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Chapter 4

Microbial Cellulase and Xylanase: Their Sources and Applications

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Abstract

Cellulose and xylan, the two major constituent of lignocellulose are the most abundant and renewable resource available on earth. Cellulose and xylan are complex substrates and their complete hydrolysis requires a variety of enzymes. Cellulases and xylanases are produced by microorganisms, algae, protozoans, crustaceans and insects however, fungal and bacterial bioconversions are economically viable. In the present chapter, remarkable collections of fungi and bacteria have been brought to the limelight that can degrade cellulose and xylan. Mode of action and brief classification of various cellulases and xylanases have been mentioned. Further, insight knowledge on use of cellulases and xylanases for bioremediation and industrial applications were also provided.

Keywords: Lignocellulose; cellulose; xylan; cellulase; xylanase; bacteria; fungi

1. Introduction

The limitation in the availability of fossil fuels and their negative impact on environment made researchers to develop new eco-friendly processes based on renewable feedstock. Lignocellulose is one among such renewable feedstock [1]. Lignocellulose is the major component of plants and referred as in exhaustible natural renewable sources on earth [2]. Lignocellulose mainly consists of cellulose, hemicellulose, and lignin; along with small amounts of pectin, protein, extractives, and ash. However, these compositions vary depending on the plant source, but typically the major part consists of cellulose, followed by hemicellulose, and lignin [3].

Cellulose is an unbranched homopolysaccharide consist of D-glucopyranosyl units [4] Hemicelluloses are branched heteropolysaccharides consist of pentoses (β-D-xylose, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α-D-galactose) and/or uronic acids (α-D-glucuronic, α-D-4-O-methylgalacturonic, and α-D-galacturonic acids). Other sugars such as α-L-rhamnose, and α-L-fucose may also be present in small amounts and the hydroxyl groups of sugars can be partially substituted with acetyl groups [5].

Lignin is a highly irregular and insoluble polymer consisting of phenylpropanoid subunits. Unlike cellulose or hemicellulose, no chains containing repeating subunits are present in lignin, thereby making the enzymatic hydrolysis of this polymer extremely difficult [2,4,6]. In order to utilize lignocelluloses chemical or biological hydrolysis are required. Chemical hydrolysis mainly includes acid, alkali and steam explosion. Recent development in biological hydrolysis, it is now known to be more effective, economical, eco-friendly as compared to the chemical-based approaches and hence biological hydrolysis are now replacing the chemical-based treatments [7].

Biological hydrolysis of lignocelluloses is complex and requires multi-enzyme system [8]. Different components of lignocellulose require different enzymes. For the efficient hydrolysis of cellulose, the action of at least three enzymes namely, endoglucanases, exoglucanases and β-glucosidase are required [9]. Xylan hydrolysis (the major part of hemicellulose) needs multi-enzyme systems, such as endoxylanases, β-xylosidases, α-L-arabinofuranosidases, and acetyl esterases [8]. Lignin degradation requires two major groups of enzymes mainly heme-peroxidases and laccases [10]. Since, various enzymes are involved in lignocellulose hydrolysis and it is difficult to include all in a single chapter hence, the present book chapter focus on enzymes involved in the hydrolysis of two major parts of lignocellulose, cellulose and xylan. It lists some fungal and bacterial derived cellulase and xylanase and their possible applications.

2. Cellulases

Cellulases (EC 3.2.1.4) are the enzymes that break the cellulose molecule into monosaccharide or shorter polysaccharides [11]. The degradation of cellulose involved two steps. In the first step, anhydroglucose chains are swollen or hydrated and in the second step, hydrolytic cleavage of susceptible polymers either randomly or endwise occurs [12].

Cellulases have been produced by microorganisms including bacteria, archaea and fungi. Cellulases were also produced by some animals, but their function in animal system are still unclear [13,14].

2.1 Enzymatic hydrolysis of cellulose

The enzymes required for the hydrolysis of cellulose are exoglucanases, endoglucanases, β–glucosidases and cellobiose phosphorylase. Exoglucanases are the type of cellulase that acts on the terminal end of cellulose chain, releasing glucose or cellobiose as the end product (Figure 1). Exoglucanase are of two types; 1,4-β-D-glucan cellobiohydrolase (EC 3.2.1.91) removes cellobiose units while 1,4-β-D-glucan glucohydrolase (EC 3.2.1.74) removes glucose units [15].

In contrast to exoglucanases, endoglucanases (EC 3.2.1.4) are responsible to initiate cleavage and hydrolyze cellulose randomly at the internal regions, releasing oligosaccharides (Figure 1) [16]. β-Glucosidases (EC 3.2.1.21) catalyze the hydrolysis of β-1-4 bonds linking two glucose or substituted-glucose molecules [16,17]. Cellobiose phosphorylase catalyzes the reversible phosphorolytic cleavage of cellobiose [18]. A list of fungi and bacteria producing different type of cellulase represented in Table 1.

2.2. Microbial source for cellulase

Utilization of cellulose in sufficient amount to provide usable energy to an organism was thought to be carried out by microorganisms [9]. A wide range of microorganisms are capable of producing cellulase. Cellulase production has been reported by both aerobic and anaerobic microorganisms. However, there is a distinct difference in cellulose degradation strategy. Usually, anaerobes degrade cellulose via complex cellulase systems exemplified by the well-characterized polycellulosome organelles. Several anaerobic species that utilize cellulose do not release measurable amounts of extracellular cellulase, and instead have localized their complex cellulases directly on the surface of the cell or the cell-glycocalyx matrix. Aerobic cellulose degraders utilize cellulose through the production of extracellular cellulase enzymes that are freely recoverable from culture supernatants [9].

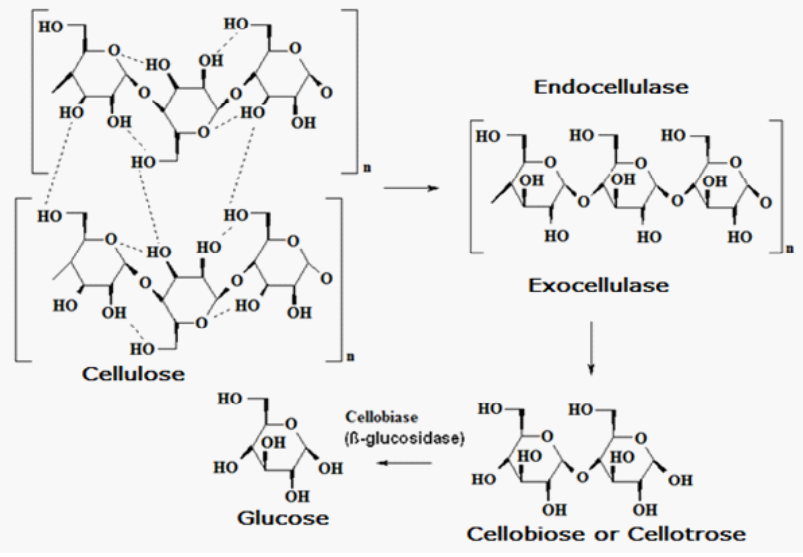

Figure 1: Enzymatic hydrolysis of cellulose.

Table 1: List of fungi and bacteria producing different class of cellulase

Fungi/bacteria	Type of cellulase	References
Aspergillus niger	Exoglucanase	[15]
Cellulomonas flavigena	Exoglucanase	[19]
Bacillus subtilis	Exoglucanase	[11]
Clostridium stercorarium	Exoglucanase	[20]
Irpexlacteus	Exoglucanase	[21]
Gloeophyllum trabeum	Endoglucanase	[22]
Thermoascus aurantiacus	Endoglucanase	[23]
Cellulomonas, Bacillus and *Micrococcus* spp.	Endoglucanase	[24]
Streptomyces misionensis PESB-25	Endoglucanase	[25]
Trichoderma	β – Glucosidases	[26]
Aureobasidium pullulans	β – Glucosidases	[27]
Penicillium decumbens	β – Glucosidases	[28]
Neocallimastix patriciarum	β – Glucosidases	[29]
Ceriporiopsis subvermispora	β – Glucosidases	[30]

2.2.1. Bacterial cellulase

Bacteria producing cellulase have been reported from various ecological niches, such as soil sample [31], dairy farm soil [32], mangrove soil [33], deep-sea sediment [34], hot springs [35], rhinoceros dung [36], pig intestine [37], gut of fish [38], etc. Cellulases have been reported by aerobic, anaerobic and facultative bacteria, Table 2 highlight some of them.

In the past few years, many cellulase producing novel strains (Table 3) including aerobic, anaerobic and facultative were added to the prokaryotes list such as gram negative, aerobic novel genus (of the family Phyllobacteriaceae) isolated from surface seashore water (*Oricolacellulosilytica*) was reported for cellulose degradation [39]. *Clostridium phytofermentans,* an obligate anaerobic novel species, isolated from forest soil was reported for cellulase activity [40]. Novel genus *Cellulosibacter* (type species as *Cellulosibacter alkalithermophilus*) isolated from coconut garden was reported for cellulase activity [41].

Cellulase for industrial applications needs to withstand various extreme conditions such as temperature and pH. Bacterial cellulases have been considered as an important industrial source as they can withstand extreme temperature and pH [42, 43]. For food industry, environmental bioremediation, and molecular biology study psychrophiles cellulase are needed [44]. Psychrophilic cellulase from *Pseudoalteromonas haloplanktis* can be used for such applications [45]. In some steps of textile industry, acidic and psychrophilic cellulases are required. Bacteria are also reported for such cellulase. Bacteria such as *Klebsiella* sp. produce cellu-

lase active at 10 °C and pH 4.5, this type of cellulase can be used in textile industries [46]. Apart from psychrophilic cellulase, bacteria also produce alkali and thermo tolerant cellulase. *Marinobacter* sp. (MSI032) isolated from the marine sponge has been reported for cellulase production. The enzyme produced by *Marinobacter* sp. (MSI032) was alkalotolerant, active at pH 9.0 [43]. Similarly, alkalotolerant cellulase from *Nocardiopsis* sp. SES28, isolated from Argentina was active at pH 8.0 and 40 °C [47]. *Bacillus mycoides* S122C show cellulase activity at 50 °C and pH 7.0 [48]. *Geobacillus pallidus* show cellulase production at 60°C [49]. Thermophilic bacterium *Caldibacillus cellulovorans* show cellulase activity at 80 °C [42].

Table 2: List of aerobic, anaerobic and facultative anaerobic bacteria producing cellulase

Bacteria	Growth condition	References
Balneomonas flocculans	Aerobic	[50]
Paenibacillus terrae ME27-1	Aerobic	[51]
Pseudomonas fluorescens	Aerobic	[52]
Paenibacillus cellulositrophicus	Facultative anaerobe	[53]
Cellulomonas uda	Facultative anaerobe	[54]
Halocella cellulolytica	Facultative anaerobe	[55]
Bacteroides cellulosilyticus	Anaerobe	[56]
Ruminococcus champanellensis	Anaerobe	[57]
Herbivorax saccincola	Anaerobic	[58]
Herbinix hemicellulosilytica	Anaerobic	[59]
Streptomyces reticuli	Aerobic	[60]
Streptomyces drozdowiczii	Aerobic	[61]

Table 3: List of novel bacterial strains producing cellulase.

Bacteria	Isolated from	References
Herbinix hemicellulosilytica	Biogas reactor	[59]
Herbivorax saccincola	Biogas reactor	[58]
Cohnella cellulosilytica	Buffalo faeces	[62]
Cellulomonas terrae	Soil	[63]
Cellulomonas composti	Cattle farm	[64]
Vibrio xiamenensis	Mangrove soil	[33]
Pseudomonas coleopterorum	Bark beetle	[65]
Bacteroides cellulosilyticus	Human gut	[56]
Ruminococcus champanellensis	Human gut	[57]
Paenibacillus cellulositrophicus	Soil	[53]

2.2.2. Fungal cellulase

Fungal cellulase have advantages over bacterial cellulase in having high yield and able to produce a complete cellulase system [66]. Cellulase producing fungi are ubiquitous and found in a wide variety of environments such as soil, decaying logs of wood, sawdust [67], forest soil [68], litter soil [69], mushroom compost [70], marine sample [71], bovine rumen [72], and even as endophytes [73]. Unlike bacterial cellulase, fungal cellulases were active over a wide range of temperatures. Psychrotolerant fungus, *Aspergillus terreus* AKM-F3 produce optimum cellulase at 15 °C [74], *Aspergillus niger* produce optimum cellulase at 30 °C [75]. *Nectria catalinensi*s cellulase activity ranged from 50 to 55 °C [76]. *Aspergillus fumigatus* M.7.1 and *Myceliophthora thermophila* M.7.7 produce cellulase at 70 °C [77].

Fungal cellulases are also active over a wide range of pH. *Penicillium citrinum* was reported for alkali stable cellulase [78]. Acid tolerant cellulase was reported by *Trichoderma reesei* [79]. Fungus with dual tolerance such as acido-thermo-tolerant cellulase by *Chaetomium thermophile* (pH 4.0-4.5 and 60°C) [80] and *Penicillium* sp. CR-316 and *Penicillium* sp. CR-313 (65 °C and pH 4.5) [68] was also reported.

3. Xylanase

Xylanases (EC 3.2.1.x) are glycosidases which catalyze the endohydrolysis of 1,4- β-D-xylosidic linkages in xylan. They are produced by a plethora of organisms including bacteria, algae, fungi, protozoa, gastropods, and anthropods [81].

There is a phenomenal increase in the use of microbial xylanase as they offer advantages over conventional chemical catalysts, including high catalytic activity, high degree of substrate specificity, high productivity, easily biodegradable, pose no threat to the environment, and are economically viable [82].

3.1. Enzymatic hydrolysis of xylan

Due to the complex structure of xylan, its hydrolysis includes different types of enzymes. Endo-xylanases are the enzymes that cleave the glycosidic bonds in the xylan backbone, bringing about reduction in the degree of polymerization of the substrate [83]. β-xylosidase (E.C.3.2.1.37) hydrolyze short xylooligomers into single xylose units. There is a significant variation in their mode of action of β-xylosidases when compared to exo-xylanases. Exo-xylanases act on the xylan backbone from the reducing end (exo-fashion) producing short-chain oligomers whereas β-xylosidases hydrolyze short xylooligomers into single xylose units [84,85,86]. α-D-Glucuronidases (E.C.3.2.1.139) cleaves the α-1,2-glycosidic bond of the 4-O-methyl-D-glucuronic acid side chain of xylan [87,86]. α-Arabinofuranosidases (EC 3.2.1.55) are enzymes known to release terminal α -1,2-, α-1,3- and α-1,5 α-L-arabinofuranosyl residues

from hemicellulose such as arabinoxylan and other L-arabinose containing polysaccharide [83]. Acetylxylan esterase (EC 3.1.1.72) acts on carboxylic ester bonds. Ferulic acid esterase and p-coumaric acid esterase cleave ester bonds on xylan [88]. Ferulic acid esterase cleaves the linkage between arabinose and ferulic acid side groups, while p-coumaric acid esterase cleaves between arabinose and p-coumaric acid [89].

3.2. Microbial source for xylanase

Horikoshi and Atsukawa in 1973 [90] reported the commercial application of xylanase obtained from alkaliphilic bacteria. However, studies on microbial xylanase started during the 1960s, but the main focus of the study was plant pathogen relation. The study suggests that, xylanase along with other enzyme degrade the plant cell wall which cause the infection [91]. Due to the wide application of xylanase, several fungi and bacteria were explored for the ability to produce xylanase.

3.2.1. Bacterial xylanase

Bacteria producing xylanase have been isolated from various environments such as soil sample [90], marine sample [92], hot-spring water [93], mushroom compost [94], poultry compost [95], human gut [96], and sheep dung [97]. Prevalence of the xylanolytic bacteria have been reported from most of the bacterial groups (Table 4).

Bacterial xylanases has been reported to be active at wide temperature range. *Pseudoalteromonas haloplanktis* TAH3A (XPH) and *Flavobacterium* sp. MSY-2 (rXFH) have been reported for producing psychrophilic xylanases [98]. *Kluyvera* sp. strain OM3 can produce high level of cellulase free xylanase at 70 °C [99]. Bacteria like *Thermonosporafusca*, *Bacillus stearothermophilus*, and *Dictyoglomus thermophilum* show an optimum xylanase activity at temperature ranging from 65 to 85 ºC [100,101,102]. The first report on xylanase from alkaliphilic bacteria, *Bacillus* sp. TAR-1 was reported by Horikoshi and Atsukawa (1973) [90]. Apart from psychrophilic thermophilic and alkalophilic, haloalkaline xylanase [92)] and acidic xylanase [103] were also reported.

Table 4: List of xylanase producing bacteria

Bacteria	References
Caldicoprobacter algeriensis	[104]
Pseudoalteromonas haloplanktis	[105]
Staphylococcus sp. SG-13	[106]
Bacillus circulans	[107]
Streptomyces actuosus A-151	[108]
Streptomyces matensis	[109]
Streptomyces sp. 7b	[110]
Bacillus licheniformis SVD1	[111]
Geobacillus thermodenitrificans	[112]
Pseudomonas sp.WLUN024	[113]
Nonomuraea flexuosa	[114]
Thermoanaerobacterium saccharolyticum NTOU1	[115]
Gracilibacillus sp. TSCPVG	[116]
Acinetobacter junii F6-02	[117]
Jonesia denitrificans	[118]
Dictyoglomus thermophilum	[102]

3.2.2. Fungal xylanase

Fungi are often selected for the production of xylanase, as their yield are much higher than bacteria and in addition they also produce several auxiliary enzymes required for the degradation of substituted xylan [83]. Unlike bacterial xylanase, fungal xylanases are active at high temperature, low temperature, acidic pH, alkaline pH and even salt tolerant [119,120,121]. *Trichoderma reesei* produces xylanase in the mesophilic range [121] while *Cladosporium* sp. produces cold-active xylanase [122]. The most thermostable xylanases that have been described so far are those derived from *Thermotoga* sp. FjSS3-B.1, *Thermotoga maritima, Thermotoga neapolitana,* and *Thermotoga thermaru*m which are active at temperatures ranging from 80 °C to 105 °C [123]. *Neocallimastix frontalis* produces xylanase active at acidic pH [124]. *Gloeophyllum trabeum* produces xylanase at high temperature (70 °C) under broad pH range (4-7) [125].

4. Application of Cellulase and Xylanase

4.1. Cellulase and xylanase in the textile industry

A lot of limitations have been imposed on the textile industry due to rising environmental pollutions caused due to chemicals used during the process. In order to combat this situation, enzymatic treatment has emerged as an eco-friendly solution [14]. Cellulase and xylanase are one among the enzymes which are extensively used in the textile industries [126,83]. In textile industry, cellulase and xylanase have been used in various steps such as, during fabric

softening [83], bio-stoning of denim garments [14,126], bioscouring [14], releasing the extra dye [126], improving textile brightness [127], and bio-bleaching [128]. The use of microbial cellulase has several advantages over traditional stone washing (which mainly include pumice stones) including high productivity, less work-intensive, safer environment, short treatment times and less wear and tear of machines [129]. Cellulase from *Penicillium occitanis* [130] and *Trichoderma reesei* found to be very efficient candidates for biostoning application [131]. Xylanase from *Bacillus pumilus, Bacillus stearothermophilus* SDX, and *Penicillium janthinellum* have been used in textile industries for de-sizing of cotton and micropoly fabrics. It also helps in lowering the wetting time of fabrics, bioscouring efficiency and reducing the weight loss of the fabrics [132, 133, 134]. Cellulase from *Trichoderma reesei* [135] and *Aspergillus niger* have been used for biopolishing of the fabric [136].

4.2. Cellulase and xylanase in paper industry

Cellulase and xylanase have been used in the paper industry to overcome the limitations of mechanical pulping processes such as refining and grinding [14,137]. Xylanase from *Streptomyces thermoviolaceus* [138], *Aspergillus sydowii* [139], *Trichoderma reesei* [121], *Bacillus pumilus*, *Aspergillus fumigates, Chaetomiumcellulolyticum, Thermomyceslanuginosus,* and *Aspergillus kawachii* have been used for bleaching [140, 83, 138].

Xylanase have been used to increase pulp brightness, removing metal cations, reducing the overall paper cost, reducing the beating time of pulp, and restoring of bonding [141]. Xylanase treatment can render the chlorine requirement [142]. Cellulase and xylanase were beneficial for deinking of different types of paper wastes. Xylanase from *Aspergillus niger* DX-23 [143] and cellulase from alkalotolerant *Fusarium* sp. [144] were suitable for deinking. Advantages of enzymatic deinking over chemical deinking includes, reduce alkali usage, improved fiber brightness, enhanced strength, higher pulp freeness, and reduced fine particles in the pulp [137].

4.3. Cellulase and xylanase in food industries

Cellulase and xylanase found applicable in many food industries. They have been used for juice clarification, improving the quality of bakery products, reducing the viscosity of nectars, alteration of fruits sensory properties, used for olive oil extraction, and during beer and wine production [137].

During the early 1930s, when fruit industries began to produce juice, macerating enzymes complex (cellulases, xylanases and pectinases) from food-grade microorganisms (*Aspergillus niger* and *Trichoderma* sp.) have been extensively used to increase the juice yield and to overcome the difficulties encountered during filtration [145].

In the baking industries, wheat (which consist hemicellulose) is the key material. The hemicellulose present in the wheat is water-insoluble and cause many problems [146]. Xylanase during baking helps to transforms water-insoluble hemicellulose into a soluble form, which helps to increase the volume and creating finer and more uniform crumbs [147]. Xylanase from *Aspergillus foetidus* showed a remarkable difference in water absorption [148]. Xylanase also improves the quality of dough in such a way that it does not stick to the machinery parts, making it more machine-friendly [147]. Xylanase also enhances the bread quality and extend the shelf life by reducing the staling rate [149].

Cellulase and xylanase plays an important role during beer and wine production [150, 151]. The first microbial enzyme used in the wine industry was a commercial pectinase from *Aspergillus*. However, in the early 1980s, it was suggested that *Trichoderma* β-glucanase could be successfully used for wine making from grapes which were infected with *Botrytis cinerea. Botrytis cinerea* deteriorate ripe grapes and produces a high molecular mass soluble β-(1,3) glucan with short side chains linked through β-(1,6) glycosidic bonds, which cause severe problem during wine filtration. *Trichoderma* β-glucanase hydrolyzes glucans that cause adverse effects during filtration of wine [145]. Endoglucanase II and exoglucanase II from *Trichoderma* showed maximum reduction in the degree of polymerization and wort viscosity [152].

In past few years, β-glucosidase has attracted considerable attention in the wine industry because of its ability to improve the aroma of wines by modifying naturally present, glycosylated precursors [153]. Apart from glycosidase, α-L arabinofuranosidase and β-D-glucopyranosidase also help to increase the aroma of wine [151].

4.4. Cellulase and xylanase in animal feed Industries

Cellulase and xylanase have been used in animal feed industries, which help to improve feed nutritional value, eliminate anti-nutritional factors present in the feed grains, degrade certain feed constituents to improve the nutritional value, and provide supplementary digestive enzymes [137]. Cellulase and xylanase as feed additive helps digestion in cows and increase milk production [154]. Xylanase from *Aspergillus niger* helps growth performance, nutrient digestibility, and non-starch polysaccharide degradation in broilers [155]. Wheat and barley based supplement with xylanase and β-glucanase showed significant increased body weight and feed efficiency of turkeys [156].

4.5. Cellulase and xylanase in treating agricultural and forest wastes

Forest and agriculture, accounts for highest lignocellulosic waste production [157]. The major components of lignocellulosic are biodegradable; however, these are unlikely to result in hazardous conditions when there is inadequate oxygen to assimilate the wastes. When

this occurs, there is considerable damage to economic activities and the environment as well [158]. Enzymatic hydrolysis offer perspectives degradation of such waste, because it is a more specific process and the products obtained are without the presence of undesirable products [159,160]. Cellulase can remove the apple pomace waste [161] while cellulase and xylanase together can degrade lignocelluloses waste (beech tree leaves) [162]. Cellulase and xylanase from *Aspergillus niger* F7 were efficient in degrading forest wastes such as *Toonaciliata, Celtrisaustralis, Cedrusdeodara* and *Pinusroxburghii* [163]. Exo-1,4-β-glucanase from *Trichoderma viride* has been efficiently used to remove orange peel waste.

5. Conclusion

Microbial cellulase and xylanase have shown their potential application in textile industries, paper industries, food industries, animal feed industries, juice industries, brewing industries, bioremediation and bio-refinery for forest and agriculture wastes. Due to immense industrial potential, microbial cellulases and xylanases represents potential candidates for research by both the academic and industrial research groups. Despite an increased knowledge of fungal and bacterial cellulases and xylanases, further studies to isolate potential cellulases and xylanases producing strains and direction for improving the process economics should be carried.

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