A brief review study on cellulase enzyme; properties, types and industrial application

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Abstract

Cellulose is an abundant natural biopolymer on earth and most dominating Agricultural waste. This cellulosic biomass is a renewable and abundant resource with great potential for bioconversion to value added bioproducts. It can be degraded by cellulase produced by cellulolytic bacteria. This enzyme has various industrial applications and now considered as major group of industrial enzyme. The review discusses application of cellulase, classification of cellulase, quantification of cellulase, the types of cellulolytic bacteria and their screening and biotechnological processes related to Cellulolytic enzymes. Cellulolytic enzymes have been studied as input for bioconversion and as a product from lignocellulose fermentation and bioethanol and biobutanol production. Cellulase production is the most important step in the commercial production of ethanol and other chemicals from renewable cellulosic material. This review is focused on current advances and innovation in cellulolytic enzymes production using plant biomass, and application of the enzymes in lignocellulose conversion to fermentable sugars for lignocellulose production.

Keyword: Cellulase, Enzyme, Cellulose, Microorganism, Bacteria, Fungi, Lignocellulose, Biofuel

Introduction

The success of the plan to convert cellulose waste into sugars and fuels depends on the successful and cost-effective production of the cellulase enzyme. Cellulase production is the most costly part of this process, accounting for about 40% of the total cost. The enzyme cellulase is used to convert cellulose to glucose. The glucose obtained is also converted to ethanol by fermentation. The enzyme cellulase is made up of many microbes, but wood-based fungi, such as trichoderma, are more suitable for cellulose. Researchers working with Alvin Rice and Mary Mandellas at the U.S. Army Laboratory in Natick, Massachusetts, found that the microbes responsible for the flaxseed contained an enzyme that hydrolyzes flaxseed. Rice and Mandellas determined the culture conditions of cellulase enzyme in aqueous medium. Despite much research and development on cellulase production in the alcohol production process. It should be noted that cellulase is made in an environment immersed in water and in fermentation tanks, similar to that used to produce antibiotics. Each fermentation tank has a volume between 38 and 178 cubic meters and is completely sterilized. The culture fluid also contains a source of carbon, salt and mixed fertilizers such as soaked corn syrup. Glucose also stimulates the growth of the organism but has no effect on cellulase production. The carbon source must contain sugar, which leads to the production of cellulase. The most important and well-known sugars that intensify cellulase production are celery, lactose and sonorose. The feed solution is sterilized with direct steam before fermentation and the enzyme strain is added to the cooled mixture of the fermentation tank. All microorganisms are able to destroy the cell wall of plants. However, aerobic and anaerobic microbes use two different pathways. Aerobic cellulose destroyers, including bacteria and fungi, with few exceptions, will produce a set of enzymes that are secreted in the extracellular environment and have an aggravating effect on cell wall destruction. Cellulose-destructive anaerobic microbes, on the other hand, form a collection of enzymes called cellosomes, which have a molecular weight of 16-16 megataltons and sometimes as much as 100 megadaltons in the case of cellosomes. The production of cellosomes was first reported in *Clostridium* thermocellum , and later in other species of *Clostridium*, other anaerobic bacteria were reported[1].

Cellulase is one of the important industrial enzymes. Cellulase is used in agriculture to break down lignocellulosic wastes, in the food industry to reduce the cellulose of food raw materials, in papermaking to soften wood. The enzyme cellulase is used in detergents for purification and color clarity. In the textile industry, it is used to soften linen and polish coarse linen fabrics. The enzyme cellulase (B-TXL enzyme from the German company ASA) is also used to produce alcohol from linters (cotton mill waste). The use of enzymes has become commercialized due to its ease of use in the recycling process, its high efficiency and its cost-effectiveness compared to the conventional deodorization method. The most effective enzymes used in this section are cellulase and lipase. By hydrolyzing the microfibrils around the main fiber, the enzyme cellulase releases toner and ink particles from the fiber surfaces and, as a result, increases the dewatering intensity in the paper machine. Cellulase production is the most expensive step in the production of ethanol from cellulosic materials. Cellulase production accounts for approximately 40% of the total process cost. For this reason, if an economical technology for cellulase production can be achieved, there will be a significant reduction in the cost of ethanol production [2].

Cellulase producing using microorganisms

Bacterial cellulase

Numerous studies on cellulase and other polysaccharides have been conducted since the 1950s. Mesophilic microbes are the major producers of these enzymes. Methods for the detection, purification and detection of cellulases are constantly evolving. Numerous microorganisms have been identified and isolated as generators of this enzyme. Enzymes produced by vibrios and Escherichia coli are active at alkaline pH. This makes them suitable for use in washing powders. Many bacillus cellulases were obtained that remained active for 18 hours at pH 4.5 to 8. Typically, cellulase is produced by bacteria in the last phase of growth. For this reason, maintaining cellulose-producing culture media is not economical for the long term. But Marinobacter produces this enzyme early in its growth. The enzyme exhibits a maximum activity at pH 9 and a temperature of 25-35 ° C and is resistant to a wide range of pH. Maintains 80% of its activity at pH 8 to 12. Among anaerobic bacteria, Clostridiums are major cellulase producers that secrete cellosomes and can hydrolyze crystalline cellulose. Endoglucanases are derived from a species of Clostridium capable of hydrolyzing CMC and soluble crystalline cellulose. For cost-effective production of this enzyme, its gene producing E. coli has been cloned. The optimum temperature and pH for its activity are 65 degrees Celsius and 6.5, respectively. A number of cellulose-producing thermophilic bacteria have been identified and isolated, but the results for hyperthermophilic bacteria show that such enzymes are less present for these bacteria. Aerobic bacteria are more limited in their production of cellulose than anaerobic bacteria[3].

Thermophilic cellulase

Thermophilic microorganisms may grow at temperatures above 100^0 C. In other words, thermophilic microorganisms survive and work at 65 to 85 degrees Celsius. But hyperthermophiles grow at temperatures above 85%. These microorganisms produce heat-resistant cellulolytic enzymes, which makes them a good choice for industrial processes. High temperatures increase the reaction rate and dissolve the substrate. In addition, microbial contamination is reduced. Thermophilic microbes are species of an ubacteria and are the major thermophilic microorganisms that destroy cellulose. These include *Rhodothermis*, *Caldibacillus* and *Cellulovans*. Cellulase production has also been reported in a number of thermophilic fungi.[3].

Fungal cellulase

Fungal cellulases have been extensively studied for use in industrial processes. The characteristics of some of them are listed in Table (1).

Table (1): Cellulase-producing microorganisms [4,3]

Microorganism		Microorganism
Acermonium cellulolyticus	Bacteria	*Clostridium thermocellum*
Aspergillus acculeatus		*Ruminococcus albus*
Aspergillus fumigatus		*Streptomyces sp.*
Aspergillus niger		
Fusarium solani		
Lrpex lacteus		
funmiculosum		
Phanerochaete	Actinomycetes	*Streptomyces Sp.*
Chrysosporium		*Thermoactinomyces sp.*
Schizophyllum commune		*Thermomonospora curvata*
Sclerotium rolfsii		
Sporotrichum cellulophilum		
Talaromyces emersonii		

Aerobic and anaerobic fungi both produce cellulose enzymes. Aerobic fungi play a major role in the production and degradation of plant materials and are found on wood, plants, soil and agricultural residues. Only a small number of filamentous fungi produce thermophilic cellulase. Hume Mola secretes several cellulases. These enzymes work at a pH of 5.5 to 9. Secretory cellulas from different fungi differ in their degree of activity. Fungal cellulas have been shown to act differently in cellulose production in terms of hydrolysis efficiency. Cellulase-producing thermophilic fungi include thermophilic *Chaetomiunb* species, many of which secrete cellulase assemblages together. The fungus Talaromyces emersonii, which has attracted the attention of biotechnology researchers in recent years, is able to produce a complex of seven enzymes including 4 exocellulases and 3 beta-glucosidases. This enzyme cocktail can hydrolyze 3-1 and 4-1 beta-glucans. Since this fungus is able to make high amounts of this enzyme complex, several attempts have been made to increase its efficiency by using inexpensive substrates such as sugarcane pulp. This complex has not only cellulite activity but also amyloolytic and hemicellulite activities have been reported. Unlike other celluloses derived from the Trichoderma family, this cellulase is also nutritionally safe. Commercially, the microorganisms Trichoderma and *Aspergillus* produce cellulose. The raw enzyme is produced by a microorganism in one process and can be used commercially. Trichoderma produces relatively large amounts of endobetagl canas and exobetaglucanas and small amounts of beta-glucocidas. When *Aspergillus* is used, large amounts of beta-glucanas and beta-glucosidas are produced, and exobetaglucanase production is very low. Cellulase enzyme production (exoglucanas and endoglucanas) has been compared in some microorganisms, including the industrial fungus *Chrysosporium* pharynx, the incomplete fungus *Aspergillus trous*, yeast, and the cellulase-degrading micrococcus. The studied fungi show higher enzyme production than other microorganisms and *Aspergillus trous* fungus isolated from the natural environment (rotten wood) is also able to produce more enzyme cellulase than the industrial strain of *Chrysosporium ferrocet* [5].

Enzymatic hydrolysis of cellulose using industrial Enzymes

In the past, acid hydrolysis has been widely used in the process of converting cellulose to ethanol. However, due to the great potential of the enzymatic hydrolysis process and the advantages that this process has over other methods, today this method has attracted a lot of attention. Most recent research on the process of converting cellulose to ethanol is based on the enzymatic process. Due to the impermeable physical structure of lignin in lignocellulosic materials as well as the crystalline structure of cellulose, enzyme access to cellulose molecules is simply not possible and the reaction rate is low. There are many different ways to convert cellulose to ethanol. But the enzymatic hydrolysis method makes the ethanol produced from mass-produced plant materials economically comparable to other liquid fuels. The following benefits can be achieved if inexpensive enzymes are used in the enzymatic hydrolysis process.

High efficiency of production and control of by-products, Simple and convenient operating conditions, so that the need to use expensive materials to build process machines is eliminated, Low energy consumption compared to other methods.

In general, enzymatic hydrolysis processes are divided into two general categories[5]:

Processes in which enzymatic and fermentation steps are performed separately and are known as SHF methods. Processes in which the fermentation and hydrolysis steps are performed simultaneously, these processes are also divided into the following two general groups:

Simultaneous process of sugar conversion and fermentation, which is known as SSF method. Simultaneous sugar conversion and fermentation process for both cellulose and hemicellulose components, which is known as SSCF method. In the SSCF process, cellulose is converted to glucose and hemicellulose to xylose and arabinose during the sugar conversion phase. During the fermentation process, both glucose and xylose will be fermented to ethanol. SSF and SSCF methods are said to be more suitable due to the simultaneous fermentation and hydrolysis process in a tank and due to the reduction of the initial cost of the devices and the reduction of the production of sugar produced during the process. A schematic diagram of the cellulose to ethanol production process, which uses an enzymatic hydrolysis process, is shown in Figure (1).

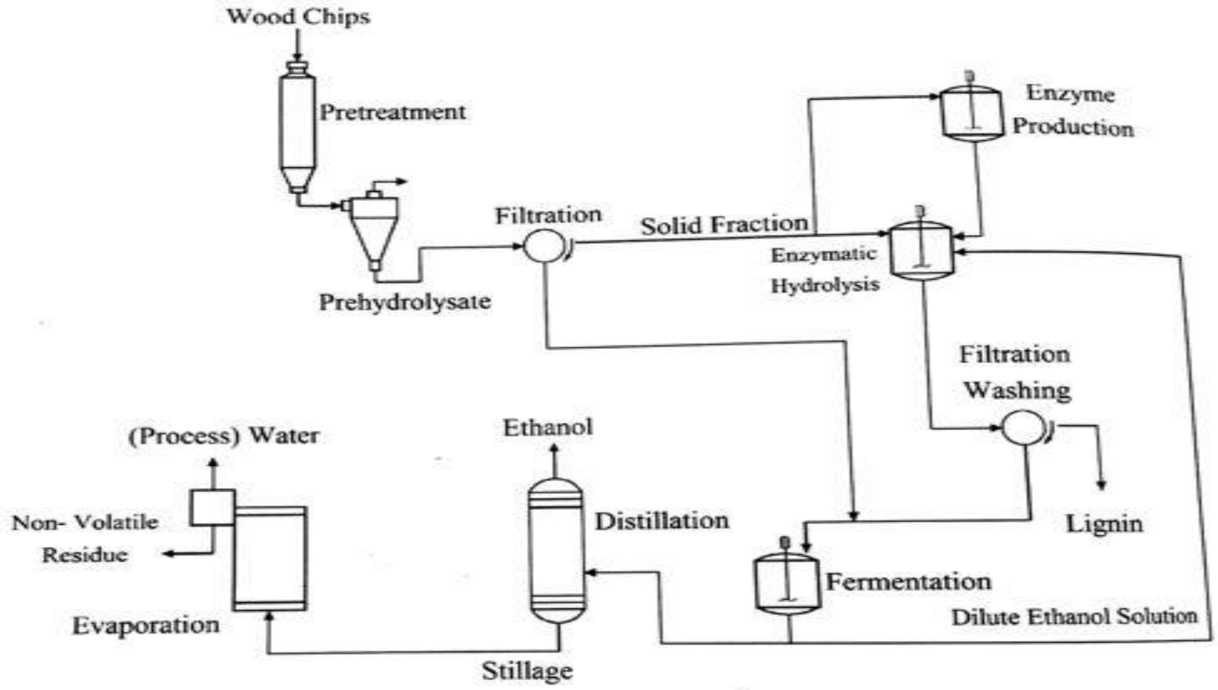

Figure1. Enzymatic hydrolysis process using industrial enzymes[6]

Conclusion

The conversion of cellulosic biomass by microorganisms is a potential sustainable approach to develop novel bioprocesses and products. Microbial cellulases are now commercially producing by several industries globally and are being widely used in food, animal feed, fuel, paper industry, textile industry and also various chemical industries. Cellulase research has been concentrated mostly in fungi but there is increasing interest in cellulase production by bacteria due to their higher growth rate and thermo stable and alkali stable properties. The development of rapid and reliable methods for the screening of cellulases from microorganisms within inhospitable environments will allow a greater number of novel bacterial cellulases to be isolated with purpose for industrial use. Our current knowledge of the production, purification ,characterization, biochemistry, molecular biology of these enzymes and of the producer bacteria is considerable. However, these novel enzymes can be further engineered using available knowledge of enzyme structure and function through rational design or, they can be improved using random mutagenesis techniques with focus on selection of ideally augmented traits through directed evolution. Moreover, improvement of bacterial cellulase activities or imparting desired characters of enzyme by protein engineering is may be another area of cellulase research. Despite the progress achieved so far for bacterial cellulases, more effort is also needed for cellulases and bacteria to have important industrial impact[6].

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