goods, such as corrugated boxes and packing materials, are frequently utilized in addition to paper.

2) Textiles: Cellulose-based fibers, such as cotton, are extensively used in the textile industry due to their excellent moisture absorption, durability, and comfort. Cellulose fibers can also be chemically modified to produce new functional fibers with improved properties. For example, cellulose acetate fibers are used in cigarette filters, and rayon fibers are used in clothing and home textiles.

3) Pharmaceuticals: Cellulose and its derivatives, such as methylcellulose and hydroxypropyl cellulose, are utilized as excipients, binders, and coatings in the pharmaceutical industry. These compounds improve drug solubility, stability, and bioavailability, making them a crucial component of pharmaceutical formulations.

4)

Food: Cellulose is used as a food additive to improve texture, increase fiber content, and act as a stabilizer. Cellulose derivatives, such as carboxymethylcellulose and microcrystalline cellulose, are also used as emulsifiers, thickeners, and fat replacers in various food products.[9]

Cellulase

Enzymes are biological molecules that are produced by all living organisms. These are commonly referred to as "biocatalysts." Since hazardous chemicals are being replaced in the industrial sector by green chemistry methods, enzymes are becoming more and more in demand. It is essential in this situation that affordable enzyme substrates are readily available to make compound production on a broad scale feasible. Plant cell walls are a crucial component of the carbon cycle because the polysaccharides they contain can be utilized by bacteria as a source of carbon and energy. As a result, scientists from all around the world are attempting to comprehend the structural characteristics of plant cell wall polysaccharides and have moved their attention to characterizing the enzymes responsible for degrading plant cell wall polysaccharides and their encoding genes. [10] It is possible to use the microbial enzymes that break down plant cell walls to create biofuels, textiles, paper, detergents, and food for both people and animals.[11] Enzymes from animals and plants are less stable than those from bacteria. They may be manufactured utilizing fermentation procedures with great consistency for less money, in less area, and with shorter production times. Additionally, process optimization is easy.[12] The main enzymes created for use in many industrial industries are those that break down plant cell walls, such as pectinase, xylanases, and cellulases. Cellulase hydrolyzes the 1,4-glycosidic linkages to cause cellulose degradation.[13]

Cellulases are used in the textile industry [14][15], in detergents [16][17], in the pulp and paper industry [18], in improving the digestibility of animal feeds[19], and in the food industry[20]. The ability to use cellulases and hemicelluloses to perform enzymatic hydrolysis of the lignocellulosic material has attracted increased interest due to worries about the scarcity of fossil fuels, the emission of greenhouse gases, and the air pollution caused by incomplete combustion of fossil fuels. [21][22] However, the cost of the enzymes required for raw material hydrolysis must be reduced, and their effectiveness in producing bioethanol must be enhanced, to make the process economically viable.[23]

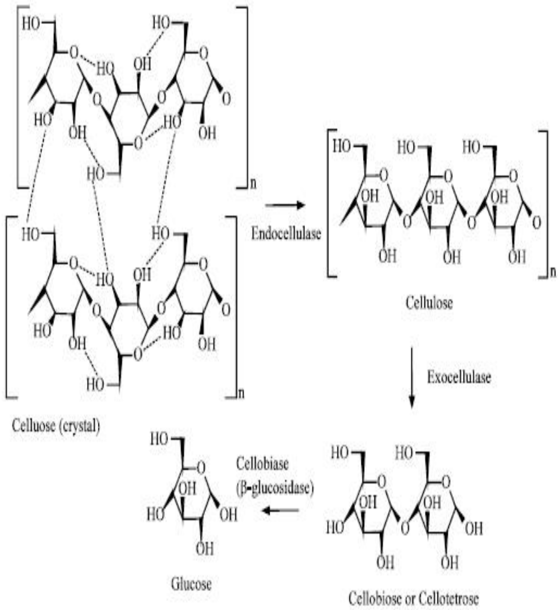

Fig 3: Mode of action of various components of cellulase

Microbial cellulase

Cellulase-producing microorganisms.

Bacteria that break down carbohydrates but cannot use proteins or lipids as growth fuel are known as cellulolytic bacteria. Bacteria that break down carbohydrates but cannot use proteins or lipids as growth fuel are known as cellulolytic bacteria.[24] While most fungi and some cellulolytic microorganisms, such as the bacteria Cellulomonas and Cytophaga, can use several different carbohydrates in addition to cellulose[25][26], anaerobic cellulolytic species can only use cellulose and/or its hydrolytic metabolites.[27][28] own specie for the efficient procedure of cellulases are *Aspergillus* and *Trichoderma.[29]* Mandel's and Reese conducted several studies to produce cellulolytic enzymes from biowaste degradation processes by many microorganisms including fungi such as *Trichoderma, Penicillium*, and *Aspergillus* species, among others.[30]

Microorganisms are primarily responsible for the majority of cellulose breakdown that occurs in nature. They address this challenge by utilizing a multi-enzyme system. Numerous extracellular enzymes with binding modules for different cellulose conformations were created by aerobic bacteria. Anaerobic bacteria contain a special extracellular multienzyme complex called the cellulosome. Cellulase preparations may degrade both natural celluloses (found in materials like filter paper) and modified celluloses like carboxymethyl cellulose and hydroxyethyl cellulose. In nature, cellulose coexists with substances like lignin, pectin, and hemicellulose. Other processes carried out by SERVA cellulases contribute to the breakdown of these elements and the deterioration of the cell wall. Cellulase is used to modify the surface characteristics of cellulosic fibers to provide the desired surface effect [31].

Table-2 Cellulose degrading microorganisms

Microorganism	Cellulose degradation mechanism	Ref.
Trichoderma reesei	Secretion of cellulase enzymes	[32]
Clostridium thermocellum	Cellulosome complex formation	[33]
Aspergillus niger	Secretion of cellulase enzymes	[34]
Cellulomonas fimi	Secretion of cellulase enzymes	[35]
Fibrobacter succinogenes	Cellulosome complex formation	[36]
Ruminococcus albus	Cellulosome complex formation	[37]
Bacillus subtilis	Secretion of cellulase enzymes	[38]

Waste from plants, or lignocellulosic, has been broken down using cellulase. Nigeria imports cellulase, an industrial enzyme, for use. Producing it from readily accessible sources, such as plant leftovers, will therefore aid in lowering importation expenses. In light of this, the purpose of this study was to evaluate Aspergillus candidus's cellulase activity on diverse agro-forestry residues used as feed substrates and to ascertain the impact of pH on cellulase activity. More focus has been placed on the generation of cellulase by different organisms during submerged state fermentation, but it has been discovered that this is prohibitively expensive due to the high cost of process engineering. Cellulose and hemicellulose can be broken down and used by many bacteria as a source of carbon and energy. The ability of *thermophilic bacteria* to create the enzymes required for substrate destruction during composting determines their capability to assimilate organic materials [39].

Cellulose application

Textile Industry

Cellulases have risen to the third position among the groups of enzymes utilized in the industry.[40]They are used in the bio-stoning of denim garments to produce a softness and the faded appearance of denim garments in place of the pumice stones that were previously employed in the industry.[41][42]They make denim appear faded by causing the cellulose fiber to release the indigo dye that was used to color the clothes. insolens cellulase is the most frequently employed enzyme in the stoning process, even though acidic cellulase from Trichoderma and proteases have both been shown to be as effective.[43] Additionally, cellulases have been utilized in defibrillation [44], softening [45], and procedures that produce regional variations in fiber color density.[46][47]

Laundry and Detergent

Cellulases, in particular EGIII and CBH I, are often used in detergents to clean clothes. Numerous reports suggest that EG III variations, particularly those from T. reesei, are appropriate for detergent use. [48,49] *T. viride* and *T. harzianum* are also industrially utilized natural sources of cellulases, such as A. niger [50]. Cellulase must be thermostable and compatible with alkaline environments as well as other formulation elements for the detergent industry. [51,52] Alkaline cellulase, in particular, enhances color vibrancy and removes dirt from textiles.[53]

Animal Feed Industry

To increase the nutritional value of forages, pectinases, hemicellulases, and cellulases are added to enzyme preparations.[46] Cellulases are used in feed processing, and this has been reported to improve feed digestibility and animal performance.[54] Additionally, cellulases break down oligosaccharides, beta-glucan, pectins, lignin, inulin, dextrins, cellulose, and arabinoxylans are examples of anti-nutritional compounds. By eliminating these chemicals, the nutritional value of feed and the health of animals are improved.[55]

Pulp and Paper Industry

Industry of Paper and Pulp

The pulp and paper industry has utilized cellulases and hemicellulases for biomechanical pulping to modify the characteristics of coarse mechanical pulp and hand sheet strength [46], and de-ink recycled fibers.[46], de-ink recycled fibers [57], and improve drainage and runnability of paper mills.[58] Cellulase Use in the paper and pulp sector is still a developing field. Mechanical or biological techniques can be used for pulping. A mechanical process produces pulp that is stiff, bulky and contains a lot of particles, whereas a biochemical approach that uses cellulose results in 20–40% energy savings.[59] Aspergillus niger and Trichoderma reseed are two common fungi whose cellulases are employed for this. It is also claimed that bacterial cellulase called CelB enhances the qualities of paper.[60]

Biofuel and Biorefineries

Cellulase breaks down the biomass into pentose or hexoses, which are then fermented to create ethanol or fuel. Cellulases are generally used in the bioconversion of renewable lignocellulosic biomass. Three stages are taken to decompose such biomass:(1) pretreatment; (2) saccharification, which involves enzymes; and (3) fermentation. According to estimates, cellulolytic bacteria can bioprocess biomass to cut the cost of the process by 40%.[61] Recently, legislation governing cellulosic ethanol was established by several countries, and their objectives include switching from starchy or cane sugars to cellulose-based materials as biomass resources.[62]This thermophilic bacteria, *Caldicelluloseruptor bescii,* can directly convert plant biomass into bioethanol, demonstrating its potential for ethanol production. Bioethanol synthesis from biomass is an important commercial use for these bacteria.[63]

Food Industry

Cellulases are used in the food industry to extract and clarify fruit and vegetable liquids, make fruit nectars and purees, and extract olive oil.[64] Many of the aforementioned goals can be accomplished in the commercial sector with the help of enzymes. However, widespread acceptance of the use of cellulases in the food industry is still a long way off.[65] Potential uses for cellulase in the food business include fungi (*Trichoderma* and *Aspergillus*).[66]

Current perspective on microbial cellulase

Overview of the synthesis of cellulolytic enzymes

Microorganisms that produce cellulolytic enzymes include bacteria and fungi. To create cellulases, a variety of bacterial and fungal strains have been employed commercially. Commercial production of cellulase has been done with expensive substrates like cellulose or carboxymethyl cellulose (CMC). As a result, researchers tried to use naturally existing biomass that is high in cellulose to substitute this pricey substrate, such as agricultural and forestry waste.[67][68][69]

Development of cellulase at the laboratory and industrial scale

Several strategies have been used to increase the cost-effectiveness and economic feasibility of cellulolytic enzyme production using sustainable approaches. These include investigating various carbon sources and pretreatment of agro-residues before their use in enzyme production. The role of various media components and media optimization can be investigated. Furthermore, different process parameters must be evaluated to increase cellulase production.[70]

Tools for statistical analysis of process parameters and media optimization to increase cellulase production.

The research demonstrates that improving the medium components and physicochemical procedures used to produce cellulase increased enzyme yield.[71]One factor at a time (OFAT) and different statistical approaches have been used in a number severaltion studies to boost cellular output.[72]To enhance the cellulase production process and evaluate the interactions of various physicochemical parameters in A. aneurinilyticus strain BKT-9 and Schizophyllum commune COC, the Response Surface Methodology (RSM), a well-known statistical technique, was examined.[73] Despite having a rotatable feature, the Central Composite Design (CCD) is a well-known statistical design for RSM that has trouble extending star points above the upper and lower bounds created for each factor in the experimental region. Nutritional and environmental parameters were optimized for bacteria found in Dal Lake, a man-made freshwater Himalayan Lake, to produce cellulase.[74] The nutritional and ecological factors for improved cellulase production with Bacillus licheniformis KY962963 were optimized using the "Plackett-Burman" design and the one component at a time technique. The study found that a moisture content of 75%, a K_2HPO_4 concentration of 2 g/L, an incubation temperature of 35 °C, and an incubation period of 3 days were the perfect circumstances for improved cellulase production.[75].

Improvement in cellulase production via a microbial fermentation process

The two primary techniques used to manufacture cellulase and other enzymes including proteases, xylanases, and pectinases utilizing diverse microorganisms are solid state fermentation (SsF) and solid submerged fermentation (SmF)[76]Solid state fermentation (SsF) and solid submerged fermentation (SmF) are the two main methods used to make cells and other enzymes such as proteases, xylanases, and pectinases using various microorganisms.[77]There are many occasions where using SsF and LCB as the substrate results in the production of cellulase at a reasonable cost. It just required simple equipment with low energy requirements, which resulted in a high enzyme yield and significantly lower processing costs. Because it promotes the search for effective substrates and allays concerns about cost, using LCB as a substrate is helpful for SsF and SmF. Since lignocellulosic plant residues serve as both a carbon source and an enzyme inducer in SsF, several methods have been developed to reduce the cost of manufacturing an enzyme.[78].

Improvement in enzyme purification techniques with enhanced specificity

Purification is the second most crucial stage in the fermentation process since it accounts for a total of 60% of the overall cost of the procedure. The downstream process (DSP) of the production of cellulase involves several traditional purification methods, including ion exchange and gel filtration chromatography, followed by precipitation and membrane filtration, either separately or in combination with other methods, each of which has its disadvantages.[79] Even though numerous effective purification techniques have been demonstrated at the laboratory scale, there are still significant problems with them. For instance, the chromatographic approach is problematic in large-scale applications since it necessitates expensive chemicals and lowers protein output. Extracellular cellulase from B. subtilis can be extracted utilizing the fundamental polymer polyethylene glycol (PEG) and the sodium citrate salt ABS (aqueous biphasic system), according to published research. The partitioning behavior of cellulases in the system is governed by their physicochemical interactions with phase-forming chemicals, which vary in their hydrophobicity, volume extension, electrochemical charges, and surface characteristics.[80]

CONCLUSION

According to the literature, the advantages of enzyme-based businesses over chemical-based ones include process safety, cheap refining costs, high yields, effective process management, and environmental friendliness. In the paper, pharmaceutical, detergent, and food sectors, enzymes, in particular cellulases, may find use. Thermostatic capacitors are frequently employed because of their durability at high temperatures. The industries that produce food, animal feed, and beverages are now those that use cellulases most successfully. We wish to emphasize once more the importance of cellulase research and its applications in the food industry. It is especially important to address the diverse needs of the food industry.

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