Chapter 12
Biodegradation of Agricultural Wastes by *Chaetomium* Species

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12.1 Introduction

Cellulose is the most abundant and renewable component of plant biomass (Srivastava et al. 2015a, b, c; Srivastava and Jaiswal 2016). It is considered as the main product of photosynthesis, and ~100 billion dry tons/year of cellulosic biomass is produced in the biosphere (Wang et al. 2016). Besides plants, some animals and bacteria also produce cellulose. However, it is the major component of the plant cell walls and abundantly available in the environment. In the lignocellulosic biomass structure, cellulose is mainly associated with hemicelluloses and lignin and sometimes with silica (e.g., rice straw, rice husk). Further, it accounts for ~35–50% of plant dry weight, whereas hemicelluloses as well as lignin cover ~20–35 and ~5–30% of plant dry weight, respectively (Zabeda et al. 2016). Cellulose is a linear polysaccharide, made up of combined units of glucose monomers bound by β-1,4-glycosidic linkage (Fig. 12.1). To release these monomeric molecules, cellulase enzymes are required for carrying out enzymatic hydrolysis. Cellulases are the combination of enzymes capable of degrading the insoluble cellulose polymer present in the lignocellulosic biomass into fermentable sugars, predominantly small chain of cellobiose and glucose molecules (Taherzadeh and Karimi 2007). Endoglucanases, cellobiohydrolases, and β-glucosidases are the main subcomponents which make the cellulase enzyme system (Thota et al. 2017). Nevertheless,

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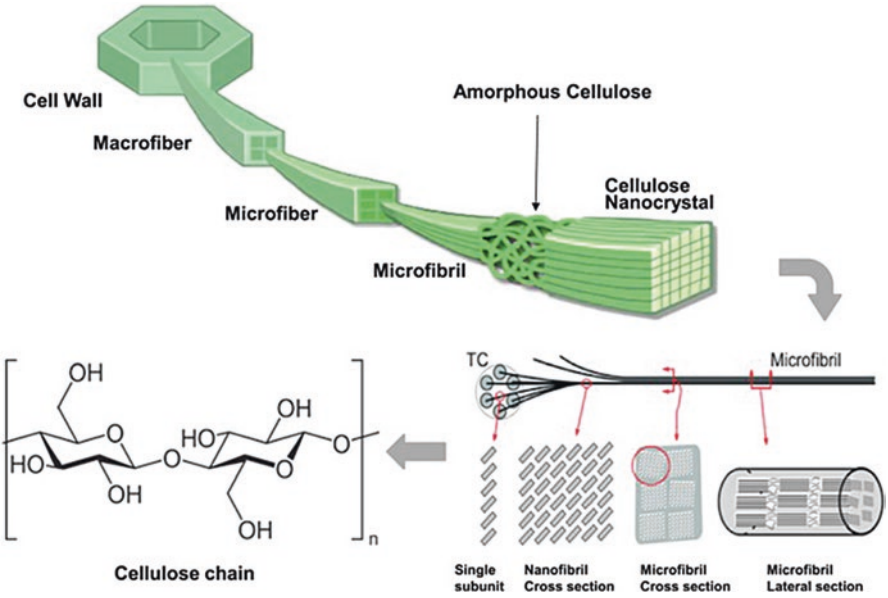

Fig. 12.1 Hierarchical structure of cellulose from wood. (Kim et al. 2015)

intensive researches are going on to achieve novel cellulase producing systems, not only to increase the yield and economic feasibility but also to expand the use of such systems by progressing toward more and more industrially flexible bacteria or fungi production systems. Additionally, many enzymatic hydrolysis researches have also been aimed at the production of enzymes with high enzymatic activity for improvement in the current technology to produce cellulase enzyme (Chandel et al. 2012; Garvey et al. 2013). Besides biofuel production, other major industrial applications of cellulases are bio-polishing; bio-stoning; bio-finishing in textile industry; starch processing; grain alcohol fermentation; malting and brewing in beer and wine industry; extraction and processing of fruit and vegetable juices in food, pulp, and paper industry (Cavacopaulo 1998; Gao et al. 2008a, b; Ibrahim et al. 2015); controlling plant pathogen and disease in agriculture as well as in household laundry detergents for improving the fabrics' softness and brightness; etc. (Wilson 2009).

This chapter deals with the production of fungal cellulases, their type, and efficiency to degrade lignocellulosic biomass. Additionally, recent advancements in cellulase production and future prospects have also been discussed.

12.2 Agricultural Lignocellulosic Wastes, Environment, and Human

Many of the agricultural lignocellulosic wastes which are produced every day in the world cause serious environmental pollution effects if they are allowed to accumulate in the agro-ecosystems or, much worse, burned for uncontrolled domestic

purposes. Every year, large amounts of lignocellulosic wastes are generated through forestry and agricultural practices, in timber industries and many agroindustries, generating environmental pollution problems by their burning on the soil surface or their incorporation into the soil matrix.

We have two different types of agro-industrial wastes, i.e., agriculture residues and industrial residues. Agriculture residues can be further divided into field residues and process residues. Field residues are residues that present in the field after the process of crop harvesting. These field residues consist of leaves, stalks, seed pods, and stems, whereas the process residues are residues present even after the crop is processed into alternate valuable resource (Table 12.1). These residues consist of molasses, husks, bagasse, seeds, leaves, stem, straw, stalk, shell, pulp, stubble, peel, roots, etc. and used for animal feed, soil improvement, fertilizers, manufacturing, and various other processes. Huge amount of field residues are generated and most of them are underutilized. Controlled use of field remains can enhance the proficiency of irrigation and control of erosion. In Middle East region, wheat and barley are the major crops. In addition to this, various other crops like rice, lentils, maize, chickpeas, fruits, and vegetables are also produced all over the world. Agricultural residues are differentiated on the basis of their availability as well as characteristics that can be different from other solid fuels like charcoal, wood, and char briquette (Zafar 2014).

The greenhouse effect is our planet's ability to pass the Sun's incoming radiation and to reflect the reradiated long wavelengths from the Earth's surface, so increasing our planet's surface and atmosphere lower-layer temperature. As a result, the melting of glaciers and snow cover intensifies, and the water level of seas and oceans increases, flooding islands and coastlines. Increase in temperature intensifies water evaporation and increases the possibility of downpour and cyclone formation in

Table 12.1 Composition of agro-industrial wastes

	Chemical Composition (%)						
Agro-industrial wastes	C	HC	L	A (%)	TS (%)	M (%)	References
Sugarcane bagasse	30.2	56.7	13.4	1.9	91.66	4.8	El-Tayeb et al. (2012) and Nigam et al. (2009)
Rice straw	39.2	23.5	36.1	12.4	98.62	6.58	El-Tayeb et al. (2012)
Corn stalks	61.2	19.3	6.9	10.8	97.78	6.40	El-Tayeb et al. (2012)
Sawdust	45.1	28.1	24.2	1.2	98.54	1.12	El-Tayeb et al. (2012) and Martin et al. (2012)
Sugar beet waste	26.3	18.5	2.5	4.8	_	12.4	El-Tayeb et al. (2012)
Barley straw	33.8	21.9	13.8	11	_	_	Nigam et al. (2009)
Cotton stalks	58.5	14.4	21.5	9.98	_	7.45	Nigam et al. (2009)
Oat straw	39.4	27.1	17.5	8	_	_	Martin et al. (2012)
Soya stalks	34.5	24.8	19.8	10.39	_	11.84	Motte et al. (2013)
Sunflower stalks	42.1	29.7	13.4	11.17	_	_	Motte et al. (2013)
Wheat straw	32.9	24.0	8.9	6.7	95.6	7	Nigam et al. (2009) and Martin et al. (2012)

**C* cellulose, *HC* hemicellulose, *L* lignin, *TS* total solids, *M* moisture

seaside regions, while on continents—droughts, heat waves, and forest fires. So lowering of the greenhouse effect is one of the main global problems today (Kazragis 2005).

The main greenhouse effect agents are carbon dioxide (CO_2) (53%), Freon, ozone, methane (CH_4), and other substances. CO_2 is formed during breathing of live organisms, activity of microbes in the soil, and also the combustion of various organic substances. CH_4 and CO_2 are formed when cellulosic matter (wood, peat moss, agricultural production waste, vegetation) decay at treatment places and landfills. From all the mentioned processes, mankind controls only the cellulose matter combustion and decay processes which should decrease as much as possible to weaken the greenhouse effect. However, at present it is unrealistic to lower the amount of CO2 formed by burning fossil fuel in industrial enterprises and heating systems.

However, the excess lignocellulosic waste is often disposed of by biomass burning, causing an environmental pollution problem through smoke and development of CO2 which is not restricted to developing countries, but is considered a global phenomenon (Levine 1996). Burning also causes almost complete losses of soil N, P, K, and S (Dobermann and Fairhurst 2002).

On the global scale, biomass burning (BB) is the main source of primary organic carbon (OC) (Bond et al. 2004; Huang et al. 2015), black carbon (BC) (Bond et al. 2013; Cheng and Yang 2016), and brown carbon (BrC) (Laskin et al. 2015). It is also the second largest source of non-methane organic gases (NMOGs) in the atmosphere (Yokelson et al. 2008; Stockwell et al. 2014). In addition, atmospheric aging of biomass burning plumes produces substantial secondary pollutants.

The increase in tropospheric ozone (O_3) in aged biomass burning plumes could last for days and even months (Thompson et al. 2001; Duncan et al. 2003; Real et al. 2007) with complex atmospheric chemistry (Arnold et al. 2015; Müller et al. 2016). Moreover, biomass and biofuel burning could contribute up to 70% of the global secondary organic aerosol (SOA) burden (Srivastava et al. 2015a, b, c) and hence influence the seasonal variation of global SOA (Tsigaridis et al. 2014). Since it produces large amounts of primary and secondary pollutants, it is essential to characterize primary emissions and photochemical evolution of biomass burning in order to better understand its impacts on air quality (Huang et al. 2014), human health (Alves et al. 2015) and climate change (Andreae et al. 2004; Koren et al. 2004; Laskin et al. 2015; Huang et al. 2016).

Open burning of agricultural residues, a convenient and inexpensive way to prepare for the next crop planting, could induce severe regional haze events (Cheng et al. 2013; Tariq et al. 2016). Among all the biomass burning types, agricultural residue burning in the field is estimated to contribute ~10% of the total mass burned globally (Andreae and Merlet 2001), and its relative contribution is even larger in Asia (~34%), and especially in China (>60%) (Streets et al. 2003), where >600 million people live in the countryside (NBSPRC 2015). Agricultural residues burned in China were estimated to be up to 160 million ton in 2012, accounting for ~40% of the global agricultural residues burned (Li et al. 2016). As estimated by Tian et al. (2011), agricultural residue burning contributed 70–80% of non-methane hydrocar-

bons (NMHCs) and particulate matter (PM) emitted by biomass burning in China during 2000–2007. A better understanding of the role agricultural residual burning plays in air pollution in China and elsewhere requires better characterization of primary emission and atmospheric aging of emitted trace gases and particles for different types of agricultural residues under different burning conditions. In the past two decades, there have been increasing numbers of characterizations of biomass burning emissions. Andreae and Merlet (2001) summarized emission factors (EFs) for both gaseous and particulate compounds from seven types of biomass burning. Akagi et al. (2011) updated the emission data for 14 types of biomass burning, and newly identified species were included. Since biomass types and combustion conditions may differ in different studies, reported emission factors are highly variable, especially for agricultural residue burning (Li et al. 2007, 2017; Cao et al. 2008; Zhang et al. 2008; Yokelson et al. 2011; Brassard et al. 2014; Sanchis et al. 2014; Wang et al. 2014; Ni et al. 2015; Kim Oanh et al. 2015; Stockwell et al. 2016; Bruns et al. 2017; Tkacik et al. 2017). Moreover, previous studies on agricultural residue burning were mostly carried out near fire spots or in chambers with low dilution ratios. Since biomass burning organic aerosols (BBOAs) are typically semi-volatile (Grieshop et al. 2009b; May et al. 2013), it is expected that measured BBOA emission factors would be affected by dilution processes (Lipsky and Robinson 2006), and BBOA emission factors under ambient dilution conditions are still unclear. Furthermore, knowledge of NMOGs emitted from agricultural residue burning is very limited. As reported by Stockwell et al. (2015), ~21% (in weight) of NMOGs in biomass burning plumes have not been identified yet. Therefore, comprehensive measurement and characterization of gaseous and particulate species emitted by agricultural residue burning under ambient dilution conditions are urgently needed.

Great attention has been drawn to SOA formation and transformation in biomass burning plumes recently, since a significant increase in mass and apparent change in physicochemical characteristics of aerosols have been observed during atmospheric aging of biomass burning plumes in both field and laboratory studies (Grieshop et al. 2009a, b; Hennigan et al. 2011; Heringa et al. 2011; Lambe et al. 2011; Jolleys et al. 2012; Giordano et al. 2013; Martin et al. 2013; Ortega et al. 2013; Ding et al. 2016a, b, 2017). For agricultural residue burning, evolution processes have not been well characterized yet. To our knowledge, up to now, there has only been a chamber study (Li et al. 2015) which has investigated the evolution of aerosol particles emitted by wheat straw burning under dark conditions. Although field studies (Adler et al. 2011; Liu et al. 2016) witnessed the evolution in mass concentrations, size distribution, oxidation state, and optical properties of aerosol particles emitted by agricultural residue burning, these changes could be also influenced by other emission sources and meteorological conditions as well. Since NMOGs emitted by agricultural residue burning are not fully quantified, it is still challenging to predict the concentration and physicochemical properties of SOA that resulted from biomass burning (Spracklen et al. 2011; Jathar et al. 2014; Srivastava et al. 2015a, b, c; Hatch et al. 2017). Bruns et al. (2016) suggested that the 22 major NMOGs identified in residential wood combustion could explain the majority of observed SOA, but it

remains unclear whether identified NMOGs emitted by agricultural residue burning could fully (or at least largely) explain the SOA formed. In addition, aerosol mass spectrometry (AMS) has been widely used to characterize sources and evolution of ambient OA (Zhang et al. 2011). Although agricultural residue burning is an important type of biomass burning in Asia and especially in China, the lack of AMS spectra for primary and aged OA from agricultural residue burning significantly limits the further application of AMS in BBOA research.

In 2017, indoor chamber experiments were conducted to investigate primary emissions from open burning of rice, corn, and wheat straws and their photochemical aging as well by Fang et al. Emission factors of NOx, NH3, SO2, 67 nonmethane hydrocarbons (NMHCs), particulate matter (PM), organic aerosol (OA), and black carbon (BC) under ambient dilution conditions were determined. Olefins accounted for >50% of the total speciated NMHC emission (2.47–5.04 g kg $^{-1}$), indicating high ozone formation potential of straw burning emissions. Emission factors of PM (3.73–6.36 g kg^{-1}) and primary organic carbon (POC, 2.05–4.11 gC kg^{-1}), measured at dilution ratios of 1300–4000, were lower than those reported in previous studies at low dilution ratios, probably due to the evaporation of semivolatile organic compounds under high dilution conditions. After photochemical aging with an OH exposure range of (1.97–4.97) × 10^{10} molecule cm^{-3} s in the chamber, large amounts of secondary organic aerosol (SOA) were produced with OA mass enhancement ratios (the mass ratio of total OA to primary OA) of 2.4–7.6. The 20 known precursors could only explain 5.0–27.3% of the observed SOA mass, suggesting that the major precursors of SOA formed from open straw burning remain unidentified. Aerosol mass spectrometry (AMS) signaled that the aged OA contained less hydrocarbons but more oxygen- and nitrogen-containing compounds than primary OA, and carbon oxidation state (OS_c) calculated with AMS resolved O/C and H/C ratios increased linearly ($p < 0.001$) with OH exposure with quite similar slopes.

In Egypt and elsewhere, large quantities of organic matter from agricultural wastes (AWs) and the removal of exotic plants (EPs) are burned without treatment of the combusts directly on agricultural fields. This practice causes important environmental issues that have been identified as major health risks for the local population by reducing local air quality and by contributing to the black-cloud phenomenon in the region (El-Askary and Kafatos 2008).

A research carried by Awasthi et al. (2010) showed that the smoke produced by crop burning could have a lasting effect on children's lung function. Professor Ravinder Agarwal, head of the University Science Instrumentation Centre at Thapar University in Patiala, India, and colleagues used portable spirometers to regularly test the lung function of children aged 10–13 and adults aged 20–35 over the course of a year. The 40 participants were healthy nonsmokers living in a village surrounded by farmland, with little traffic and no industry within 10 km (Awasthi et al. 2010). Children's force vital capacity (FVC) dropped from a mean 98% in August 2008 to 92% in July 2009. Mean FVC dipped as low as 88% in October and November, when farmers burned their rice crop residue, and in April and May, when they burned wheat stubble. The children's mean lung function remained

significantly lower throughout the test period. The mean lung function of the adult study participants declined during the burn seasons as well, but largely returned to original levels by the end of the study (Awasthi et al. 2010). Decreases in lung function correlated with increases in the concentration of particulate matter, which exceeded India's national air quality standards during the burn season (Awasthi et al. 2010). Small particles (PM2.5 and PM10)—which make up the majority of the smoke produced by crop burning—were more closely associated with decreases in lung function than suspended particulate matter (SPM), which can contain particles 100 μm or larger (EPA 1999). The findings linking seasonal burning with health issues "coincide with the anecdotal evidence that have been recorded in the Canadian prairies," notes Kate Letkemann, environmental issues coordinator of The Lung Association, Manitoba, and a member of the provincial Crop Residue Burning Advisory Committee. Argawal's work "builds a relationship between pulmonary function tests and the concentration of SPM, PM10, and PM2.5," notes Shijian Yang of the School of Environmental Science and Engineering at China's Shanghai Jiao Tong University. But he would like to see further research that looks closely at the dose–effect relationship between lung function and crop residue burning. Yang's work has shown that the peak concentration of PM10 and its duration may be more important than average concentrations for estimating the health effects of burning crops (Yang et al. 2008).

Cellulose-containing waste can be reprocessed without emitting CO_2 or CH_4 by manufacturing building materials, thermal as well as acoustic insulating composites. Wooden waste has been used for these purposes for a long time. At present it is recommended to use other cellulose raw materials—straw, reeds, boon, peat moss, and other materials. The utilization of straws given composites, containing straws, Portland cement (in some cases—construction gypsum and sand and polymeric additives—vinyl acetate (e.g., polyvinyl acetate PVA) or cellulosic materials (e.g., carboxymethylcellulose (CMC) dispersions. The utilization of reeds gives composites containing reeds, Portland cement, and PVA or CMC. The utilization of boon or chaff gives composites containing boons or chaffs, anhydrite or aluminate cements, and PVA or CMC. Optimal composition composites distinguish themselves by good physico-mechanical as well as thermal and acoustic properties and can find applications as building materials as well as thermal and acoustic insulating materials.

12.3 Lignocellulose Biodegradation

12.3.1 Cellulolytic Enzymes

Most known cellulolytic enzymes are produced and excreted by filamentous fungi, among which *Trichoderma reesei* has received special attention for its hyperenzymatic production capabilities (Vinzant et al. 2001; Sun et al. 2008a, b). It has been subsequently used as a model organism for investigations of enzymatic hydrolysis

mechanisms. Proteomic analysis of these enzymes with high-resolution two-dimensional gel electrophoresis revealed many glycoside hydrolases with this species (Vinzant et al. 2001; Sun et al. 2008a, b), which can be divided into two main groups: cellulases and hemicellulases. Cellulases are enzymes that hydrolyze cellulose, a linear polysaccharide molecule composed of repeated β(1 → 4) linked D-glucopyranosyl (Glc) units. Multiple types of cellulases have been discovered, including at least two exo-β-glucanases, or cellobiohydrolases (CBHs; EC 3.2.1.91) (CBH I and CBH II), four endo-β-glucanases (EGs; EC 3.2.1.4) (EG I, EG II, EG III, EG V), and one β-glucosidase (β G; EC 3.2.1.21) (Lynd et al. 2002).

Hemicellulases are enzymes that degrade hemicelluloses, a group of polysaccharides and one of major plant cell wall components. Unlike cellulose, which is composed entirely of glucosyl moieties linked by only β-1,4-glycosidic bonds, various types of sugar moieties linked by different bonds, intramolecular architecture, and intermolecular interactions can be found within hemicelluloses. Hemicelluloses also differ from the major plant cell wall structural component cellulose in their much smaller polysaccharide chains. Hemicelluloses can serve to cross-link cellulose microfibrils by interconnecting them as well as linking cellulose molecules to other cell wall components (Kumar et al. 2008). Common hemicelluloses include β-glucan (different from cellulose), xylan, xyloglucan, arabinoxylan, mannan, galactomannan, arabinan, galactan, and polygalacturonan. Corresponding to these hemicelluloses, various types of hemicellulases exist, each specific to a kind of hemicellulose (Polizeli et al. 2005; Collins et al. 2005; Kumar et al. 2008). These enzymes can be clustered into two groups: hemicellulases that attack the polysaccharide backbone and those that attack the side chains.

Xylan, whose structure differs from plant to plant, is the second most abundant polysaccharide in herbs and hardwoods, demanding the collaboration of a group of enzymes during their degradation. Multiple enzymes including endo-β-xylanase (EC 3.2.1.8), β-xylosidase (EC 3.2.1.37), α-glucuronidase (EC 3.2.1.139), α-L-arabinofuranosidase (EC 3.2.1.55), and acetylxylan esterase (EC 3.1.1.6) act synergistically in this process (Fig. 12.2). Endo-1,4-xylanases cleave internal β-1,4-xylosidic bonds on the xylan polysaccharide backbone. Unlike EGs, whose cleavage sites are random, endo-1,4-xylanases recognize specific bonds for cleavage on the basis of polysaccharides properties such as chain length and branching levels (Polizeli et al. 2005). Endoxylanases were initially classified into two groups by their ability to hydrolyze the 1,3-α-L-arabinofuranosyl branching points of arabinoxylans: hydrolyzing and nonhydrolyzing endoxylanases, which have different pI values and molecular weights (Wong et al. 1988). However, these patterns were shown to account for only 70% of all endoxylanases, and a classification system of all glycoside hydrolases (glycoside hydrolase families) is better recognized now (Collins et al. 2005). Products from xylan degradation by endoxylanases are a mixture of β-D-xylopyranosyl oligomers of various lengths, which serve as substrates for β-xylosidases that subsequently hydrolyze them to xylose from the nonreducing end of these oligomers (Polizeli et al. 2005). Multiple other enzymes are also involved in xylan degradation, primarily due to the complex nature of these polysaccharides: α-L-arabinofuranosidase cleaves the α-glycosidic

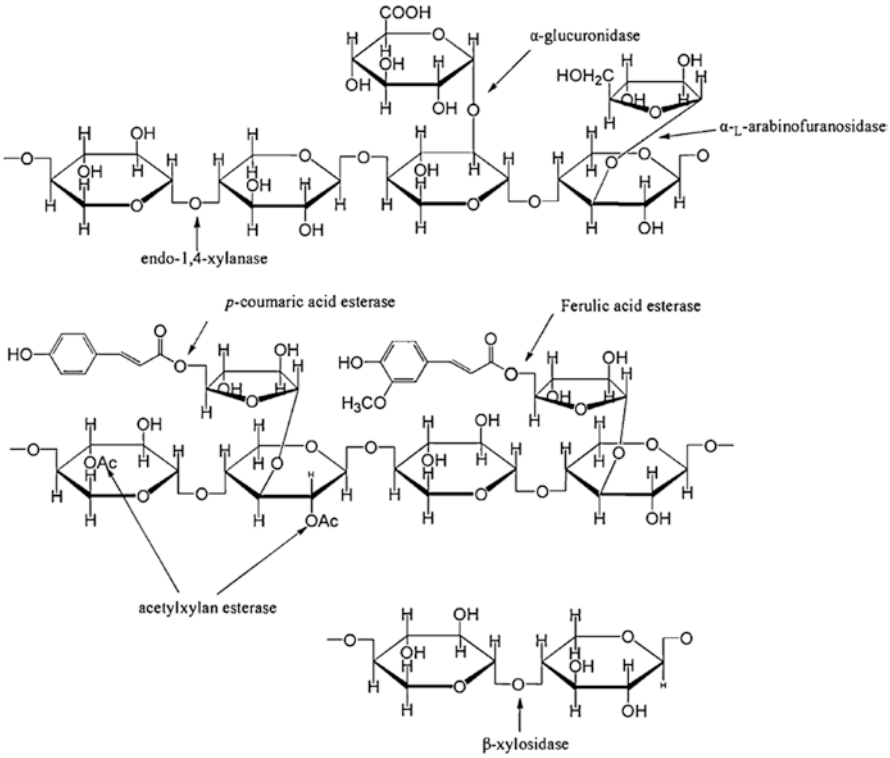

Fig. 12.2 Chemical structure and degradation of hemicellulose

bonds between arabinose and xylose moieties in xylan; α-glucuronidases cleave the α(1 → 2) bonds linking the (methyl) GlcU units in xylan (Kumar et al. 2008); acetylxylan esterase removes the O-acetyl groups at the 2- and 3-positions of β-D-xylopyranosyl residues; ferulic acid esterase (EC 3.1.1.73) cleaves the ester bond between the arabinose and ferulic acid side chains; and p-coumaric acid esterase (EC 3.1.1.73) cleaves the ester bond between the arabinose and ρ-coumaric acid (Polizeli et al. 2005).

12.3.2 Fungal Cellulases

Cellulase enzyme plays a key role in the hydrolysis of cellulosic substrate and converts it into monomeric sugars. For the effective hydrolysis of cellulosic substrate, three types of synergistically acting subcomponent enzymes are essential: endoglucanases (EG), exoglucanases (CBH), and beta-glucosidase (BGL). Cellobiohydrolases or exoglucanases which attack the crystalline ends of cellulosic substrate produce cellobiose, while endoglucanases divide glycosidic

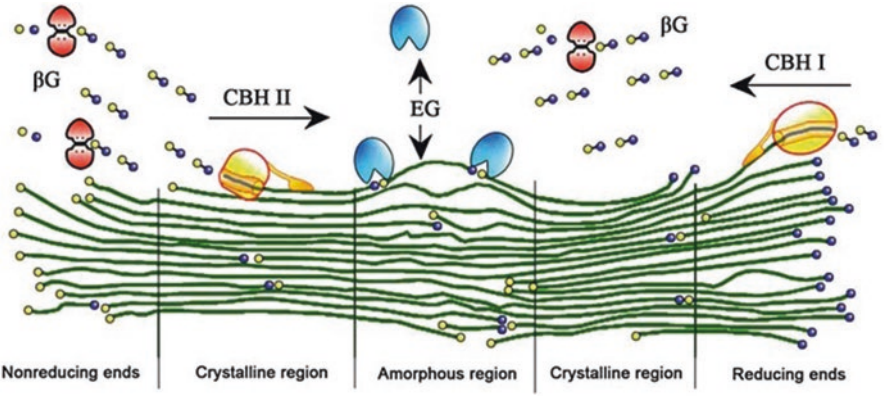

Fig. 12.3 Mechanism of cellulose biodegradation. CBH cellobiohydrolase (or exo-β-glucanase), EG endo-β-glucanase, β-G β-glucosidase

bonds within the amorphous part of the cellulosic substrate (Zhang and Lynd 2004; Yoon et al. 2014). Further, the liberated cellobiose is sliced by β-glucosidases (BGL) and releases glucose molecules (Liu et al. 2012; Wang et al. 2013). Figure 12.3 shows all three components and functions of cellulase responsible for enzymatic hydrolysis of agriculture waste. Cellulase enzymes are widely distributed in nature, and fungi are known as the potential producer of cellulase. Additionally, cellulolytic fungi have the major advantages to utilize secretory pathways as well as the production of high yields of protein. Intense research on other fungi like *Penicillium*, *Acremonium*, and *Chrysosporium* are underway for the potential production of cellulase. Table 12.2 summarizes cellulase production from different fungal species using variety of wastes via solid-state fermentation (SSF) and submerged fermentation (SmF).

12.3.3 Production of Fungal Cellulases

Fungal cellulase production can be carried out by two methods: (i) solid-state fermentation (SSF) and (ii) submerged fermentation (SmF) (Pandey 2003; Singhania et al. 2009; Bansal et al. 2012a, b). In case of SSF, solid substrates are used such as agriculture waste of rice straw, wheat bran, sugarcane bagasse, etc. for the production of cellulases (Xia and Cen 1994; Subramaniyam and Vimala 2012; Cherian et al. 2016). SSF involves the fermentation process carried out in absence or nearly in absence of free water using solid substrate. On the other hand, SmF involves fermentation in presence of water. Moreover, SmF uses primarily free molecules soluble in water as liquid substrates, like molasses in broth (Subramaniyam and Vimala 2012). The main advantage of SSF technique is easy recycling of cheap waste material and less cost, whereas SmF offers ease of purification and product recovery (Pandey et al. 2000; Couto and Sanromán 2006). Further, SSF is mainly

Table 12.2 Ratio of different used biomass for cellulase production

Fungi	Type of agricultural waste	Cellulase productivity	Fermentation process (SmF/SSF)	References
Aspergillus terreus	Rice straw	FPase: 10.96 IU/g	SSF	Narra et al. (2012)
Aspergillus fumigatus ABK9	Wheat bran: rice straw(1.1:1)[a]	CMCase: 826.2 IU/g FPase: 102.5 U/g β-glucosidase: 255.16 IU/g	SSF	Das et al. (2013)
Aspergillus protuberus	Rice husk	β-glucosidase: 26.06 IU/g	SSF	Yadav et al. (2016)
Trichoderma asperellum SR1–7	Wheat bran	FPase: 2.2 IU/gds CMCase: 13.2 IU/gds β-glucosidase 9.2 IU/gds	SSF	Raghuwanshi et al. (2014)
Aspergillus niger N402	Wheat straw	FPase: 24 IU/g	SSF	Pensupa et al. (2013)
Aspergillus fumigatus NITDGPKA3	Rice straw	CMCase: 64.18 IU/gds FPase: 3.1 IU/gds	SSF	Sarkar and Aikat (2012)
Myceliophthora thermophila JCP 1–4	Sugar cane bagasse: wheat bran (1: 1)[a]	CMCase: 357.51 IU/g β-glucosidase: 45.42 IU/g	SSF	Pereira et al. (2015)
Aspergillus nige KK2	Rice straw	FPase: 19.5 IU/g CMCase: 129 IU/g β-glucosidase: 100 IU/g	SSF	Kang et al. (2004)
Aspergillus niger NCIM 548	Wheat bran: corn bran, kinnow peel (2:1:2)[a]	FPase: 5.54 IU/g	SmF	Kumar et al. (2011)
Aspergillus fumigatus P40M2	Soybean bran	CMCase: 160.1 IU/g	SSF	Delabona et al. (2012)
Aspergillus ellipticus	Wheat straw	FPase: 117.25 IU/g CMCase: 725.11 IU/g β-glucosidase: 29.65 IU/g	SSF	Agrawal and Matkar (2016)

(continued)

Table 12.2 (continued)

Fungi	Type of agricultural waste	Cellulase productivity	Fermentation process (SmF/SSF)	References
Penicillium echinulatum 9A02S1	Sugar cane Bagasse	FPase: 12.5 IU/g	SmF	Camassola and Dillon (2014)
Trichoderma viride VKF3	Sugar cane Bagasse	CMCase: 33 IU/g FPase: 10.09 IU/g	SSF	Nathan et al. (2014)
Rhizopus oryzae CCT 7560	Rice husk and rice bran	CMCase: 5.1 U/g FPase: 2.3 IU/g	SSF	Kupski et al. (2014)

[a]Ratio of different used biomass for cellulase production

favorable for microorganisms which require less moisture content, while due to high water activity, SmF is suited to bacteria for cellulase production (Babu and Satyanarayana 1996). Additionally, SSF has been known for fermentation in Asian and Western countries since the ancient time (Ryu and Mandels 1980; Zhuang et al. 2007; Swain and Ray 2007). However, SSF has gained importance in Western countries after the discovery of penicillin via SmF technique in the 1940s. Though, in the last two decades, SSF has gained attention because of many biotechnological advantages like high fermentation ability, more stable end product, subordinate catabolic repression, as well as cost-effective technology (Sukumaran et al. 2009; Kasana et al. 2008; Liang et al. 2010; Dhillon et al. 2013). In the past 10 years, interest in SSF has been renewed because microorganisms including genetically modified organisms (GMO) may produce cellulase effectively through the SSF (Chahal 1983). Additionally, cellulase production via SSF is preferred over SmF because of two to three times higher enzyme production, high protein rate, and direct accessibility of dried fermentable solids as source of enzyme which easily eliminate the cost involved in downstream processing (Sánchez 2009; Hendriks and Zeeman 2009; Sadhu and Maiti 2013; El-Bakry et al. 2015).

12.3.4 Source of Fungal Cellulase Production

Degradation of cellulose via fungal cellulase is well documented, and several cellulose degrading fungi like *Aspergillus niger*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum*, *Scopulariopsis brevicaulis*, *Stachybotrys chartarum*, *Verticillium cycolsporum*, and *Chaetomium hamadae* have been investigated for cellulase production (El-Morsy 2000; Luo et al. 2005). Further, various fungi have also been screened for the cellulase production based on their habitat (El-Morsy 2000; Luo et al. 2005). In one of the study by Maria et al. (2005), twenty-nine different fungal isolates were reported for cellulase

production. Moreover, most of these fungal isolates produce endoglucanases where most of them belong to *Ascomycetes*. Kathiresan and Manivannan (2006) isolated seven fungal species such as *Acremonium* sp., *Alternaria*, chlamydospore, *Aspergillus* sp., *Fusarium* sp., as well as *Pestalotiopsis* sp. from the southwest coast of India and used for cellulase production. Among all these fungi, *Aspergillus* sp. was found to be efficient cellulase producers.

Besides *Aspergillus* sp., *T. reesei* are also known as prominent cellulase producers that possess a complete cellulase system (Rasmussen et al. 2010). Though, action of fungi is different in terms of degradation of lignocellulosic biomass in decaying pattern and the structural changes found in the degraded substrates, category belonging to white-rot fungi (WRF) degrades all the components of biomass, namely, lignin, cellulose, and hemicellulose by colonizing themselves on the lignocellulosic substrate (Kuhad and Singh 2007). On the other hand, fungi belonging to category brown-rot fungi (BRF) favorably degrade the cellulosic component and hemicellulosic part with the modified lignin part (Schwarze et al. 2000; Schmidt 2006). Apart from the fungi type, production of cellulases might also be dependent on the initial amount of cellulose, hemicellulose, and lignin present in biomass (Liu et al. 2014). Figure 12.4 shows different subcomponents of lignocellulosic biomass and their obtained product as sugars after enzymatic hydrolysis. A separate group of fungi are responsible for degrading these biomass via bioconversion reaction of cellulases.

12.3.5 Chaetomium' *Cellulases*

Eriksen and Goksøyr (1977) cultivated *Chaetomium thermophile* var. *dissitum* in a liquid medium with cellulose. They recorded that taxon produced extracellular cellulolytic enzymes. By concentration of the culture filtrate, followed by ion-exchange chromatography on DEAE-Sephadex A-50 and gel filtration on Biogel P-100, three electrophoretically pure components were obtained. Of these, one was a typical C, enzyme (endoglucanase) causing rapid decrease of the viscosity of carboxymethylcel-

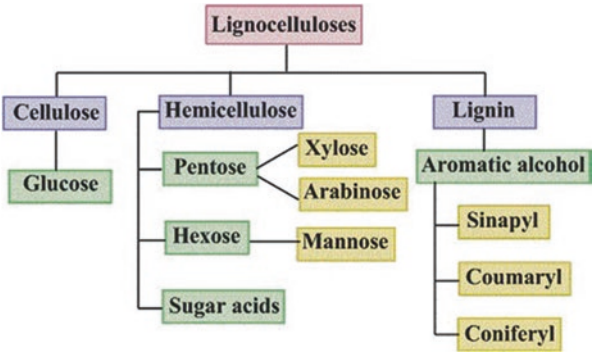

Fig. 12.4 Schematic representation showing the contents present in the lignocelluloses. (Sajith et al. 2016)

lulose solutions, while showing low effect on native cellulose. The other was active toward native cellulose but had little effect on the viscosity of carboxymethylcellulose. It is concluded that this enzyme is an exoglucanase (CI enzyme), possibly a cellobiohydrolase. The third component showed only cellobiase (P-glucosidase) activity and had no effect on cotton or carboxymethylcellulose. The three components, when mixed, showed synergistic effects on highly ordered cellulose. The endo- and exoglucanases were characterized with regard to molecular size and isoelectric point (pl). Both cellulases had pl near 4.55, but their molecular weights were different: 67000 (exoglucanase) and 41,000 (endoglucanase). The effect of temperature on the activity of the cellulases was examined with both cotton and carboxymethylcellulose as substrate. Arrhenius activation energies, Q_{10}, and temperature optima for the different reactions were determined.

The cellulolytic properties of a *Chaetomium crispatum* strain were investigated by Geeraerts and Vandamme (2008). They found that the cellulolytic enzyme complex, i.e., exo-1,4-β-glucosidase (EC 3.2.1.74), endo-l,4-β-glucanase (EC 3.2.1.4.), and 8-glucosidase or cellobiase (EC 3.2.1.21), displayed optimal activity at pH 5.0 and 25 °C. Although carboxymethyl-celluloses are the usual pseudo-substrates for this enzyme complex, those with a high degree of substitution gave rise to poor growth and low cellulase activity. Insoluble crude cellulosics such as newsprint, recycled paper, rice, and flax straw were substantially solubilized at 28 °C within 3–5 days of fermentation. A study of the cellulase-complex formation during the growth cycle revealed that β-glucosidase was produced mainly intracellularly in the early exponential phase, while the overall exo-l,4-β-glucosidase and endo-1,4-β-glucanase formation gradually increased during the total fermentation cycle. The mycelial protein of *Chaetomium crispatum* grown on crude cellulosics displayed a favorable amino acid pattern, indicating its potential value as a source of single-cell protein (SCP).

Soni et al. in 1999 studied the distribution pattern of cellulases in the extracellular and cell-associated fractions of *Chaetomium erraticum* varied depending upon the cultural conditions. The extracellular fractions revealed two forms each of endoglucanase (EG I, EG II) and β-glucosidase (β-Glu I, β-Glu II) under static and shake conditions. However, the appearance of an additional form of endoglucanase, EG III, and β-glucosidase, β-Glu III, in static intracellular and cell debris fractions showed a relation of cellulase production with the perithecia development in this fungus. The maximal production of enzymes was observed at 37 °C, pH range 5.0–10.0 in the presence of 1–2% carboxy methyl cellulose (CMC) in static cultures (for exoglucanase and β-glucosidase) and shake cultures (for endoglucanase).

Chaetomium globosum has been a well-known potential antagonist of several seed- and soilborne fungi. Eight isolates of *C. globosum* were obtained from different sources and were identified by morphological characters. *C. globosum* isolates were examined for the presence of extracellular proteins, cellulases, and antifungal metabolites in culture filtrate by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDSPAGE), thin-layer chromatography, and high-performance liquid

chromatography by Shanthiyaa et al. (2014). Variation in the mycelial protein of *C. globosum* isolates was noted in the SDSPAGE analysis. Different *C. globosum* isolates that showed more number of bands in protein profile were further screened for the production of cellulases in culture filtrate. Cellulase activity of *C. globosum* isolates revealed that maximum activity was observed in the isolate Cg-6 after 11 days of incubation, while Cg-2 had least activity.

Yadav and Bagool (2015) used books and important documents in storage having moldy appearance to analyze and isolate cellulolytic fungi. They found that most dominated genus was *Chaetomium* with 13 different species and were screened for their cellulase producing capability by the filter paper degradation ability. Eight *Chaetomium* isolates were chosen for exoglucanase and endoglucanase enzyme activity assay on the basis of percentage loss of filter paper. Five *Chaetomium* species were selected as potentially able to secret high exoglucanase and endoglucanase cellulases. *Chaetomium dolichotrichum*, *C. funiculosum*, *C. globosum*, *C. anguistispirale*, and *Chaetomium* sp. were found very good producer of total cellulase and endogluccanase during study.

Recently Cuomo et al. (2015) sequenced a strain of *Chaetomium globosum* (CBS 148.51) isolated from stored cotton in Washington, DC. This strain is commonly used in testing paper and polymers for fungal resistance (Gu and Gu 2005). Three size-selected libraries were constructed from genomic DNA. These included a 4-kb plasmid, a 10-kb plasmid, and a 40-kb fosmid library; each library was paired-end sequenced using Sanger technology. The resulting 568,566 reads were assembled using Arachne version 3.0, with an average of 8.9 sequence depth in the final assembly. Based on the assembly, the genome size was estimated to be 34.3 Mb with a GC content of 55.6%. The assembly was organized in 1245 contigs, which are linked by paired-end reads into 37 scaffolds. The average base is found in a scaffold of N50 size 4.72 Mb and a contig of N50 size 50.76 kb. The assembly is highly contiguous; the largest 8 scaffolds account for 98% of the assembly bases. This genome sequence of *C. globosum* will serve as an important reference for further studies of the basis of its cellulose specificity, for genes that enable human infection and for further comparative studies with other fungi.

Flannigan and Sellars (1972) evaluated 30 thermophilous fungi for their ability to produce CMCase and found that 16 were able to degrade cellulose. Similarly, Rosenberg (1978) tested 21 species of thermophilic and thermotolerant fungi for their cellulolytic activity. It was observed by the author that *Chaetomium thermophile* var. *coprophile*, *Chaetomium thermophile* var. *dissitum*, *Humicola grisea*, *Humicola insolens*, *Myriococcum albomyces*, *Sportotrichum thermophile*, *Malbranchea pulchella*, *Allescheria terrestris*, *Allescheria fumigatus*, *Talaromyces thermophilus*, *Torula thermophila*, *Thielavia thermophile*, and *Chrysosporium pruinosum* showed positive cellulolytic activity. On the contrary, Jain et al. (1979) observed that *Humicola lanuginosa*, *Mucor meihei*, *Malbranchea pulchella* var. *sulfurea*, and *Talaromyces dupontii* did not degrade cellulose and filter paper when used as a sole source of carbon, but showed filter paper-degrading activity when grown on wheat straw. Based on these observations, they suggested that these fungi

have some specific requirements for cellulose production that were fulfilled by growing on wheat straw. *Humicola lanuginosa* (syn. *Thermomyces lanuginosus*) was unable to synthesize cellulose, as observed by Chang and Hudson (1967), Fergus (1969), and Deacon (1985). However, some recent reports (Lee et al. 2014) indicate that *Thermomyces lanuginosus* is able to produce cellulase.

Of the 15 thermophilc fungi tested by Srivastava et al. (1981), 6 were found to be celluloytic. They observed variations in the decomposition of cellulose by these fungi. Some of the fungal isolates were high decomposers, while others were weak decomposers. Thermophilic fungi such as *Mucor miehei*, *Mucor pusillus*, and *Rhizopus rhizopodiformis*, which were considered secondary sugar fungi, are confirmed to be moderately cellulolytic (Johri and Pandey 1982). Tong and Cole (1982) observed *Thermoascus aurantiacus* as the most active cellulose producer among the several thermophilic fungi evaluated by them. Sandhu et al. (1985) screened 98 strains of thermophilous fungi for their ability to produce cellulases. Most of these fungi belonged to the genera *Acremonium*, *Aspergillus*, *Chaetomium*, *Penicillium*, *Thermoascus*, and *Thielavia*. They also revealed that cellulases produced by true thermophiles were more stable than those produced by thermotolerant and microthermophiles.

Chaetomium thermophilum var. *coprophilum* produced large quantities of extracellular as well as intracellular b-glucosidase when grown on cellulose or cellobiose (Venturi et al. 2002). The purification and characterization of cellulases from *Humicola grisea* and *Aspergillus fumigatus* have been studied by Takashima et al. (1996) and Ximenes et al. (1996).

van Noort et al. (2013) investigated the genome of *Chaetomium thermophilum* and observed that there are fewer genes that encode complex carbohydrate-degrading enzymes, in particular, in thermophilic mold genomes than their mesophilic counterpart. The genome of *Chaetomium thermophilum* encodes three CDHs, while those of *Chaetomium globosum* and *Neurospora crassa* encode only two, which depicts a higher cellulolytic ability of the former.

12.4 Mechanisms of Cellulase Synthesis

Apart from the development of economically feasible systems for cellulose degradation, there has also been continuing interest in understanding the mechanisms of cellulase synthesis and production to identify feasible approaches for increasing cellulase production (Li et al. 2010).

Sun et al. (2008a, b) investigated the proteome profiling map of the cellulases secreted by Trichoderma reesei Rut C-30 using two-dimensional gel electrophoresis. CBH I and CBH II were found to represent about 37% of the total extracellular proteins, and the CBH II concentration produced with nonpretreated rice straw powder was about threefold higher than that with alkali-treated straw. This interest-

ing result suggests that the synthesis of CBH II is controlled by other factors aside from cellulose. Sun et al. (2008a, b) reported the differences in the composition and expression levels of *P. decumbens* cellulases under induced and basal conditions. The basal cellulase in *P. decumbens* was demonstrated to be composed of CBH I, CBH II, EG I, EG II, and bG, whereas two EGs were expressed only under induction conditions. Furthermore, the basal and induced EGs from *Penicillium decumbens* were encoded by different genes.

To understand the mechanism of the two transcription repressors Cre1 and ACE I in T. reesei, Su et al. (2009) developed a new strategy wherein a plasmid that encodes a chimeric transcription activator containing the DNA binding domains from Cre1 and ACE I and the effector domain from the activator ACE II was constructed and transformed into T. reesei. The recombinant strain had higher cellulase activity than its parent strain and had a different colony appearance. The results also provide an overview of the set of genes that might be regulated by Cre1 or ACE I. These results contribute to further understanding the regulatory roles of these two repressors in cellular pathways and provided a new method for strain improvement through genetic manipulation.

Liu et al. (2008) studied the differences in gene sequences of CBH I gene (cbh1) from wild-type and mutant *P. decumbens* strains and found that the mutant strain JU-A10 is a multiple mutant of the wild-type strain in the sequences upstream of the gene. The enhanced CBH activity of the mutant may be due to a single-base mutation of the upstream sequence of cbh1, which affects the transcription regulation of the mutant instead of the protein-coding sequences. This discovery suggests the critical role of the promoter region of cellulose-encoding genes, which is helpful in constructing hyperproducing strains of *P. decumbens*. An apparent abolishment of glucose repression was also identified in strain JU-A10, with enhanced observed cellulase and hemicellulase production in glucose-containing media. Genomic analysis of this strain revealed a single nucleotide deletion at the +1205 position in the creA gene, which encodes a carbon catabolite repressor protein. This frameshift mutation changes the amino acid sequence downstream from the site of the mutation (unpublished data). Numerous other mutations have also been identified from this mutant strain through genomic analysis, in addition to the changes in the creA gene.

Another focus for Chinese scientists is identifying inducers for cellulolytic enzyme production, which could potentially benefit the cellulase industry. Wang et al. (1995) observed that the concentrations of ATP and cyclic AMP (cAMP) influence cellulase production. Cellulase synthesis is repressed by high concentrations of intracellular ATP, whereas exogenous cAMP increases cellulase synthesis. The effects of wheat bran on the hydrolysis of extracellular biomass were investigated in *P. decumbens* by Sun et al. (2008a, b). The soluble cello-oligosaccharide composition of wheat bran was shown to be one of the most significant factors in cellulase production. This significant discovery may be critical in the cellulase industry because wheat bran, as an inducer in cellulase and xylanase production, is inexpensive.

12.5 Molecular Weight of Fungal Cellulases

The MW of cellulase produced by different fungal species may vary from 12 kDa to 126 kDa (Parry et al. 1983; Bai et al. 2013). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) is the most commonly used method for judging the apparent MW of enzymes (Joo et al. 2010; Lee et al. 2010; Ramani et al. 2012). Fungal cellulase may be of monomeric (Naika et al. 2007) or dimeric (Chaabouni et al. 2005) in nature. Cellulase produced by T. viride was purified to homogeneity using DEAE-sepharose column and the MW was estimated as 87 kDa by SDS-PAGE (Yasmin et al. 2013). P. pinophilum MS 20 produced a monomeric cellulase with MW of 42 kDa, which appeared as a single band on SDS-PAGE gel (Pol et al. 2012). It was reported that A. awamori VTCC-F099 and Fomitopsis pinicola produced monomeric thermo active cellulase with a MW of 32 kDa (Yoon et al. 2008; Van Tuan Nguyen 2010). The cellulase produced by *A. niger* revealed a MW of 60 kDa on SDS-PAGE gel (Baraldo et al. 2014). In all the aforesaid studies, the purified celluase appeared as single band upon SDS-PAGE, indicating that the cellulase produced by these fungi are active in solution as monomers or homodimers, consequently migrates through the SDS-PAGE gel according to their MW so as to segregate as single band. In contrast, some studies reported the identification of hetero-dimeric cellulases or isoforms which appeared as separate bands upon SDS-PAGE. For instance, *A. niger* Z10 produced two cellulase bands on SDS-PAGE gel with MWs 50 and 83 kDa (Coral et al. 2002a, b). Similarly, another strain of *A. niger* is also reported as producing dimeric cellulase with MWs of 23 and 36 kDa, whereas *A. fumigatus* produced dimeric cellulase with MWs of 21 and 32 kDa. Kaur et al. (2007) reported 40 and 50 kDa isoforms of cellulase produced by Melanocarpus sp. MTCC 3922 with MW as judged by SDS-PAGE.

12.6 Factor Affecting Cellulase Production

Several fermentation conditions play fundamental roles on cellulases production, among which fermentation method, carbon source, nitrogen source, pH, temperature, salt/metal ions effect, incubation time, aerations, and fungal species (Norouzian 2008; Okoye et al. 2013; Saini et al. 2017).

12.6.1 Fermentation Method

Fungal cellulases have been produced through solid-state fermentation (SSF) and submerged fermentation (SmF). In SSF, the fungal species is grown on one or more solid substrate such as rice straw, wheat bran, corn husk, cassava cake, or sugar cane bagasse without or very low water content. The grown microorganism utilized the solid substrate steadily and slowly thus under SSF condition, the microorganism

can be grown for long period of time, for instance, for several days (Ahmed et al. 2017a, b). The high productivity, cheap substrate utilization, and low energy requirement are the advantages of SSF. Moreover, under SSF conditions, there is minimal water output as well as lack of foam up which makes it economically feasible (Faisal and Benjamin 2016). SSF shortcomings are limited to heat generation and lack of knowledge on automation (Ahmed et al. 2017a, b, Shweta 2015; Soccol et al. 2017). SSF has been utilized for cellulase production from several fungal species such as *lichtheimia romosa* (Garcia et al. 2015), *Phaffomycetaceae* (Cerda et al. 2017), Dipodascaceae (Cerda et al. 2017), *Trichoderma citrinoviride* AUKAR04 (Periyasamy et al. 2017), *Humicola insolens* MTCC 1433 (Singla and Taggar 2017), among many others. On the other hand, in SMF, free-flowing liquid like molasses and/or broths supplemented with different nutrients is used to cultivate microorganisms. The enzymes including cellulase and metabolic byproducts are secreted into fermentation medium, and medium supplements or nutrients are rapidly utilized and a continuous supply is needed. SMF has several advantages such as simplicity of sterilization, heat and mass transfer, process monitoring (pH, temperature, and soluble molecules) and automation, and extraction and recovery of enzymes and bioactives (Ahmed et al. 2017a, b). Several cellulase enzymes have been produced by SMF from different fungal species including *Aspergillus flavus* (Gomathi et al. 2012), *Aspergillus niger* FC-1 (Jiang 2013), and *Aspergillus niger* (Reddy et al. 2015), among many others.

12.6.2 Carbon Source

Carbon source is the major factor affecting the cellulases production, attributing to the fact that cellulases are inducible enzymes that are expressed by cells in response to different carbon source present in the fermentation medium (Saini et al. 2017; Zhang et al. 2017). For instance, optimal cellulase production from *Hypocrea jecorina* QM6a, QM9414, and RUTC-30 was attained in medium containing microcrystalline celluloses as the sole carbon source (Dashtban et al. 2011). *Penicillium* sp. produced the highest cellulase activity on lactose-containing media among different carbon sources tested such as sarbose, maltose, sucrose, lactose, dextrose, galactose, cellobiose, and CMC (Prasanna et al. 2016). Expression of different cellulase isoforms in response to carbon source has also been reported (Amore et al. 2013). For example, *Aspergillus terreus* expressed four endoglucanase (EG) isoforms in the presence of rice straw as solid substrate or corn cobs as liquid substrate. Similarly, supplementation of fructose and cellobiose to corn cobs medium upregulates at least one of EG, while adding mannitol, ethanol, and glycerol selectively suppressed the expression of three EG isoforms. Similarly four isoforms of β-glucosidase (βG) were expressed in presence of corn cob containing medium, and addition of glucose, cellobiose, mannitol, fructose, sucrose, or glycerol repressed one or more βG isoforms (Nazir et al. 2010). *Aspergillus fumigatus* Z5 grown on culture media containing glucose, avicel, and rice straw secreted 61, 125, and 152 proteins, respectively. Proteomic analysis suggested that glycoside hydrolases

including cellulases and hemicellulases were overexpressed on rice straw and avicel-containing media compared to glucose used as carbon sources (Liu et al. 2013). The molecular mechanisms by which the expression of these different isoforms are regulated and different carbon sources influence the quantity and isoforms expression are not well established which hampered genetic engineering of these fungi for industrial purpose (Coradetti et al. 2012). Thus understanding the mechanisms of cellulase expression hold a critical significance for enhancement of cellulase enzymes production and have been investigated in *Aspergillus* and *Trichoderma* (Gautam et al. 2011). These fungi produce extracellular cellulase enzymes when they are grown on media containing plant polymers, or short oligosaccharides as an energy source, and when cultivated on media containing easily metabolizable sugar such as glucose, the expression of these enzymes is repressed. Carbon catabolite repression is considered the most acceptable mechanism to repress cellulase production when grown on easily metabolizable sugars (Amore et al. 2013).

Recently, Zhang et al. published a study demonstrating that *Rhizopus stolonifera* host a gene which encodes for cellobiose synthetase (CBS) to synthesize cellobiose from uridine diphosphate glucose (UDPG). CBS was found to play a fundamental role in expression of cellulase gene through the induction of the cellobiose-responsive regulators CLR1 and CLR2 and thus inducing the transcription of cellulase genes. The authors suggested that minimal constitutive expression of cellulase may be driven by cellobiose synthesized by CBS from carbohydrate metabolites (Zhang et al. 2017).

12.6.3 Nitrogen Source

Another important factor that affects protein secretion in fungi is nitrogen. Different nitrogen source can be included in fermentation medium for cellulase production. Among organic nitrogen sources that can be used are peptone, yeast or beef extract, and tryptone or soybean meal. Inorganic nitrogen sources like ammonium sulfate, ammonium chloride, and ammonium hydrogen phosphate can also be used as a nitrogen source (Ahmed et al. 2017b; Kachlishvili et al. 2006). Optimum cellulase activity was achieved from *Penicillium* sp. when cultivated on yeast extract containing medium (Prasanna et al. 2016). *Trichoderma reesei* showed optimum production of cellulase when cultivated on *Parthenium* biomass containing ammonium molybdate, peptone, or yeast extracts as nitrogen source (Saini et al. 2017).

12.6.4 pH and Temperature

Optimization of parameters such as pH and temperature is also of crucial significance for enzyme production since these physicochemical parameters affect the growth of microorganism hence the bioactive production. The optimal cellulase production from *Penicillium* sp. was attained on Czapek-Dox medium at pH 5.0 and

30 °C (Prasanna et al. 2016). Similarly, optimum production of celllulase by *Aspergillus tubingensis* KY615746 was achieved at pH 4 and temperature of 30 °C (El-Nahrawy et al. 2017).

12.6.5 Incubation Time

Myceliophthora heterothallica produced the highest endoglucanase on SSF containing wheat bran or sugarcane occurred at 192 hours and on SmF containing cardboard at 168 hours (da Teixeira et al. 2016). Optimal production of carboxymethylcellulase (CMCase) from *Aspergillus hortai* under SMF was achieved after 96 hours (El-Hadi et al. 2014).

12.7 Statistical Approach for Optimization of Cellulase Production

Optimization of cellulase production is a critical process for efficient and cost-effective cellulase production. Traditionally optimization of cellulase production is carried out by employing One Variable at A time (OVAT) approach. OVAT involves varying one parameter at a time keeping other factors constant. OVAT is regarded as a laborious technique and time-consuming and misleading approach because these parameters are independent and OVAT tends to ignore the interactions between them, in addition to extensive time needed to perform a large number of experiments.

Statistical approaches such as surface response methodology and Plackett-Burman design are efficient approaches employed for optimization of fermentation parameters (Shajahan et al.; Singh et al. 2014). Several studies have employed statistical methods for optimization of cellulase production. Cellulase production from *Trichoderma reesei* was optimized using Plackett-Burman design of 9 nutrients for their influence on cellulase secretion using Response Surface Methodology (RSM). The study demonstrated that the optimal concentration of avicel, soybean cake flour, KH2PO4, and CoCl2·6H2O for cellulase production were 25.30 g/l, 23.53 g/l, 4.90 g/L, and 0.95 g/l, respectively (Saravanan et al. 2012). In another study, using statistical Full Factorial Design (FFD), optimal cellulase production from *Penicillium funiculosum* ATCC11797 was achieved on culture media containing avicel (10 g/l) as carbon source, urea (1.2 g/l), yeast extract (1.0 g/l), KH_2PO_4 (6.0 g/l), and $MgSO_4$. $7H_2O$ (1.2 g/l) with an agitation speed of 220 rpm and aeration rate of 0.6 vvm. These conditions resulted in activities of 508 U/l for FPase, 9,204 U/l for endoglucanase, and 2,395 U/l for β-glucosidase which are 3.6–9.5 times higher than production using nonoptimized conditions (de Albuquerque de Carvalho et al. 2014). Cellulase production from *Trichoderma reesei* RUT C-30 was optimized employing a two-stage statistical design, namely, fractional factorial design and response surface Box-Behnken design, on wheat bran and cellulose under SSF. This approach resulted in a 3.2-fold increase in CMCase production to

959.53 IU/gDS (Idris et al. 2017). The statistical approaches for optimization of fermentation conditions are considered efficient because the interactions of multiple variables are taken into consideration and the number of experiments needed to be performed is reduced to minimum (Ahmed et al. 2017a, b; Shajahan et al. 2017; Singh et al. 2014).

12.8 Application of Cellulases

Cellulases, over many decades, are used in various industrial applications, securing the third rank among enzymes annual sale and expected to exceed the protease in the near future (Menendez et al. 2015). Cellulase enzymes have got tremendous applications in different industries including biofuel production, paper and pulp industry, detergent industries, animal feeds among others.

12.8.1 Biomass Hydrolysis and Biofuel Production

Cellulase along with other enzymes is used in the hydrolysis of biomass into sugar and other chemicals. Hexoses or pentoses are then fermented to bioethanol or other fuel (Sun and Cheng 2002).

With the rapid increase in world population accompanied by increased demand of energy, depletion of fossil fuel, and enhanced greenhouse effect from traditional fuel, there is crucial need to develop or search for cheap, renewable, and sustainable sources of energy. Thus cellulases are involved in biofuel productions and minimization of energy crisis and environmental pollution (Horn et al. 2012; Sharada et al. 2014). However, the bioconversion of pretreated cellulose-based materials at the industrial level into fermentable sugars employs a mixture of enzymes for complete hydrolysis, the cost of which is very high, making biorefining processes economically unfeasible. Thus the search of biocatalysts such as cellulases with novel properties exemplified by high thermostabilty, acidophilicity, and high solvent tolerance could help to overcome the cost hurdles. Cellulases application in biomass hydrolysis and biofuel productions is currently the subject of numerous studies supported by different agencies across the world (Budihal et al. 2016; Srivastava et al. 2015a, b, c).

12.8.2 Paper and Pulp Industry

Cellulases are used in the paper and pulp industry which has expanded significantly in the last decades from 320 to 395 million tons (Przybysz Buzała et al. 2016). Pulping process can be achieved either through mechanical or biomechanical manners.

Mechanical pulping such as refining and grinding of the woody raw material results in pulps containing a high content of fines, bulk, and stiffness. On the other hand, biomechanical pulping employing enzymes such as cellulases results in around 20–40% energy savings during refining making the process economically feasible and significantly improved hand-sheet strength properties (Demuner et al. 2011; Sharada et al. 2014). It has also been reported that the addition of cellulases enhanced the bleachability of softwood kraft pulp and improved the final brightness score comparable to that of xylanase treatment (Kuhad et al. 2011).

12.8.3 Waste Management

Cellulase can be used in waste management. For instance, cellulases are used in the conversion of cellulosic municipal solid wastes to desirable chemicals and energy. Cellulases benefits in minimizing the effect of cellulose waste on our environment and driving the conversion of the pollutants to an alternative source of energy and chemicals thus displacing our growing dependence on fossil fuels (Bayer et al. 2007; Gautam et al. 2011; Kuhad et al. 2011).

12.8.4 Animal Feed Industry

Cellulase has great potential to be used in the animal feeds industry. Cellulase can be used in the pretreatment of agricultural silage and grain feed to enhance nutritional value and performance of animals (Kuhad et al. 2011). Similarly, addition of cellulase, along with other enzymes, can eliminate anti-nutritional factors present in the feed grains such as arabinoxylans, cellulose, dextrins, inulin, lignin, pectins, β-glucan, and oligosaccharides by degrading them. This in turn enhances the nutritional value and improves animal's health and performance (Asmare 2014; Murad and Azzaz 2010; Sharada et al. 2014).

12.8.5 Laundry and Detergent Industry

Cellulases are also used in the laundry and detergent industry which is one of the most popular markets for enzymes sale accounting for 20–30%, with lipase and proteases as major enzymatic components. An innovative approach recently adopted in this industry is the use of alkaline cellulases, protease, and lipase results in a crucial improvement of color brightness and dirt removal from the cotton blend garments (Juturu and Wu 2014b; Olsen and Falholt 1998).

12.8.6 Textile Industry

The most successful and popular application of cellulases is the textile industry. Cellulases are used in textile wet processing such as finishing of cellulose-based textiles, biostoning of jeans, and biopolishing of cotton and other cellulosic fabrics in order to improve hand and appearance (Arja 2007; Duran and Duran 2000; Juturu and Wu 2014b).

12.8.7 Wine and Beverage Industry

Cellulase enzymes along with glucanase can be used to improve both quality and yields of the fermented products such as wine and beverages. For example, during wine production, cellulase, pectinases, glucanases, and hemicellulases are used to improve color extraction, skin maceration, must clarification, filtration, and finally the wine quality and stability. Addition of β-glucosidases can increase the aroma of wines by hydrolyzing glycosylated precursors into their aglycones and glucose (Araujo et al. 2008; Kuhad et al. 2011).

12.8.8 Other Applications

Cellulases have also been applied in agriculture where they are used to hydrolyze the cell wall of plant pathogens thus controlling the plant infection and diseases. Many cellulolytic fungi including *Trichoderma* sp., *Geocladium* sp., *Chaetomium* sp., and *Penicillium* sp. are known to play a key role in agriculture by enhancing the seed germination, rapid plant growth and flowering, improving root system, and increasing crop yields (Behera et al. 2016; Kuhad et al. 2011). Cellulases have also been used for the improvement of the soil quality (Phitsuwan et al. 2013). In addition, cellulases are used in food processing during fruit and vegetable juices manufacturing to improve extraction (Sharada et al. 2014; Zhang and Zhang 2013).

Furthermore, applications of cellulases along with macerating enzymes have been found to increase extraction of olive oil under cold processing conditions and to improve its antioxidants and vitamin E contents (Aliakbarian et al. 2011; Sharma et al. 2015). Moreover, humans are known to poorly digest cellulose fiber, and taking a digestive enzyme product containing cellulases like Digestin helps to relieve digestive problems such as malabsorption (Gurung et al. 2013; Sharada et al. 2014). Finally, an interest in applying cellulases enzymes in chemical analysis such as diagnostic and food analysis has been considered (Li et al. 2012).

12.9 Biodegradation of Agricultural Biomass

Bioconversion of lignocellulosic biomass via cellulase enzyme is also known as enzymatic hydrolysis or saccharification. For effective conversion of cellulose into monomers, a complete cellulase system is required (endoglucanase, exoglucanase, and β-glucosidase) to act synergistically. Biomass, which undergoes pretreatment before the enzymatic hydrolysis can be easily converted into sugars, effectively (Megan et al. 2013; Jose and Arnold 2014). Moreover, released sugars can undergo fermentation to produce biofuel. Although pretreatment process removes lignin effectively, the hemicellulose and cellulose part is converted by the synergistic action of hemicellulases and cellulases (Dashtban et al. 2009). Enzymatic hydrolysis of lignocellulosic biomass is divided into primary hydrolysis and secondary hydrolysis. Primary hydrolysis generally takes place on the surface of substrate, and cellobiose is released due to the catalytic action of exo- and endoglucanases. The cellobiose is further converted into glucose via β-glucosidase in secondary hydrolysis (Zhang et al. 2006).

12.10 Biofuel Production from Biomass Waste Degradation

Over the past decade, several research groups have focused on biofuel production using biomass-based process as cost-effective technology. Cellulosic biomass to sugar conversion is the key step for biofuel production, and efforts have been made to explore the technology related to biomass conversion via cellulase such as improving the efficiency of cellulase-producing microorganisms and cellulase efficiency (Garvey et al. 2013). Figure 12.5 explains the efficient destruction of biomass via cellulase to release sugar and produce biofuels. Additionally, non-efficient cellulase is incapable to release sugars from this biomass and hydrolysis becomes incomplete (Fig. 12.5). Although commercial cellulases are manufactured from native microorganism, economic viability seems far away. Currently, for efficient enzymatic hydrolysis of biomass, different cellulases (EG, CBH, and BGL) have been achieved from different microbial sources, which makes the process economically sustainable. Therefore, intense research is underway to find out novel cellulase-producing systems in order to increase the flexibility of available microbial strain, yield, and processing. The current enzymatic hydrolysis research is aimed to improve the production of cellulase via screening of effective microorganisms that would provide new opportunities to face this challenges (Peterson and Nevalainen 2012; Chandel et al. 2012).

The fermentable sugars obtained from the effective hydrolysis via cellulase enzymes are used for the production of renewable energy (Fig. 12.6). In addition, fermentable sugars can be converted into bioethanol, biohydrogen, and methane through different processes with the help of specific microorganisms. Effective production and yield of biofuels are directly dependent on the amount of fermentable

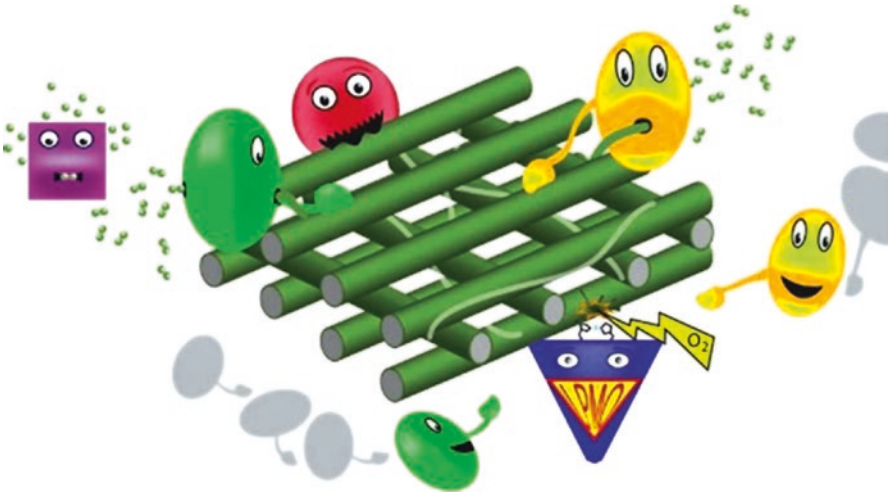

Fig. 12.5 Cartoon depiction of enzymatic cellulose deconstruction. Processive reducing (*orange*) and nonreducing end (*green*) cellulases move along the cellulose fibers liberating cellobiose units. The cellobiose released is then converted to individual glucose sugars by β-glucosidases (*pink*). Endo-acting cellulases (*red*) introduce chain breaks that the exo-acting enzymes can act upon. All of the aforementioned enzymes use hydrolytic mechanisms, while the new players in this process, LPMOs (*blue*), further potentiate their action using an oxidative mechanism to introduce further chain breaks on which the processive cellulases can initiate further degradation (Hemsworth et al. 2015)

sugars present in reaction medium, and the fair sugar amount is directed by viable cellulase system. Apart from cellulase system, the targeted process can be improved by removing the structural weakness of biomass via effective pretreatment strategies for biofuel production.

12.11 Conclusion

Continuous and significant studies have been made to improve the production and efficiency of cellulase enzyme for low-cost biofuel production. Although cellulase enzymes have versatile industrial applications, improvement in efficiency, reduction in cost, and energy consumptions are always selective parameters for the bioconversion of cellulosic biomass into biofuels. Isolation, screening, and cultivation of thermophiles/thermotolerant fungi for obtaining thermophilic/thermostable cellulase systems at commercial scale to produce biofuels are still a roll-back factor. Low cell yield and submerged production processes of enzyme are also cost-intensive for biomass to biofuel conversion processes. Limited knowledge of protein engineering and its implementation are also adding an extra obstacle in the overall bioconversion process apart from the purification of enzyme and end products separation. In

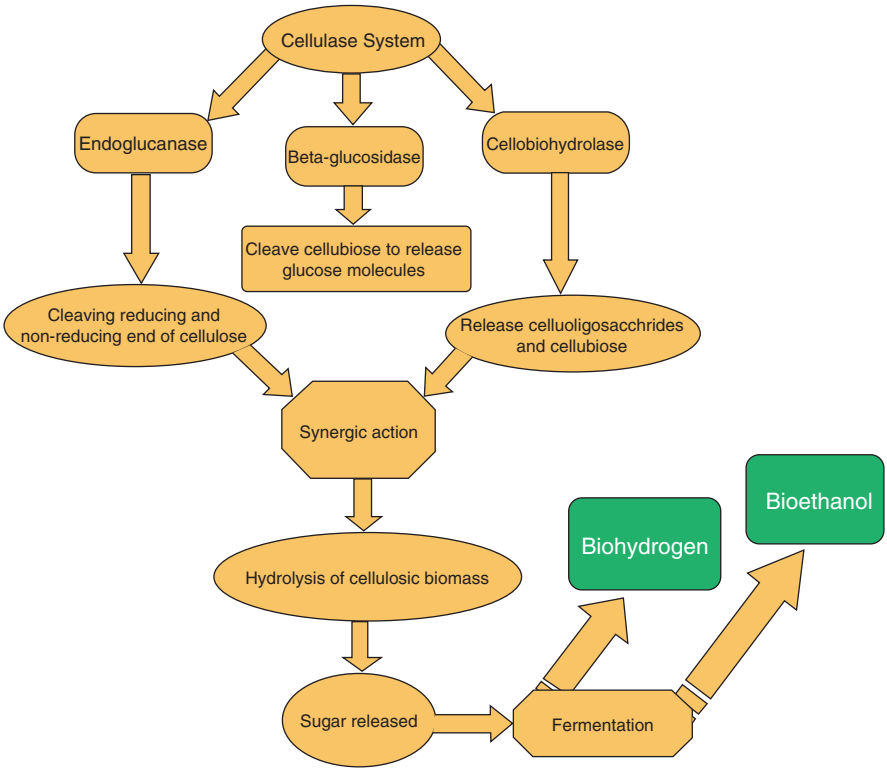

Fig. 12.6 Role of cellulases in complete biofuel production processes

view of the above issues, there is need of more and vast research for the production and optimization of cost-effective cellulases from potential fungal strain using SSF and biomass as a low-cost process. Additionally, research on more thermostable cellulase enzymes, their stability, and protein engineering may also support to achieve novel and more economical processes for biofuel production compared to the existing process technologies.

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