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Enhanced Production of Microbial Cellulases as an Industrial Enzyme - A Short Review

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Abstract: Enzymes are biocatalysts that exist with the ability to degrade complex molecules. Enzymes play a major role in every individual's life and for commercial purposes. Various microbes can produce high-yield enzymes. Microbial cellulases have an integral part in our biosphere because cellulose is present in abundance on our earth. Cellulose is a major component of every plant cell, and it is responsible in plant cells to maintain processes like photosynthesis. The methodology for this review paper involved an extensive literature review to gather information on the production and industrial applications of microbial cellulases. Relevant data was collected from scientific journals, research papers, and reputable online databases and was thoroughly analyzed to identify key concepts and emerging trends. The primary motive of this study was to compile all the numerous applications of cellulases as well as to describe some strategies to modify microbial cellulases with the help of genetic engineering and other tools in brief. This study has involved describing the structure, role, and mode of action, economic importance, and strain improvement of cellulases. Cellulases have applications in various sectors such as pharma, food, brewery, paper, pulp, textile, detergent, and many more. This study mainly focuses on microbial cellulases retarding chemical cellulases with their versatile behavior.

Keywords: *Cello dextrin, Cellulases, Degrade, Glucosidase, Hydrolysis, Photosynthesis*

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1. Introduction

Cellulose is the major component of most biological components, especially plant bodies. Cellulose also can be defined as an abundant biomolecule found in the biosphere. Cellulose is the component present in all cells of a plant that is essential for the photosynthesis process of that plant. The main source of cellulose in the plant cell is usually present in the microfibrils region of the plant cell, about 4-20 nm in diameter and approximately 3900 nm elongated in size (Bhatt 2000). In the meantime, cellulose plays a more complex role. Particularly since cellulose is acknowledged as an affordable raw material, its practical uses in the industrial sector have grown considerably more intricate. This has created a vast platform for researchers using multidisciplinary ways to study cellulose. The hydrolysis of cellulose is one such area. Cellulases typically carry this out in nature. The hydrolysis of cellulose is catalyzed by cellulase.

Recent data on the enzyme market indicates that the healthcare, textile, pulp and paper, food, and beverage industries are the main ones where cellulase enzymes are being used more and more. Its extensive use in the food and beverage industry includes the processing of coffee, the creation of wine, and the production of fruit juice. It is widely used to make cleaning and washing agents and laundry detergents in other industrial uses. Additionally, cellulase is becoming widely acknowledged as a potent substitute for currently prescribed antibiotics in the management of Pseudomonas biofilms. Thus, the remarkable development of cellulases' ability to combat germs resistant to antibiotics will solve issues in the healthcare industry. Cellulases have another essential property because it is known as the most crucial to maintaining the earth's carbon cycle for both marine and plant systems. Cellulases are the most important class of enzymes as it's able to degrade cellulosic components present in abundance in the biosphere. Cellulases can break down the chains of cellulosic enlarged components with numerous repetitive polymers. Cellulases are known as polysaccharides with beta 1-4 linkage with glycosidic bonding. There are mainly three components present in the group of cellulaes β-D glucosidase or cellobiase, 1,4-β-gluconase and exoglucanase. Exoglucanases are usually known for cleaving the cellulose chain at the end, and the non-reducing end is the target site most of the time. From crystalline, cellulose exoglycanase can split elementary fibers into simple and small structures. (Nowak et al., 2005)

Numerous microbial enzymes can be prepared in industries with fermentation technology. Cellulases have a major role in industrial enzymes as they can degrade complex sugar and make them simple fermented bio-molecules. Lignin is a molecule present in large amounts in plant cells and raw biomaterial present in the environment as lignocelluloses. So cellulose is required to hydrolyze these lignocellulosic molecules into simple cellulose or glucose chains. Most of the time, from an industrial point of view, the substrate is the cheapest and most effective for enzyme actions. In simpler words, we can say that the most suitable enzymes that can fit in the environment as commercial fields should be cheap, easily available, and have less effort to utilize an enzyme process. But raw materials that come in industries to fermentation have main components such as hemicelluloses, lignin, and long-chained cellulose or cellobiose. (Gomashe et al., 2013)

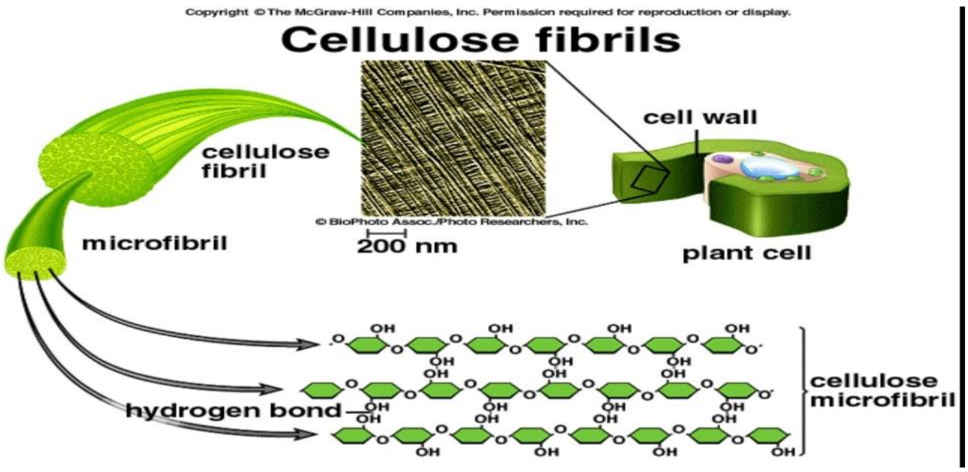

Figure 1: Cellulose is the major component of a plant cell in the plant cell wall (Jawhari,2021)

The main problem during hydrolysis is the hydrolysis of lignocelluloses into monosaccharides and small molecules of sugars. Due to high cost, industries do not prefer costly enzymes for commercial purposes. Cellulases can be isolated from the places where microorganisms secrete their enzymes to degraded plant molecules such as wooden borer microbes etc. Although a large number of microorganisms produce cellulases, such as bacteria and fungi, every year, demand for industries increases, and there is a necessity for microbial enzymes that can produce enzymes and also cheapest (Chang et al., 2011). Some studies have found that fungus is a rich source of cellulose and species included Trichoderma, Penicillium, and Aspergillus. There are numerous microbial species responsible for producing cellulases, but the main species are Cellvibrio, bacillus, pseudomonas, aspergillus, micrococcus, cellulomonas, etc. Many researchers concluded that microbial cellulases are an important part of industrial enzymes. Cellulases also can be produced by dairy animals like sheep and cows. However, the yield of the microbial enzymes to give products without by-products depends upon factors such as incubation period, pH, substrate, temperature, inoculum size, etc (Varghese et al., 2011)

Although a large number of studies have already been done on cellulases, this study indulged to express the quality of microbial cellulases rather than chemical cellulases. Despite of availability of numerous data regarding isolated strains of bacteria and fungus-producing cellulases, this study relies upon characteristics, mechanism action of cellulases, and strategies involved in gene manipulation. The main objective of this study is to express and evaluate a wide range of microbial cellulases, including their sources, availability, mode of action, and global purchase and demand. The specificity of the study focuses on summarizing huge applications for industries as well as the environment also, the techniques could be used to enhance the production of cellulases to fulfill industrial goals and further future tasks for bioremediation

1.1. Different types of cellulases with their mode of action

Usually, three types of enzymes are found in nature with their mode of action.

Exoglucanases (E.C.3.2.1.9.1.): These are the group of cellulases that mainly target the endpoint or ending chain of the complex polymers of celluloses or lignocelluloses. Exoglucanases hydrolyze the end part of the long-chained cellulose and break it down into simple saccharides or glucose molecules. Exoglucanases are also defined as their biochemical structure with the linkage of 1,4-β-Glucan Cellobiohydrolases. After degradation, they release water molecules.

Endoglucanases (E.C.3.2.1.4): These are the cellulases that cut mostly in internal oligo saccharides and release water at the end of the reaction. Endoglucanase can be defined as their linkage of 1,4-β-D-Glucan as its biochemistry.

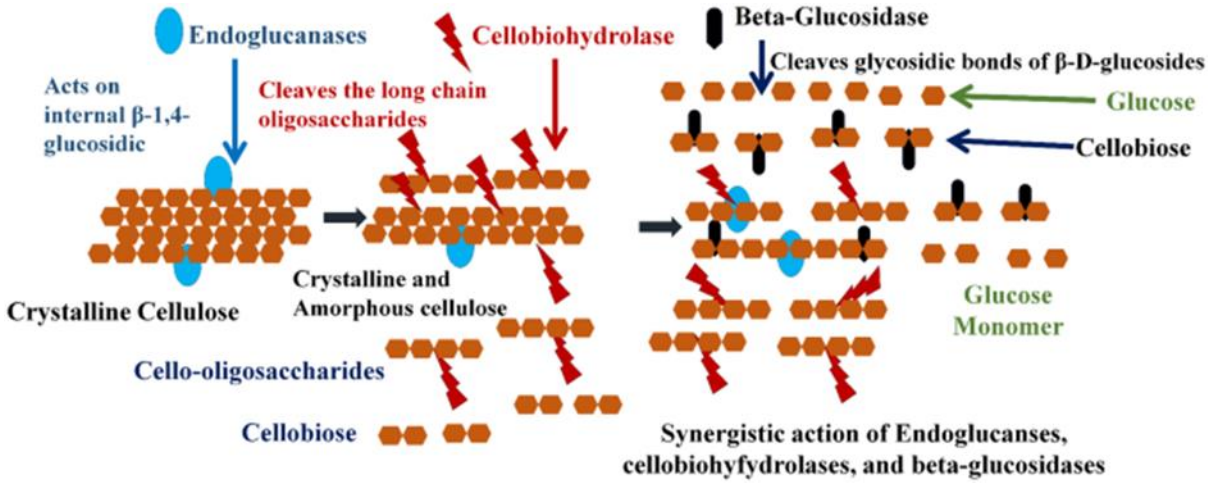

Figure 2: Mechanism of cellulases enzymes (Bhardwaj et al., 2021)

B-D-Glucoside or glucohydralases (E.C.3.2.1.2.1.): These third class of cellulases enzymes also belong hydrolases category. The main function of this glycohydrolase is to cut or cleave non-reducing ends of cellodextrins and cellobiose (Immanuel et al ., 2006).

1.2. Microbial cellulases

There are several microbes known for producing cellulases, but some microbes are popular for producing huge amounts of cellulases at a time. Both aerobic and anaerobic microorganisms are involved in producing microbial cellulases. Usually, bacteria and fungi contribute to microbial enzyme production (Singh et al., 2021). Usually, industries prefer microbial strains that can produce cellulases as an extracellular secretion of the cell. Within a microbe, cellulase enzymes can either be produced by cell-bound or extracellular organelles. But the most prominent microbes involved in producing are bacillus, clostridium, cellulosomes, echinococcus, streptomyces, penicillum, etc. Bacillus is defined as the best-suited microbe for enhancing the production of cellulases in process. Bacillus spp involved are following B agardherans, B circulans, B amyloliquifacuens, B thurigienesis, Paenibacillus, B Aeromonas and fungus spp involved, Penicillium, Trichoderma reesei, Aspergillus niger and many more (Margey et al.,2018).

Table 1: Microorganism and their sources of producing cellulases (Singh et al., 2016)

S.N.	Source of bacteria	Type of bacteria	Species
1	Water, Soil, Compost, sludge and Rumen	Aerobic mesophilic bacteria	1. *B. subtilis* 2. *B. Brevis* 3. *B. thurigienesis* 4. *B. cereus* 5. *Cellulomonas Femi* 6. *C. japonica* 7. *Cytophaga Hutchison* 8. *Paenibaillus polymyxa* 9. *Pseudomonas flurorescens* 10. *Pseudomonas putida*
2	Hot Springs and composts	Aerobic thermophilic bacteria	*Acidothermus cellulolyticus*

			Thermobifida fusca
3	Sewage, compost, food fermenter, mud and rumen	Anaerobic mesophilic bacteria	1. *Acetivibrio cellulolytic* 2. *Bacteroides cellosolve* 3. *Clostridium cellulolyticum* 4. *C. just* 5. *C.cellulovorans* 6. *C. papriosolvans* 7. *C. phytofermentus* 8. *Ruminococcus albus*
4	Hot spring sewage compost and mud	Anaerobic thermophilic bacteria	1. *Thermotoga maritime* 2. *Rhodothermus marinus* 3. *Clostridium thermocellum* 4. *C. stercoraium*

Table 2: Types of fungus and their species to produce cellulases (Pal, 2021; Sarwan, 2021)

S.N.	Source of fungus	Type of fungus	Species of fungus
1	Soil, wood rot, and compost	Aerobic mesophilic	1. *Aspergillus niger* 2. *A .oryzae* 3. *A . eidolons* 4. *Rhizopus oryzae* 5. *Agaricus bisporus* 6. *Coprinus truncorum* 7. *Geotricum candidum* 8. *Tricholadium canadense* 9. *Trichoderma reset* 10. *T. longibrachaitum*
2	Soil , must , compost	Aerobic thermophillic	1. *Thielavia terrestris* 2. *Chaetomium thermophillum* 3. *Paecilomycus thermophile* 4. *Corynascus thermophilus*
3	Rumen and Faeces	Anaerobic mesophilic	1. *Piromyces equi* 2. *P. E2* 3. *Orpinomyces joyonii* 4. *O. PC-2*

1.3. Mechanisms to produce cellulases

Cellolytic enzymes are meant to degrade long-chained cellulose such as lignocelluloses and hemicelluloses. At the end of the cellulolytic reaction, water molecules are released as an end product. Studies suggested that there are two types of cellulases exocellulases and endocellulases. Based on their criteria to cleave polymers of cellulose they are divided into categories of cellulases. Cellobiose, cellodextrins, lignocelluloses, and hemicelluloses can be cleaved by exoglucanase and endoglucanase to reduce either non-reducing end or internal glycosidic bonds and produce water as a by-product. Here Fig 1. shows the enzyme action of cellulases (Verma 2012).

1.4. Factors affecting the production of cellulases

During the fermentation process of several products, enzyme action can be disturbed by factors like

a. Temperature
b. pH
c. Inoculum age
d. Inoculation size
e. Incubation time
f. Incubation temperature
g. Carbon source
h. Nitrogen source
i. Fermenter volume

So all these factors are responsible for either disturbing or enhancing the fermentation process in the industries. Temperature is an important factor, as suggested, that enzymes work at a specific temperature. Enzymes also have optimum pH to maintain their stability. Similarly, inoculum age and size are also responsible for the fermentation process. Carbon and nitrogen sources are required here to maintain stability and as a substrate in the fermentation of cellulolytic reaction. The Volume of the fermenter is also recognized as a crucial factor in maintaining enzymatic reactions. Therefore all of the above components must be optimized for the cellulolytic fermentation process (Sadhu 2013).

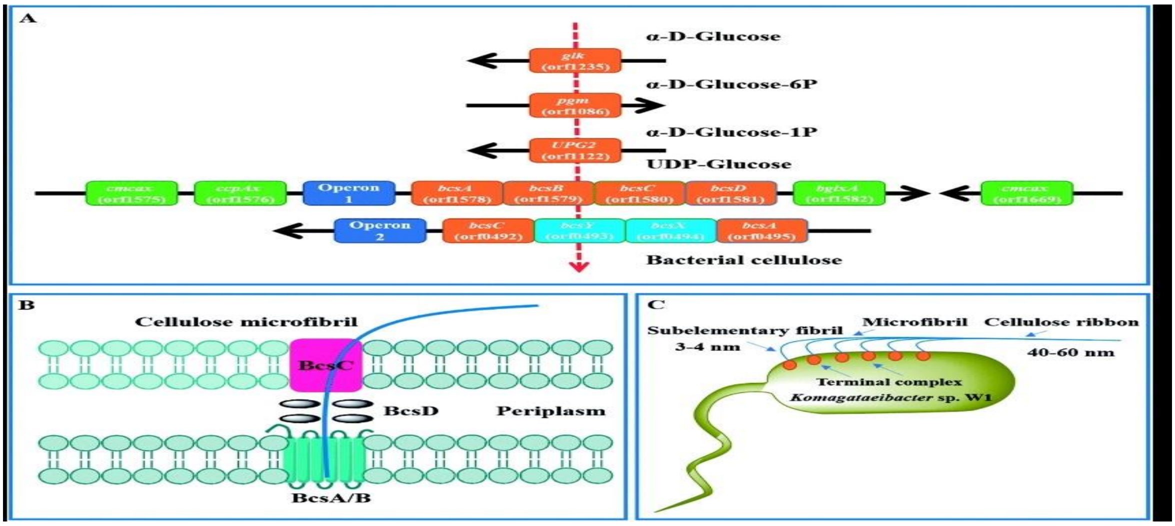

Figure 3: The genetic regulations of cellulose biosynthesis in bacterial cells for conversion of cellulosic mass into glucose by cellulases (Wang et al., 2017)

2. Materials and methods

The methodology for this review paper involved an extensive literature review to gather information on the production and industrial applications of microbial cellulases. Relevant data was collected from scientific journals, research papers, and reputable online databases, and was thoroughly analyzed to identify key concepts and emerging trends. The synthesized information was organized based on specific themes, critically evaluated for accuracy and relevance, and compiled to develop a comprehensive review paper. The draft was reviewed, revised, and finalized to ensure clarity and coherence, incorporating proper citations and references.

3. Results and discussion

3.1. Worldwide status of cellulases

The importance of cellulases in the global enzyme market can be defined as both two figures in which we can say that the demand for microbial cellulases, as well as other industrial enzymes, has been increasing day by day and year by year. In this competitive and rising period, the challenges of industrial enzymes also became very high. Nowadays, not only in the paper and food industry but in every industry like pharma, bioethanol production, and juice industries, every fermentation requires high-yield industrial enzymes with the lowest by-products and higher stability factor.

If it’s discussing the economy related to commercial enzymes, the modified and stable enzymes catch everyone's curiosity, and the economy of the country is completely dependent upon enzymes, and enzymes are the main part of industries like paper, food, brewery, textile, detergent, and many more.

According to recent studies, the global market of enzymes was assigned to produce enzymes about 5 billion USD in 2016, but it has increased by about 7 billion USD in 2021, as results concluded by Business Communication Company Research. Therefore, from the data observed with BCCR, it is proved that not only industries microbial cellulases are also an integral part of the economy of a nation (Islam 2018).

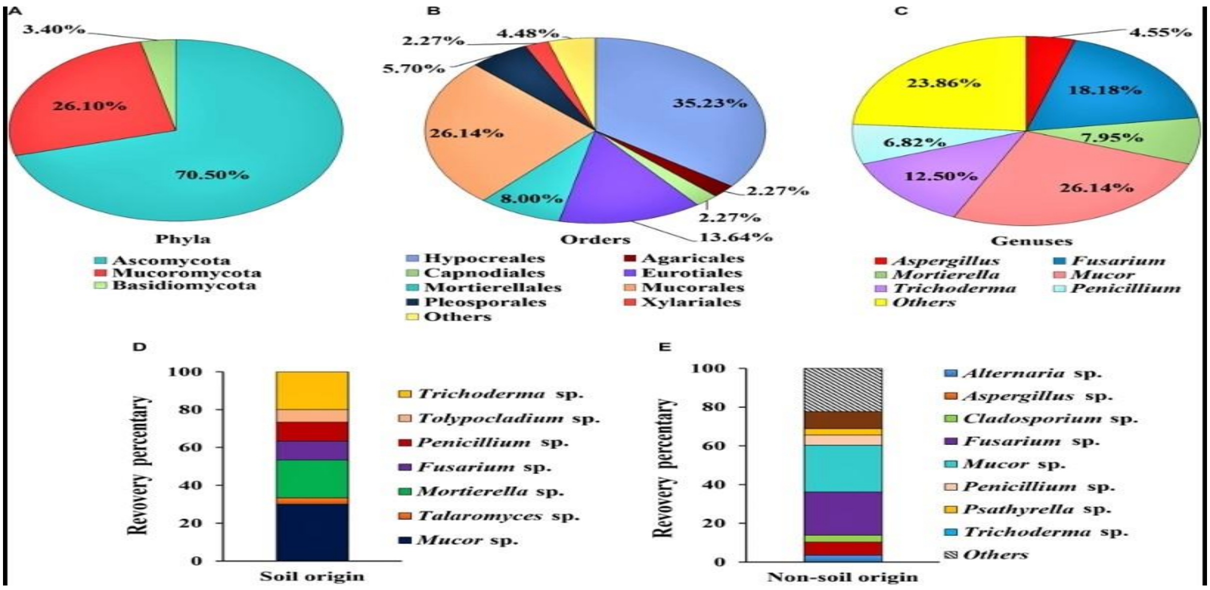

Figure 4: Role Of Cellulases in Global Economy (Li et al., 2020)

3.2. Removal of biofilms with cellulases

Biofilms can be defined as groups of microbial cells that abruptly differentiate and make an aggregate of microbial cells. These biofilms are mainly aggregates of pathogenic strains of fungus and bacteria. Cellulases have larger applications for inhibiting and removing biofilms. According to some studies (Estmat, 2021), the activity of preparing biofilm formation can be decreased with the help of enzymes like cellulases. Different strains available producing cellulases are also responsible for degrading biofilms. Their studies concluded that different concentrations of microbial strains like Penicilium aeruginosa successfully remove biofilms from the medium.

Cellulases are enzymes that can hydrolase beta 1-4 glycosidic links between two polysaccharides. According to some studies, biofilms can be removed with cellulases-producing strains of microorganisms like S. aureus and P. aeruginosa (Jiang 2020).

3.3. Other Applications of cellulases

There is a large number of applications of cellulases, and hence, it is part of almost every industry of the world. Table number 3 shows that cellulases play a major role in fulfilling the demands of industries. Bacterial and fungus strains can produce cellulases, giving greater yields, like Aspergillus, Trichoderma, Bacillus, Penicillium, Rhinococcus, etc. Table 3 will be able to tell that not for fermentation, although cellulases a crucial enzymes for the food industry, bread industry, pharma industry, dye industry, brewery industry, paper industry, animal feed industry, fabric industries, protoplast production, and biofuel production (Subramaniyam 2012)

Table 3: Industrial Applications of Cellulases (Chapman et al., 2018)

S.N.	Type of Industry	Use of Enzyme for degradation
1	Food industry	cellulosic materials
2	Bread industry	Wheat and grams
3	Paper industry	Hemicelluloses and lignocelluloses degradation
4	Fabric industry	Cotton fabric to improve texture
5	Dye industry	Dye effluents
6	Brewery industry	Wheat, rice, barley
7	Animal feed industry	Cereals
8	Pulp industry	Hemicelluloses
9	Agriculture industry	Cell wall of plant pathogens
10	Biofuel industry	Saccharification of lignocelluloses
11	Sewage treatment	Effluents of celluloses like straw, rice, paper
12	Detergent industry	Cellulose for modifying cellulose fibrils
13	Pharma industry	Components of cellobiose
14	Protoplast	Cell walls of plant cell
15	Pollution treatment	Lignocelluloses and hemicelluloses

3.4. Strain improvement of microbial enzymes

Every year, demands of commercial sectors for higher production of fermentation have come up with a new microbial strain. With the effect of the current scenario, industries require a microbial enzyme, which is the cheapest resource as a substrate and minimum cost with higher output. But when talking about the stability and longevity of an enzyme doubts arise in researchers' minds. In various processes of fermentation, cellulases and other enzymes lose their activity just in the first reaction or treatment. Therefore all these troubles and problems lead to strain modification of the microbial enzymes. Protoplast fusion of microbial cells to enhance the yield of an enzyme is not a solution to this problem. several Hence many researchers have been working with several techniques like mutant preparation and with the help of gene medication like site-directed mutagenesis. The purpose of modifying the strain of microbe is to accomplish demand commercial practices (Pandey 1999).

Strain improvement by mutations: The mutation is the classical technique to improve the strain of fungus, yeast, and bacteria. Mutations with the practice of random mutations are the oldest technique and are still acceptable to alter genes.

Mutations with random mutations involved catabolic repression in the end product of cellulose production. After 30 cycles of mutation, the product with enhanced production can be obtained. Most of the time productions by mutants are 4 times higher than wild strains of the microorganisms producing cellulose. There are several examples of different microbial strains that are successfully used for enhancing the production of cellulosic fermentations (Peterson et al., 2012)

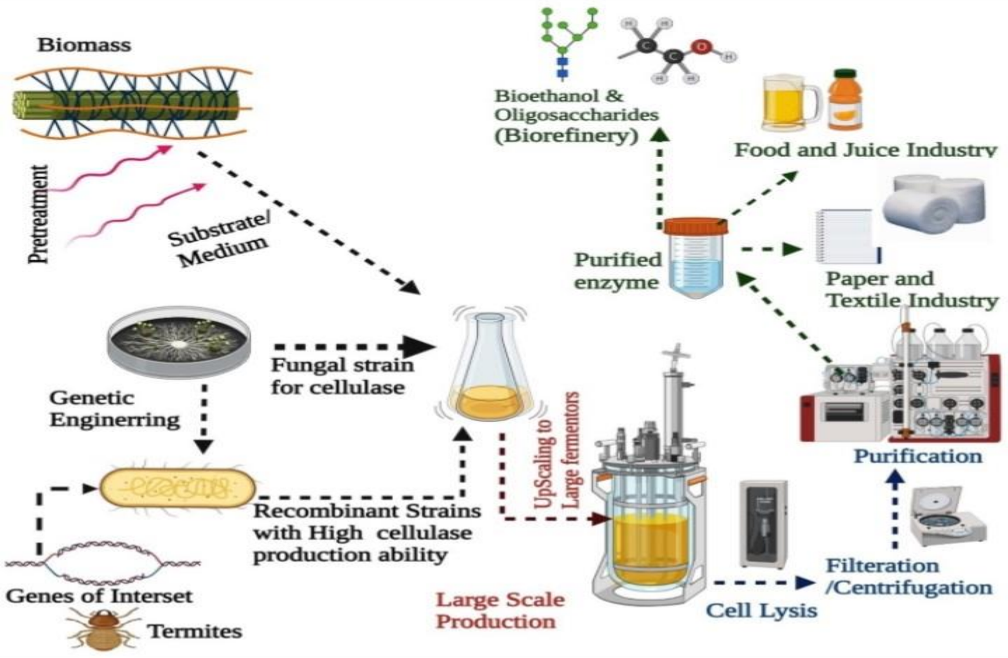

Figure 5: Large-scale productions of cellulases in industries (Bhardwaj et al., 2021)

Enhance production with genetic engineering: Genetic engineering is a different approach to inserting or deleting genes in an organism. Genetic engineering is an achievement for the alteration of genes in microorganisms like fungi and yeasts. Bacillus strain can also be altered with the help of genetic engineering and can enhance the fermentation processing. The main knowledge is required for genetic engineering is the whole genome of the organism, and by knowing the complete genome, it will easier to manipulate target genes. For improving the production with microbial strain the target genetic make-up is required. The biggest complexity that comes up in gene manipulation is the regulation of glycosylation and the existence of introns. Fungus is described as the most prominent host in genetic engineering, and some species have enough tendencies to play a vital role in increasing the production of lignocellulolytic pathways and regulations like Aspergillus, Pseudomonas, and Trichoderma, etc (Singh et al ., 2017)

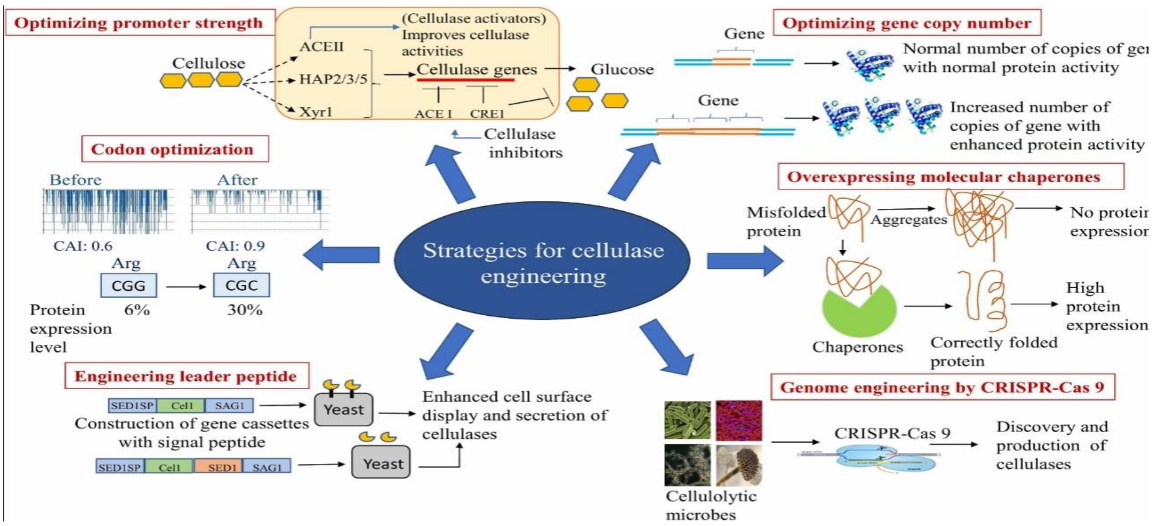

Figure 6: Different strategies of genetic engineering for manipulate microbial cellulases (Dadwal et al .,2020).

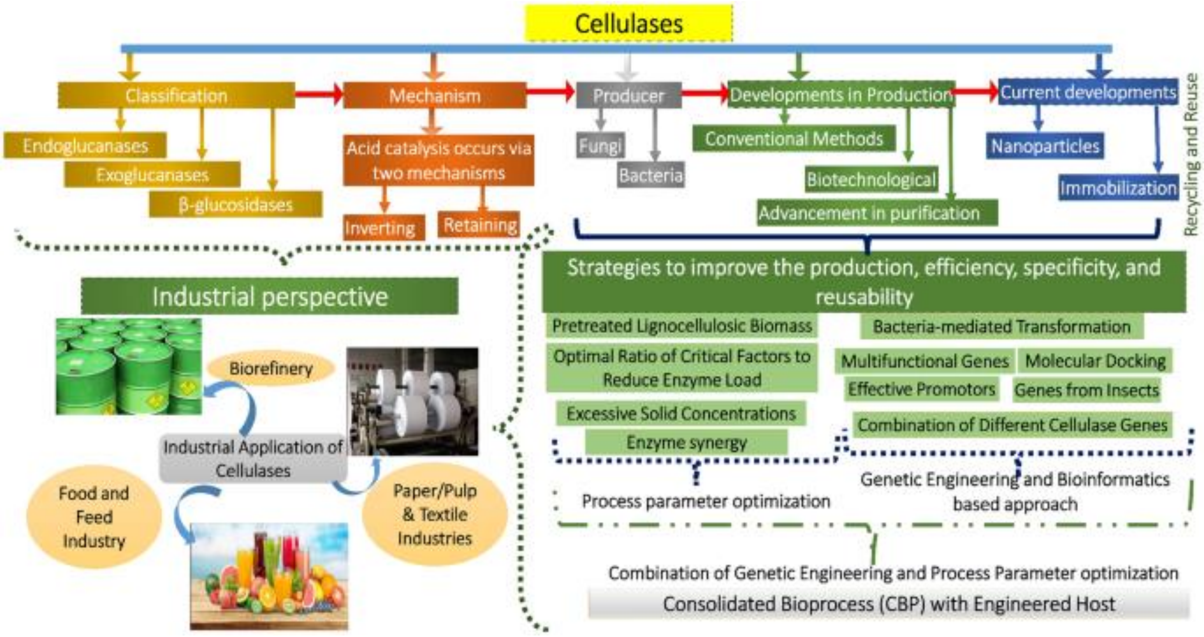

Figure 7: A complete overview of cellulases including applications and strain improvement strategies (Bhardwaj et al., 2021)

Enhance production with site-directed mutagenesis: Other than genetic manipulations, site-directed mutagenesis can make changes in specific target genes and modifications in amino acids. Genetic engineering site-directed mutagenesis also has an achievement with genetic manipulation, and individuals should keep all the knowledge regarding the modifications in specific amino acids. So, in site-directed mutagenesis, the target amino acids are primers with their active sites to be targeted and make changes in protein regulations. Mostly microbial strains in site-directed mutagenesis like Aspergillus, Trichoderma, Clostridium, and many more. In site-directed mutagenesis (Singh et al., 2017).

5. Conclusion

The way cellulases are currently being applied is amazing and garnering interest from all around the world. It has already achieved unparalleled success in the global market. Because of their enormous potential for producing cellulases, microbes are a topic of research for cellulase synthesis. It's evident, though, that more productive species exist in the ecosystem now that scientists haven't yet discovered. It is far more crucial to do additional research and comprehend the obscure processes behind the action of these enzymes. The preference is for microbial cellulases because of their potential uses in numerous sectors. Their business endeavors are growing every day. The increasing need for microbial cellulase is being met by an increasing number of scientific studies that are needed to provide knowledge.

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