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Importance of Microbial Cellulases and Their Industrial Applications

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Abstract :Cellulases are the enzymes that have several industrial applications in worldwide. These cellulases comprises of three main different enzymes are ,β- glucosidase, exoglucanase and endoglucanase are responsible for conversion of cellulosic biomass into glucose.Recent studies on cellulases revealing, cellulases are main ingredients to convert glucose from lignocellulose. As comparable with chemical cellulases , microbial enzymes are cheaper resources for industries and isolated from soil . Cellulose is a biopolymer of plant microfibrils and has to be degraded in the form short linked glycosidase. Submerged fermentation is the best suited fermentation for the production of cellulases on industrial level. The conversion of glucose from cellulose has to be pass through from different challenges during production. So, there are several studies on cellulases activity on cellulose but still not confirmed how many enzymes are responsible for conversion of cellulose into oligosaccharides.The scenario of the cellulases focusing upon three major aspects 1. Decreasing the cost of substrate and increasing the productivity, 2.producing a energy crop highly dedicated for biofuel production and 3. optimizations of enzyme utilization on pilot scale without any bi-products.

INTRODUCTION

Cellulose can be defined as the main structural polymers present in the plant cell wall in the form of micro-fibrils. There are several small units of cello-biose and glucose linked together and form a cellulosic polymer. There is the most important product of the world called enzymes. These enzymes can be carried out from the cheapest source , the soil for the various crucial aspects in the favor of mankind. Enzymes are being used on different stages and for perspectives like food , environment ,detergents in industries. Recent studies on Enzymes concluded large number of applications of Industrial enzyme at very low cost. The biggest issue to be resolve nowadays , is the procurance of energy for the human being .cellulose is the main component of the plant cell wall and found in the form of waste at various sites.

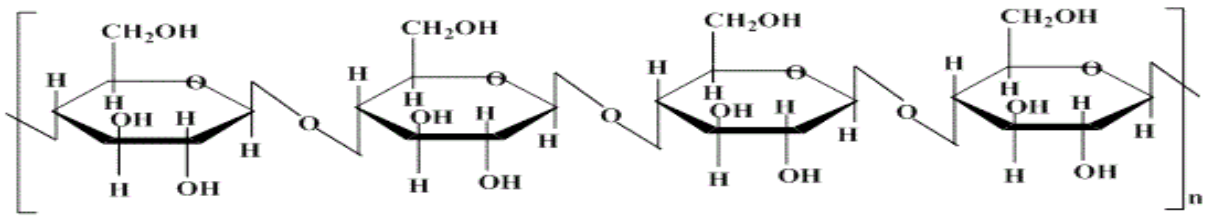

Figure 1.This picture representing the biochemical structure of polymeric cellulose with hydroxyl groups.

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The cellulose can be used food as well as great source of energy for Biofuels .Breakdown of cellulose takes place in the form of glucose through acid hydrolysis and enzymatic hydrolysis. Enzymatic hydrolysis is preferable during processing because it made very less by products and reaction under mild conditions. In the 20thcentury ,Cellulases is most preferable enzymes family at commercial values. *Aspergillusniger* and *Trichoderma sp* is most suited fungus for Industrial purposes. There are large number of group of microorganism responsible for degradation of these complicated linked polymers. Microbes have to be gone through several modifications and challenges for degradation for complex bio-molecules. There are two types of enzymes produced by microorganisms extracellular and bound liknkedendocellular enzymes during digestion of these cellulosic structure. Cellulase can be defined as group of hydrolyticenzymes have an ability to degraded insoluble form of cellulose in soluble sachharides .Cellulases consist of structurally and functionally small units called domains.Previous studies suggests that cellulases isolated from different sources always have different properties than others. The main difference exist in isolates are of their polymeric chains in their structures.

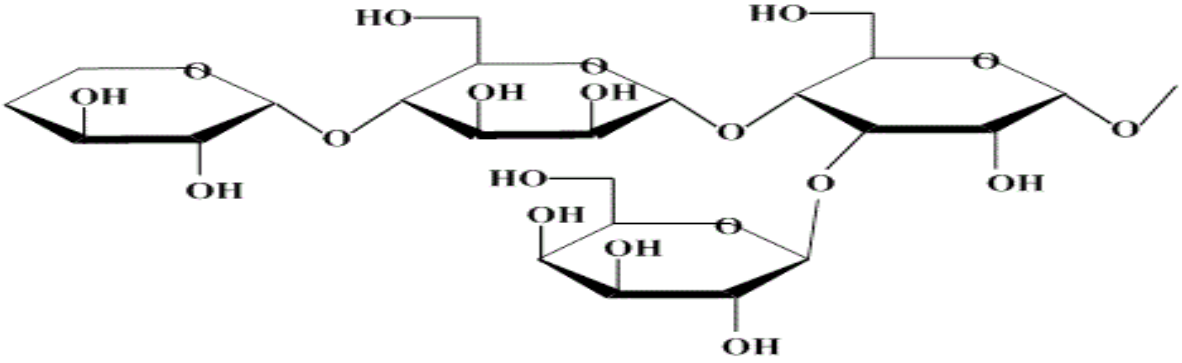

Figure 2. This picture represents the structure of lignocelluloses.

Classification of cellulases:

Cellulases are enzymes ,that have complex structures consist of exo-1,4-β-D-glucanase(exoglucanase ,EC3.2.1,91) , endo – 1,4-β-D-glucanase (endoglucanse, EC.3.2.1.4) and β-D-glucosidase(β-Dglucosideglucanhydrolase , EC 3.2.1.21).

Exoglucanase:

Exoglucanase also known as cellobiohydrolase having linkages exo-β-1,4-D glucanase has main function to split chain structute in cellulose to make cellobiose units with reducing ends . This enzyme can't hydolyseendo membranes of cellulose fibres only degrade outer crystalline structures.

Endoglucanase:

Endoglucanase(endo-β-1,4-D-glucanase) It is also called CMCase or hydolysescarboxymethyl cellulose . These endoglucanase directly react upon cellulosic long chains and converted them into cellobiose(disaccharides) and glucose.

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B-glucosidase:

β-glucosidase is the hydrolytic enzyme responsible for completion the step hydrolysis by the cleavage of cellobiose and remove oligosaccharides from non-reducing ends and formation of aryl β –glucosides.

Fungal celluloses:

Insoluble Celluloses arehydrolysed by microbial enzymes mainly ,endoglucanase, exoglucanase and β-glucosidase to convert into soluble form or simple oligosaccharides/glucose. Around 80% of cellulose digestion is done by fungi with several spp. Fungi secrets these enzymes to degrade biomass in the process bioremediation as well as producing these enzymes for commercial purposes as comparable to chemical enzymes. The fungi members involved in cellulose degradations are *ascomycetes, basidomycotas, deuteromycota* and *chytrids*. The cellulolytic fungi spp are following *aspergillus, penicillum, chaaetomium, trichoderma, fusarium, stachybotys, clasdosporuim, alternaria, acremenuim, ceratocysts, mycrothecium, humicola*, etc.

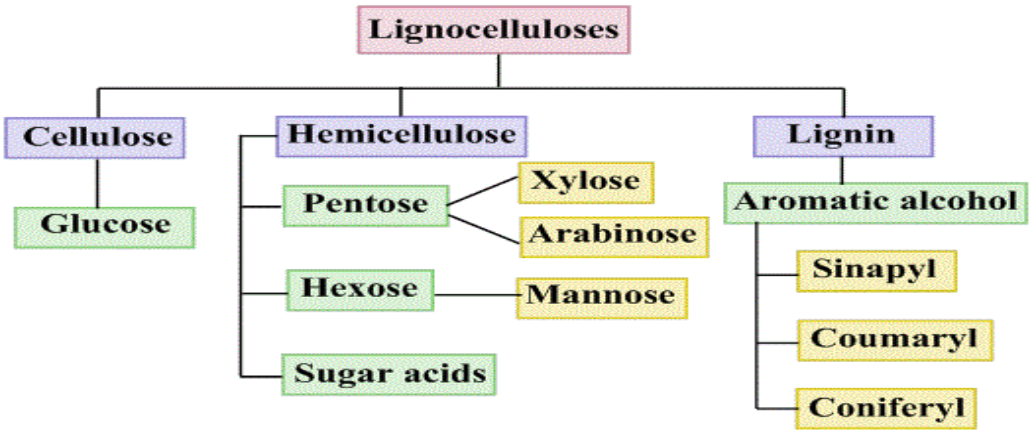

Figure 3. This figure represents the main components present in Lignocellulose

Ethanol production by lignocelluloses:

The plant material usually contains cellulose, hemicelluloses and lignin not glucose and starch. The plant micro-fibrils composed with main materials are , Hemicellulose , cellulose and glucose in the form of crystals to protect plant cells from water and enzymes. Lignin is the most complex molecule among all of these(Schubert,2006). The conversion of hemicelluloses and cellulose takes place into bioethanol production. The bio fuels produced by first generation technology involved distillation and fermentation of starch rich crops and sugars in cellulose. The conversion of lignin into sugars is the most resolvable task through out the process. The modern second generation technique involving fermentation and distillation process of bioethanol by taking raw material like straw, energy crops and forest wastes like dead leaves etc.

Cellulose degradation for Biofuel production :

Continuously depletions of fossil fuels and non renewable resources lead to increasing demand of other Renewable resources. The higher demand of these alternative resources have responsible production of bio fuels. So , in the production of biofuel involves degradation of

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cellulose , hemicelluloses and lignin. This bioconservation is depend upon synergins , product inhibition and stability of lignocellulose biomass. Cellulose degradation totally depends upon higher enzyme concentration and low requirement of equipments. Cellulose degradation of biofuel production takes place with the help of submerged fermentation. Enzyme components lead to hydrolysis of lignocelluloses substrate can directly used SSF as a enzyme product. Celluloses have main efficient function here is to reduce production cost of SSF and low energy rate. SSF provides a suitable environment to fungi during fermentation for easy cultivation. Lignocellulosic are the biomass that present in the huge amount in the environment and great source as substrate for biofuel production.

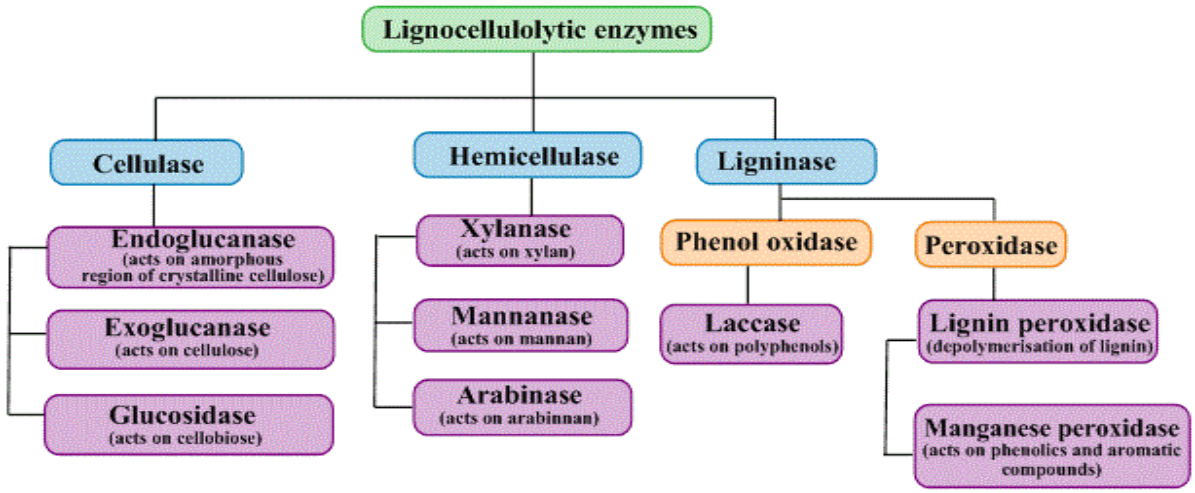

Figure 4.This figure representing the number of enzymes responsible for Biofuel production.

Non cellulase proteins developed from cellulase.

There are several protein formation during enzyme secretion for cellulose degradation in cultivation. Cellulase enzyme also responsible for non cellulase proteins. There are two types of cell proteins formed during cultivations one is expansions (plant protein) and other one is fungi protein called swollenin. Recent studies proved that expansin can be secret by*Bacillus subtilis* and *T. fusca*.

Modeling of cellulase activity.

There are large number of mechanisms from previous studies to elaborate cellulases activity on cellulose but still there is no appropriate mechanisms that can explain actual mechanism of thsescellulases on cellulosic biomass to sugar production.

Cellulosic engineering

The engineering of cellulase depends upon three major tasks for a great scenario of these enzymes are - To increase cellulasethermostability to lead higher activity, effective impact on crystalline cellulose and a rational design . There are large number of thermostable enzymes already exist but have low activity on celluloses and only some sequence known.

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Designing cellulosomes

A new product form during hydrolysis of crystalline cellulose in soluble glucomeric structures are called cellulosomes. These cellulosomes are actually ahybrid structure. Its an another approach to the researchers to create these cellulosome into hybrid scaffoldin . These scaffoldinare hybrid molecules having cohesions which can bind to another organisms with different site specificity. Benefit of the scaffoldinto attaching with appropriate cellulosomes for controlling the utility of enzymes. But there is no exact composition and geometry not known yet. There is a bacterial *spp* that is know to produce these domains called *T. fusca.*

Serial number	Types of industries	Applications
1	Biofuel	• To preserve high quality fodders • To produce food having high energy properties of animal food • To increase nutritional level of ruminant • To produce fermented products like ethanol, organic acids and solvents • To produce single cell proteins and lipids with microbial substrate
2	Detergent and Laundary	• To remove tough protuberance from cotton fibers • To remove sticky deposited ink stains • To improve color brightness • To increase dirt removal ability
3	Textile industry	• Preferable over pumic stones for bio stoning • To reduce unrequited indigo dye from denims • Defibrillation and softening of colored fibers. • Degradation of protruding fiber for better finishing
4	Fermentation	• To enhance color extraction from grapes • To increase malting and mashing procedure in wine making • To improve quality of beer
5	Agriculture	• To control over on plant pathogens and plant diseases • To reduce the dependency on mineral fertilizer and improve soil quality

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		• Improve the flowering of plants • Enhancing the seed germination process
6	Paper and pulp industries	• Biomechanical pulping preferable over mechanical pulping • Bio characterization of fibers • To reduce bitterness of citrus fruits • To produce soft paper and paper towels • To produce biodegradable cardboards
7	Food and animal feeding	• To extract olive oil • To extract and clarify fruit juices and vegetables • To extract cartenoids during food color productions • To release antioxidants from fruits
8	Others	✓ To produce cellulosomes and reduce biomass waste.

Table no.1 .cellulase from bacterial origin and their applications : A review (ShwetaAggarwal , 2012)

Microorganismsinvoledcellulase production:

1. Cellulolytic bacteria : There are large number of microorganisms involved in cellulose degradation . The group of bacteria than utilize carbohydrates with cellulose are namely, *cellumonas*and *cytophaga*. These cellulolytic bacteria utilize carbohytrates and produce hydrolytic enzymes.(Sukumaran et al;2005)

2. Clostridia species:

The bacteria of this group is totally an anaerobic microbes and aerotolerant, with the spore formulation behavior. It usually exists in bacillus form and easily available in soil. This bacterial falls in both categories gram positive as well as gram negative bacteria.(Wells et al; 1996). The species involves in this group are *Clostridium thermocellulm*, *Clostridium cellulolyticum, Clostridium stercorarium, Clostridium acetobutylicum etc.*

3. Cellulomanas species:

The members present of this group usually does not form spores, they are gram negative and mesophillic bacteria. They exists in rod shapes and higher G+C content about 72%. This group responsible for producing extracellular cellulases. The members of this group are of following name ,*Cellulomonasfimi , Cellulomonasuda , Cellulomonasflavigena*and *Cellulomonasfermentans.*

4. Bacillus species :

The microbes present in this group of *Bacilaceae* are endo spore forming bacteria . These are gram positivebacteria , secreting large number of enzymes like , amylase, protease ,β-

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glucanase and hemicellulases. Examples of these groups are *Bacillus subtilis, Bacillus polymyxa , Bacillus licheniformis , Bacillus cereus etc.*

5**. Thermonosporaspecies** :
The group involved bacteria exihibit as thermophiles , and aerobic bacteria. These microbes secrets extracellular cellulase and β-endoglucanases. *Thermonosoprafusica*and *Thermonosporacurvata.*

6. Acetivibro species:
The member of this group utilizecellulobiose and salicin only for degradation . thespp involved in this group is *Acetivibriocellulolyticus*. These microbes are usually in gram negative form and mesophilic bacteria.

7. Ruminococcus species:
The member of these group are called rumen bacteria . These group of bacteria usually degrade cellulose fibers . ruminococcus group show their cellulobio -hydrolase acitivity by forming a cellulosome like complex after degradation . The members involved in this group are following *R. albus and R.flavefacians*

CONCLUSIONS

According to the above data ,cellulases are the main source of biofuels and several industrial applications. The demand of cellulases increasing day by day because of their low cost and easy availability in the environment .Though there are several modifications and challenges have to face to these microorganisms to fulfill requirements on large scale production. Cellulases are effectively help to reduce biomass waste on large scale and helping in bioremediation . These cellulases are thermostable, thermophilic and addition of some co-factor can enhance the production of large scale . Therefore, on the commercial point of view these cellolytic enzymes are very much valuable and cost effective, but slight modifications and engineering can support a better scenario of these microbial enzymes.The scenario of the cellulases focusing upon three major aspects 1. Decreasing the cost of substrate and increasing the productivity, 2.producing a energy crop highly dedicated for biofuel production and 3. optimizations of enzyme utilization on pilot scale without any bi-products.

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