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A Comparative Study of Cellulase Production: Minireview

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Abstract

Cellulose is one of the most abundant polymers on earth. It is present in huge amount in the various wastes of agricultural produce. Cellulase enzyme can be produced by using these wastes. Cellulase enzyme has been produced by a wide range of microbial flora such as fungi and bacteria. Production conditions for the production of cellulase enzymes has been optimized using one factor variable at a time approach. Various production conditions such as carbon source, nitrogen source, pH, temperature, incubation temperature and inoculum size have to optimized for maximum enzyme production. This review describes the production of cellulase enzyme using various agricultural wastes, which are reported in the literature till now. In this, we have mentioned the various microbial species, which are able to produce cellulase under optimum conditions. In this we have also discusses the comparative production of cellulase enzyme using submerged and solid-state fermentation. In today's world, due to wide role of industrial enzymes in commercial market makes them more popular and we have to focus on more research towards their production using cheap substrates. These substrates are available annually and also environmentally friendly to use. The above-mentioned methods are very useful for the production of cellulase by reducing disposal problem of these agro-wastes. These substrates are more efficiently consumed by a potential strain like microbes, plants, etc. for production of cellulase enzyme. By this review, we are trying to explore the more potent source of cellulase production both in terms of increased productivity as well as quality.

Keywords: Cellulose; Cellulase production; Fermentation conditions; Microbial sources; Solid state and submerged fermentation; Agrowastes.

INTRODUCTION

One of the most abundant polymer present on earth is cellulose. It is the one of the most important renewable resource and primary product of photosynthesis produced in the atmosphere (100 billion dry tons/year). Cellulose is also present in paper, paperboard and fabric formed of cotton, linen and other plant fibres. Cellulose is tasteless, odorless and hydrophilic in nature. It is made up of several D-glucose units linked with β (1→4)- glycosidic bonds and also the main component of green plants cell wall [1], various algae and oomycetes. A few species of microbes such as bacteria produce cellulose to make biofilms. The process of cellulose hydrolysis breakdown cellulose polymer into its monosaccharide form i.e. glucose residues but due to the tight linkage among cellulose molecules, cellulolysis is a very tough process as compared with other polymeric units. Cellulose is converted into useful products by cellulase enzyme. Cellulase enzyme production was done by microbes either in cell associated or free form, which absorb the insoluble cellulose [2]. Various microbes have been reported in the literature for the production of cellulase enzymes such as bacteria and fungi [3]. The enzymatic hydrolysis of cellulose using cellulase enzyme needs combined action of three enzymes i.e. cellobiohydrolase, endoglucanase or carboxymethycellulase (CMCase) and β-glucosidases [4]. Apart from microorganisms some dairy animals like cow and sheep also have microbial flora just like bacteria, which can be used to produce cellulases. As cellulose is found in huge amount in the agricultural waste products, so these agrowastes can be utilized by cellulolytic microorganisms as their sole carbon source [5].

In various industries, cellulases are widely used to increase the product quality and quantity such as in textile industry for softening of cotton and finishing of denim, in laundry detergents for color care, cleaning, and anti-deposition, in the food industry for mashing, in the pulp and paper industries for deinking, drainage improvement and fiber modification and also in pharmaceuticals [6,7]. Apart from above mentioned applications cellulase enzymes are also use in the production of biofuels.

Production of ethanol from various lignocellulose materials is one of the new techniques to produce renewable resources of fuel [8]. Therefore, in order to commercialize these technologies, the interest is continuously rising for producing biofuels by utilizing the cheap biomass materials such as molasses or other starchy plant sources. The production of ethanol using lignocelluloses requires hydrolysis, which can be acidic or enzymatic. Acidic hydrolysis leads to the release of toxic wastes and not easy to convert the biomass in reducing sugars, on the other hand enzymatic method is more efficient in generation of reducing sugar and also ecofriendly in nature [9].

Therefore, this review discussing about the production of cellulase enzyme, due to abundance of cellulose on earth and also in the various wastes of agricultural produce. Cellulase enzyme has been produced by a wide range of microbes and production conditions has to be optimized using one factor variable at a time approach. Various production conditions such as carbon source, nitrogen source, pH, temperature, incubation temperature and inoculum size have to optimized for maximum enzyme production. So, this review describes the production of cellulase enzyme using various agricultural wastes, which are reported in the literature till now. In this, we have mentioned the various microbial species, which are able to produce cellulase under optimum conditions. In this we have also discusses the comparative production of cellulase enzyme using submerged and solid-state fermentation. In today's world, due to wide role of industrial enzymes in commercial market makes them more popular and we have to focus on more research towards their production using cheap substrates. These substrates are available annually and also environmentally friendly to use. The above-mentioned methods are very useful for the production of cellulase by reducing disposal problem of these agro-wastes. These substrates are more efficiently consumed by a potential strain like microbes, plants, etc. for production of cellulase enzyme. By this review, we are trying to explore the more potent source of cellulase production both in terms of increased productivity as well as quality.

Cellulase producing microorganisms

A wide range of fungal and bacterial groups are able to produce cellulase [10]. Various bacterial species like *Pseudomonas, Cellulomonas, Bacillus, Micrococcus, Cellovibrio* and S*porocytophaga* [11] have been reported in literature. Some of *Bacillus* species such as, *B. agardherans, B. circulans* and *B. subtilis* were also able to produce cellulolytic enzymes [12] *Bacillus amyoliquefaciens* [13]and *Bacillus thuringiensis* [14] have also been well documented in literature.

Some anaerobes also able to degrade cellulose such as *C. thermocellum, C. cellulolyticum* and *C. cellulovorans* [15]. In various anaerobic species *Bacillus* strains, *B. agaradhaerens* JAM-KU023, *B. subtilis* DR, *B. pumilus* EB3 and *Brevibacillus* JXL are able to produce endoglucanase [16,17]. Some mesophilic species such as *Cytophaga hutchinsonii, Cellvibrio fulvus, Cellvibrio gilvus, Erwinia carotovora* and *Paenibacillus campinasensis* [18] have been also well studied by some workers. Archaebacterial species i.e *Pyrococcus horikoshii* and *Thermotoga neapolitana* are also have ceellulase producting ability [19]. Among cyanobacteria like *Oscillatoria, Phormidium, Nostoc* and yeasts mainly *Cryptococcus* sp. are also well documented [20].

Fungi are the most potent source of cellulase production [21]. Several species of fungus like *Penicillium funiculosum* [22], *Aspergillus niger* [23], *Sclerotium rolfsii* [24], *Penicillium pinophilum* [25], *Penicillium sp.* CR-316 [26], *Penicillium sp.* [27-30], *Trichoderma reesei* [31, 32], *Fusarium oxysporum* [33] *Humicola* sp. [34], *Gloeophyllum trabeum* [35], *Melanocarpus* sp. [36] and *Ascomycota* [37, 38] are well reported in literature. Six species of Trichoderma for cellulase production have been reported by Chandra *et al*. [39]. Thermophilic fungus such as *Chaetomium thermophilum, Humicola insolens, Humicola grisea, Myceliopthora, thermophila, Talaromyces emersonii* and *Thermoascus aurantiacus* have been widely used for commercial production of cellulase [40].

Various organisms including protists, mollusks, insects, nematodes, crustaceans and annelids have been also utilised for cellulase producing capabilities [41, 42]. Insect gut of some organisms are also a source of cellulase enzyme production [43, 44].

Production of cellulases using fermentation

In enzyme technology the most critical step is the production of enzymes, because this step decides the overall cost of the process. So, the main goal is to focus on the production step and make the process economically viable. Fermentation is the well-known method used for production of enzymes. In this method, microbes are used for the conversion of polymers into their reducing form i.e monomers. Every microorganism has some unique requirements for maximum production of enzyme titre, which have to optimized using either 'one-variable-at-a-time' or statistical approach [45].

Parameters affecting production of cellulase

Various growth parameters such as fermentation method, carbon source, nitrogen source, pH, incubation temperature and incubation period widely affect the enzyme productions, so they need to be optimized first [46].

Fermentation method

Two types of fermentation methods i.e Submerged (SmF) and solid-state fermentation (SSF) are employed for enzyme production. In submerged fermentation nutrient broth or liquid medium is used to dissolve all the essential nutrients and microbial source used for production of enzyme utilize these nutrients. Enzyme is produced inside the fermentation medium along with the used medium, which can be separated from the enzyme by centrifugation. This method has advantages such as easy sterilization, monitoring of parameters and quick down streaming process [7]. Various cellulase enzymes have been produced from fungal and bacterial species using SmF such as *Aspergillus flavus* [47], *Aspergillus Niger* FC-1 [48], *Aspergillus niger* [49], *Bacillus subtilis* BS05 [50], *Bacillus amyloliquefaciens* UNPDV-22 [51], *Bacillus sp*. SMIA-2 [52], *Brucella* and *B. licheniformis* [53] and *B. licheniformis* RT-17 [54] respectively. On the other hand, solid state fermentation uses little moisture just to make the nutrients soft. The main advantage of using this method is the reusability of solid wastes and high enzyme titre [55]. Cellulase enzyme produced by using SSF from various fungal and bacterial sources such as *lichtheimia romosa* [56], *Phaffomycetaceae* and *Dipodascaceae* [57], *Trichoderma citrinoviride* AUKAR04 [58], *Humicola insolens* MTCC 1433 [59] and *B. cereus* [60], *Bacillus* and *pseudomonas sp.* [61] respectively.

Carbon source

Carbon source is the major prerequisite for the production of enzyme and whole enzyme production depends on it [46, 62]. Various agrowastes such as wheat bran, rice bran, corn stover, wheat and rice straw, cotton stalks, corncob and sugarcane baggase, are commonly used as carbon source. Prasanna *et al*. [29] reported maximum cellulase activity by *Penicillium sp*. using carbon sources such as sorbose, maltose, sucrose, lactose, dextrose, galactose, cellobiose and CMC. Zhang *et al*. [62] mentioned that *Rhizopus stolonifer* gene encodes for cellobiose synthetase (CBS) to synthesize cellobiose from uridine diphosphate glucose (UDPG) and CBS have important function in expression of cellulase gene by inducing cellobiose-responsive regulators i.e. CLR1 and CLR2. So, cellobiose can be synthesised from carbohydrates using this CBS and helps in the expression of cellulase.

Nitrogen source

The second main constituent of fermentation medium is nitrogen source as they induce the protein synthesis, which is essential for extracellular enzymes production. Peptone, yeast, tryptone, ammonium sulfate, ammonium chloride, ammonium hydrogen phosphate are the organic and inorganic sources of nitrogen [21]. Maximum cellulase activity has been obtained on yeast extract and combination of ammonium molybdate, peptone or yeast using *Penicillium* and *Trichoderma reesei sp.* respectively [29, 46].

pH and incubation temperature

The pH and temperature of production medium have important roles on the production of enzymes. Optimization of pH and temperature has to be done because they can directly affect the enzyme production, as high or low pH and temperature results in low enzyme activity. Prasanna *et al*. [29] have been reported 5.0 and 30˚C of pH and temperature respectively, to attain maximum cellulase activity from *penicillium.* Similarly, pH 4 and temperature 30˚C was also found to be optimum for cellulase production from *Aspergillus tubingensis* KY615746 [63].

Incubation period

It is defined as the time period taken by microorganism to synthesize enzyme using the production medium nutrients. It also depends on the type of fermentation and microorganism used for production. SSF takes longer time in comparison to SmF and similarly bacteria require lesser time for cultivation in comparison to fungal sources. Activity of enzyme ceases after reaching at optimum time period, this may be due to the exhaustion of nutrients in fermentation medium. Ahmed *et al.* [21] has been reported 192 h and 168 h for SSF and SmF respectively, using *Myceliophthora heterothallica* containing wheat bran or sugarcane.

CONCLUSION

The increased demand of industrial enzymes significantly raises the utilisation of natural products and cellulase is one of them occupying a great level. The main hurdle in the commercialization is the overall production cost due to the scarcity of economic viable process. This problem can be solved only by using cheap agrowastes, but this needs more exploration in order to attain increased enzyme productivity and quality. This review describes the various potential sources of cellulase and their utilisation using cheap lignocellulose wastes in order to attain maximum enzyme titre.

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