Chapter 21

Cellulase in Waste Management Applications

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1 INTRODUCTION

The recognition that environmental pollution is a worldwide threat to public health has given rise to a new massive industry for environmental restoration. Despite the massive utilization of lignocellulose materials, there are still ample cellulose-containing raw materials and waste products that are not exploited or that could be used more efficiently. The problem in this respect is, however, to develop sustainable processes that are economically profitable. Biological degradation, for economic and ecological reasons, has become an increasingly popular alternative for the treatment of agricultural, industrial, organic, as well as toxic wastes. These wastes have been insufficiently disposed leading to environmental pollution (Chandra et al., 2007).

Lignocellulose is the most abundant plant cell wall component of the biosphere and the most voluminous waste produced by our society. It consists of 70% moisture and 30% solid; of which holocellulose accounts for 65.5%, lignin 21.2%, ash 3.5%, hot water-soluble substances 5.6%, and alcohol–benzene soluble 4–1% (Sjöström, 1993). Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation (Saranraj et al., 2012). Cellulose-containing wastes may be agricultural, industrial, or urban in origin, and sewage sludge might also be considered a source of cellulose, since its cellulosic content provides the carbon needed for methane production in the anaerobic digestion of sludge.

Biological degradation with enzymatic hydrolysis of cellulosic biomass requires low volumes of chemicals and are conducted at mild conditions, in comparison with chemical hydrolysis. Moreover, chemical hydrolyzates need to be detoxified before carrying out fermentation. Therefore, enzymatic hydrolysis of lignocellulosic substrates is an efficient process (Rodhe et al., 2011). A variety of microorganisms take part in enzymatic hydrolysis of cellulose with the aid of a multienzyme system. Cellulases are inducible enzymes synthesized by a large diversity of microorganisms including fungi, bacteria, and actinomycetes during their growth on cellulosic materials. These microorganisms can be aerobic, anaerobic, mesophilic, or thermophilic (KOO, 2001; Kubicek, 1993). But, relatively few fungi and bacteria produce high levels of extracellular cellulase capable of solubilizing crystalline cellulose extensively (Johnson et al., 1982; Wood, 1985, 1989). Cellulase production by different organisms in fermentation has received more attention and is found to be cost-prohibitive because of the high cost of process engineering. Therefore, its production using readily available sources will help reduce importation costs. A portion of pretreated biomass can be used to feed a fungus or other organisms that produce cellulase that can then be added to pretreated solids to release glucose from cellulose (Johnson et al., 1982). Cellulases are responsible for the hydrolysis of the β-1,4-glucosidic bonds in cellulose. They are members of the glycoside hydrolase families of enzymes, which hydrolyze oligosaccharides and/or polysaccharides (Henrissat and Davies, 1997). In nature, complete enzymatic hydrolysis of cellulose is mediated by a combination of the three main types of cellulases: (1) *endo*-1,4-β-glucanase (CMCase), (2) cellobiohydrolase or exoglucanases (Avicelase), and (3) β-glucosidase (cellobiase), which act synergistically in the hydrolysis of cellulose (Nizamudeen and Bajaj, 2009).

Municipal solid waste (MSW) contains high amounts of cellulose, which is an ideal organic waste for the growth of most microorganisms as well as composting by potential microbes. MSW is composed of 40–50% cellulose, 9–12% hemicelluloses, and 10–15% lignin on a dry-weight basis. Unscientific disposal causes an adverse impact on all components of the environment and human health. A large number of microorganisms have been found in MSW. MSW is suitable for composting because of the presence of high percentages of organic matter (Rani and Nand, 2000; Gautam et al., 2010a).

Numerous industrial and agricultural wastes generated due to agricultural practices and food processing, such as rice straw, yam peels, cassava peels, and banana peels, represent one of the most important energy resources. These waste products can potentially be bioconverted into value-added products through the action of enzymes (Nfor et al., in press).

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Lignocellulosic biomass from agricultural residue is a renewable resource that stores energy from sunlight in its chemical bonds (McKendry, 2002). It contains a high proportion of cellulosic matter, which is easily decomposed by a combination of physical, chemical, and biological processes (Sabiiti et al., 2005). It has great potential for the production of affordable fuel ethanol by enzymatic hydrolysis and microbial fermentation because it is less expensive than starch (e.g., corn) and sucrose (e.g., sugarcane) producing crops and available in large quantities (Zheng et al., 2009). Saccharification of lignocellulosic biomass produces environment-friendly bioethanol-biofuel and other platform chemicals (Bajaj et al., 2014).

Waste recycling has been advanced as a method for preventing environmental decay and increasing food supply. The potential benefits from a successful recycling of lignocellulosic wastes are enormous. Cellulose and hemicellulose are sugar-rich fractions of interest for use in fermentation processes, since microorganisms may use the sugars for growth and production of value-added compounds, such as bioethanol, animal feed, compost, flavor, bioactive compounds, organic acids, and others. Nature solves the problem of removing recalcitrant plant cell wall material from the environment through the action of broad consortia of bacteria in the various cellulosic ecosystems, but over extended time periods. But the development of scientific and/or engineering approaches to the cost-effective conversion of plant cell wall biomass to biofuels is more beneficial (Bayer et al., 2007).

This chapter focuses on a broad view of cellulase systems emphasizing on their catalytic activity over the cellulosic biomass. Here, a comprehensive discussion on cellulosic biomass waste management with the enzymatic degradation of waste by cellulase and recycling of biomass applying cellulase enzyme toward the production of some value-added products are discussed.

2 CELLULASE

Cellulase is a class of enzyme that catalyzes the hydrolysis of cellulose. Cellulase is a multiple enzyme system consisting of *endo*-1,4-β-D-glucanases and *exo*-1,4-β-D-glucanases along with cellobiase (β-D-glucosideglucano hydrolase). Cellulases are expressed by a wide spectrum of microorganisms in nature. Screening and isolation of cellulase-producing microbes from nature is one of the important ways to get novel cellulases. These newly screened microbes are sources of new cellulase genes with diverse properties. Microorganisms that have cellulytic abilities (Kuhad et al., 2011) are listed in Table 21.1 and cellulase-producing bacteria are listed in Table 21.2.

TABLE 21.1 Microorganisms having Cellulolytic Abilities

Microorganisms	Examples
Fungi	***Soft-rot fungi***
	A. niger; Aspergillus nidulans; Aspergillus oryzae; Aspergillus terreus; Fusarium solani; Fusarium oxysporum; Humicola insolens; Humicola grisea; Melanocarpus albomyces;Penicillium brasilianum; Penicillium occitanis; P. decumbens; T. reesei; Trichoderma longibrachiatum; T. harzianum; Chaetomium cellulyticum; Chaetomium thermophilum; Neurospora crassa; Penicillium fumigosum; Thermoascus aurantiacus; Mucor circinelloides; Penicillium janthinellum; Paecilomyces inflatus; Penicillium echinulatum; Trichoderma atroviride
	Brown-rot fungi
	Coniophora puteana; Lanzites trabeum; Poria placenta; Tyromyces palustris; Fomitopsis sp.
	White-rot fungi
	Phanerochaete chrysosporium; Sporotrichum thermophile; Trametes versicolor; Agaricus arvensis; Pleurotus ostreatus; Phlebia gigantea
Bacteria	***Aerobic bacteria***
	Acinetobacter junii; Acinetobacter anitratus; Acidothermus cellulolyticus;Anoxybacillus sp.*; B. subtilis; B. pumilus; Bacillus amyloliquefaciens; B. licheniformis; Bacillus circulan; Bacillus flexus; Bacteriodes* sp.*; Cellulomonas biazotea; Cellvibrio gilvus; Eubacterium cellulosolvens; Geobacillus* sp.*; Microbispora bispora; Paenibacillus curdlanolyticus; Pseudomonas cellulosa; Salinivibrio* sp.*; Rhodothermus marinus*
	Anaerobic bacteria
	Acetivibrio cellulolyticus; Butyrivibrio fibrisolvens; C. thermocellum; Clostridium cellulolyticum; Clostridium acetobutylium; Clostridium papyrosolvens; Fibrobacter succinogenes; Ruminococcus albus
Actinomycetes	*Cellulomonas fimi; C. biazotea; C. uda; Streptomyces drozdowiczii; Streptomyces lividans; Thermomonospora fusca; Thermomonospora curvata*

TABLE 21.2 Native Cellulase-Producing Microorganisms Isolated from Different Sources

Enzymes	Source of Microorganisms	Isolated Microorganisms	References
Cellulosomes (multienzyme complexes)	Droppings of elephant	*C. thermocellum CT2*	Harish et al. (2010)
	Agriculture soil	*Cellulomonas* sp. *TSU-03*	Sangkharak et al. (2011)
	Hot-water spring	*Anoxybacillus flavithermus, Geobacillus thermodenitrificans, Geobacillus stearothermophilus*	Salah et al. (2007)
	Salt pans	*Halomonas caseinilytica, Halomonas muralis*	Sahay et al. (2012)
	Vinegar waste	*Acetobacter pasteurianus, Acetobacter oboediens, Gluconacetobacter xylinus, Gluconacetobacter hansenii, Gluconacetobacter europaeus, Gluconacetobacter intermedius, Gluconacetobacter entani*	Avdin and Aksoy (2009)
	Persimmon vinegar	*Gluconacetobacter* sp. *RKY5, G. intermedius TF2*	Wee et al. (2013)
	Empty fruit bunch, palm oil, mill effluent, compost	*Geobacillus pallidus*	Baharuddin et al. (2010)
	Ripe olives	*Cellulomonas flavigena*	Patel and Vaughn (1973)
Endoglucanase	Compost	*A. terreus M11*	Gao et al. (2008)
	Soil near rotten wood	*Fusarium chlamydosporum HML 0278*	Qin et al. (2010)
	Soil	*Cellulomonas* sp. *YJ5*	Yin et al. (2010)
	Gut of silk worm	*B. circulans, Proteus vulgaris, Klebsiella pneumonia, Escherichia coli, Citrobacter freundii, Serratia liquefaciens, Enterobacter* sp. *Pseudomonas fluorescens, Pseudomonas aeruginosa, Aeromonas* sp. *Erwinia* sp.	Anand et al. (2010)
	Wood waste from saw mill	*Aspergillus* sp. *Penicillium* sp. *Fusarium* sp., *Botrytis cinerea*	Chinedu et al. (2005)
	Rice bran	*T. reesei* QM9414	Rocky-Salimi and Hamidi-Esfahani (2010)
	Rice straw	*Myceliophthora* sp. IMI 387099	Badhan et al. (2007)
	Corn Cob	*Fusarium oxisporum* F3	Panagiotou et al. (2003)
	Wheat straw and bran	*A. niger*38	Jecu (2000)
	Wheat bran and straw, corn cob, reed straw, sugarcane bagasse	*A. terreus M11*	Gao et al. (2008)
Exoglucanase	Soil near rotten wood	*F. chlamydosporum HML 0278*	Chinedu et al. (2005)
	Gut of silkworm	*B. circulans, P. vulgaris, K. pneumonia, E. coli, C. freundii, Serratia liquefaciens, Enterobacter* sp. *P. fluorescens, P. aeruginosa, Aeromonas* sp. *Erwinia* sp.	Anand et al. (2010)
β-Glucosidase	Compost	*A. terreus M11*	Gao et al. (2008)
	Soil near rotten wood	*F. chlamydosporum HML 0278*	Qin et al. (2010)
	Wheat bran	*Aspergillus sydowii BTMFS 55*	Madhu et al. (2009)
	Wheat bran, soy bran, soy peel, corncob, and corn straw	*Th. aurantiacus CBMAI 456* and *Aureobasidium pullulans ER-16*	Leite et al. (2008)

3 CLASSIFICATION OF CELLULASE

According to the carbohydrate-active enzymes database, complete hydrolysis of cellulose to glucose is mediated by a combination of the three main types of cellulases (Zhang and Zhang, 2013): (1) endoglucanases (EG; EC 3.2.1.4), (2) exoglucanases/cellobiohydrolases (CBHs; EC 3.2.1.91), and (3) β-glucosidase (BG)/cellobiase (EC 3.2.1.21).

3.1 Endoglucanase

endo-Glucanase (*endo*-1, 4-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4), often called CMCase, hydrolyzes carboxymethyl cellulose or acid-swollen amorphous cellulose, soluble derivatives of cellulose such as carboxymethyl cellulose (CMC), cello-oligosaccharides due to which there is a rapid decrease in chain length along with a slow increase in reducing groups. Endoglucanase also acts on cellodextrins, the intermediate products of cellulose hydrolysis, and converts them to cellobiase and glucose (Wood, 1989; Sharada et al., 2014).

3.2 Exoglucanase/Cellobiohydrolases

Exoglucanase or cellobiohydrolases (1,4-β-D-glucancellobiodehydrolase, EC 3.2.1.91) degrades cellulose by splitting off the cellobiost units from the nonreducing end of cellulose polysaccharide chains, liberating either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as major products. Cellobiohydrolase does not degrade cotton promptly, but can affect considerable saccharification of microcrystalline substrates such as Avicel, amorphous celluloses, and cello-oligosaccharides. However, they are inactive against cellobiose or substituted soluble celluloses such as CMC (Sharada et al., 2014; Sadhu and Maiti, 2013).

3.3 β-Glucosidase/Cellobiase

β-Glucosidases (β-glucosideglycosyl hydrolase or cellobiase) hydrolyze cellobiose or cello-oligosaccharides to glucose and are also involved in transglycosylation reactions of β-glucosidic linkages of glucose conjugates. They complete the process of cellulose hydrolysis by cleaving cellobiose and removing glucose from the nonreducing ends of oligosaccharides (Sharada et al., 2014; Coughlan and Ljungdahl, 1988).

4 PRODUCTION OF CELLULASE

Successful utilization of cellulosic materials as renewable carbon sources is dependent on the development of economically feasible process technologies for cellulase production. Large numbers of microorganisms are capable of degrading cellulose; fungi and bacteria are the main cellulase-producing microorganisms. Various bacteria, actinomycetes, and filamentous fungi produce extracellular cellulases when grown on cellulosic substrates though many actinomycetes have been reported to have less cellulase activity than molds. Cellulases are inducible enzymes that are synthesized by microorganisms during their growth on cellulosic materials (KOO, 2001).

Fermentation is the technique of biological conversion, which has been widely used for the production of cellulase. Over the years, fermentation techniques have gained immense importance due to their economic and environmental advantages.

4.1 Solid-State Fermentation

Solid-state fermentation utilizes solid substrates, such as bran, bagasse, paddy straw, other agricultural waste, and paper pulp (Subramaniyam and Vimala, 2012). The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as cheaper substrates. Solid-state fermentation is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content. However, it cannot be used in fermentation processes involving organisms that require high water activity, such as bacteria (Babu and Satyanarayana, 1996).

4.2 Submerged Fermentation

Submerged fermentation (SmF) utilizes free-flowing liquid substrates, such as molasses and broth (Subramaniyam and Vimala, 2012). This fermentation technique is best suited for microorganisms, such as bacteria, that require high moisture content. An additional advantage of this technique is that purification of products is easier.

The major steps involved in cellulase production are as follows:

1. Selection of potent strain as cellulose source.
2. Selection of waste as substrate, for example, lignocellulosic agricultural waste.
3. Enrichment of lignocellulosic waste in carbon content by process of pretreatment.
4. Fermentation growth of selected strain utilizing the pretreated lignocellulosic waste.
5. Harvesting the biomass cultivated after fermentation.
6. Downstream processing of extracellular enzymes and recovery of cellulase.

So there are three main stages for the production of cellulase. They are "prefermentative stage," where the pretreatment of substrate and medium preparation is done, followed by "fermentative stage" for cultivation of organism, and finally "postfermentative stage" involving downstream processing and product recovery.

5 CATALYTIC MECHANISMS OF CELLULASE

The complete cellulose hydrolysis to glucose is mediated by a combination of the three main types of cellulases. Between the three components of cellulase, endoglucanase acts on CMC, causing random scission of cellulose chain yielding glucose and cello-oligosaccharides. Exoglucanase acts on microcrystalline cellulose (Avicel), imparting an *exo*-attack on the nonreducing end of cellulose, liberating cellobiose (cellobiohydrolase) as the major product. β-Glucosidases hydrolyze cellobiose to glucose. All these cellulases release glucose as the end product (Karmakar and Ray, 2011) (Fig. 21.1).

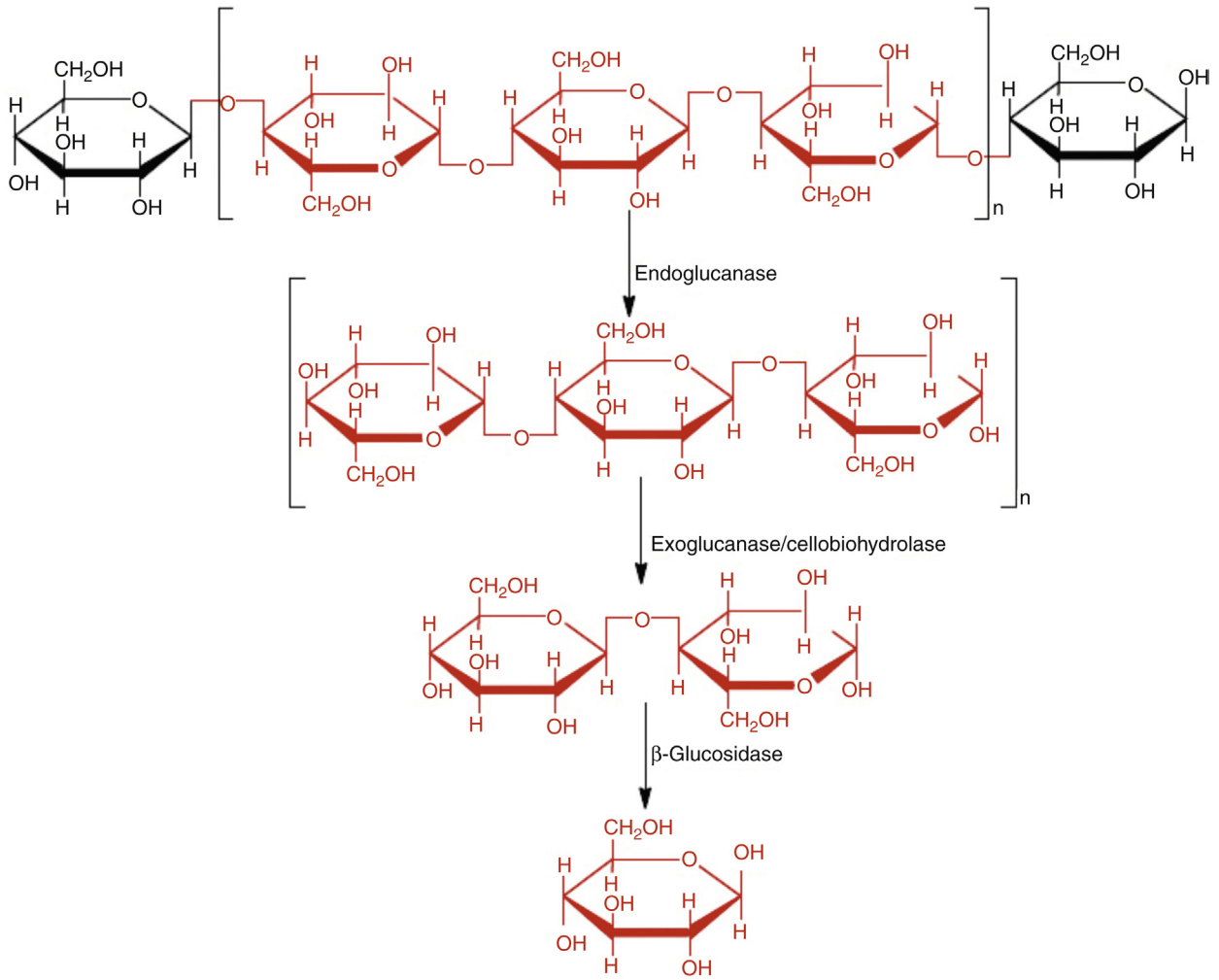

FIGURE 21.1 Principal cellulase sites of action on the cellulose polymer liberating glucose. *From Juturu and Wu (2014) ©2014, with permission from Elsevier.*

FIGURE 21.2 Sources of MSWs.

6 CELLULASE IN MSW TREATMENT

Municipal solid waste is categorized commonly as the "trash" or "garbage," which generally refers to household wastes, including similar wastes from offices, commerce, shops, and retailers, but excludes the industrial, constructional, and hazardous wastes. The sources consist of durable materials, such as tires and furniture and nondurable materials, such as newspapers, plastic accessories, containers, and packaging, (milk cartons and plastic wrappers), and other wastes (garden wastes and food) (as shown in Fig. 21.2).

6.1 Composition and Statistics of MSW

MSW is comprised of 40–50% cellulose, 9–12% hemicellulose, and 10–15% lignin on a dry-weight basis (Rani and Nand, 2000; Gautam et al., 2010a). As the world hurtles toward its urban future, the amount of MSW, one of the most important by-products of an urban lifestyle, is growing even faster than the rate of urbanization. The World Bank report for 2012 estimated that globally about 3 billion residents generate 1.2 kg/capita/day (1.3 billion tons per year) of MSW. By 2025 this will likely increase to 4.3 billion urban residents generating about 1.42 kg/capita/day of MSW (2.2 billion tons per year). With the increasing population, the deposition of MSW has become a public health problem because of the dearth of suitable locations for waste disposal near urban centers and the transformation in the composition of waste (Benito et al., 2003; Chroni et al., 2009).

6.2 Municipal Waste Management Proposals

The overall goal of waste management is to collect, treat, and dispose waste using the most economical means available. Irrational disposition of wastes cause unfavorable impressions on all the components of the environment and human health. Their conversion into useful products may modify the problems they cause (Rathnan et al., 2012).

The eminent methods of waste management are as follows: landfill – waste is deposited in a specially designated area; incineration – a process of combustion designed to recover energy and reduce the volume of waste going to disposal; sewage treatment – a process of treating raw sewage to produce nontoxic liquid effluent, which is discharged to rivers or the sea and a semisolid sludge; recycling – refers to the recovery of materials from products after they have been used by customers; and composting – usually comprises an aerobic, biological process of degradation of biodegradable organic matter (Rushton, 2003).

Landfilling needs a large amount of space while incineration causes a huge environmental problem including large costs of fuel and energy. So, recycling and composting are considered the most promising sectors for waste management process as they use beneficial microorganisms for a sustainable environment. The waste management hierarchy is shown in Fig. 21.3.

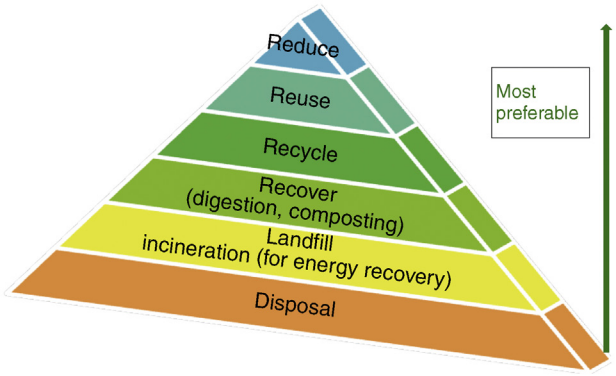

FIGURE 21.3 Waste management hierarchy.

6.3 Cellulase as a Potential Trigger for MSW Management

It is proved by many biological studies that only a few strains are capable of secreting a complex of cellulase enzymes, which have practical application in the enzymatic hydrolysis of cellulose as well as bioconversion of organic MSW. Microorganisms can produce a variety of enzymes like cellulase under appropriate conditions. Catalyzed by their diverse enzyme-mediated reactions, microorganisms perform their metabolic processes rapidly and with remarkable specificity that lead to the intensive exploration of natural microbial biodiversity to discover enzymes (Gautam et al., 2012).

Extracellular cellulases are more activated in depolymerizing the cellulosic substrates. Many cellulolytic organisms include fungal species: *Trichoderma*, *Humicola, Penicillium,* and *Aspergillus* (Gautam et al., 2009a) are capable of degrading cellulose producing large quantities of extracellular cellulases. More than 14,000 fungi are listed that are active against cellulose and other insoluble fibers. From these, to produce cellulolytic enzymes in organic waste degradation process, most common experimental studies were carried out with *Trichoderma* sp., *Penicillium* sp., and *Aspergillus* spp. (Brown et al., 1987; Gautam et al., 2010b; Mandels, 1975). *Trichoderma harzianum* (Gowthaman et al., 2001; Kumar et al., 2009; Macris et al., 1985; Wilson, 2011) and *Trichoderma koningii* (Wood and Bhat, 1988; Wood and McCrae, 1982) were studied among *Trichoderma* spp. Gram-positive and Gram-negative bacteria, including *Bacillus subtilis, Bacillus* spp., *Clostridium thermocellum, Cellulomonas* spp., *Pseudomonas* spp., *Proteus, Ruminococcus* spp., *Streptomyces* spp., *Serratia,* and *Staphylococcus* spp. produce many cellulases, which are mainly bound to their cell wall and capable of hydrolyzing native lignocellulose preparations to any significant extent (Wood and Bhat, 1988; Gautam et al., 2010c).

6.4 Composting the Green Technology

Composting is an environmentally approvable technology because of its recycling effectiveness of organic wastes discharged from industrial and municipal plants or livestock farming. As the costs of chemical fertilizers have increased, the world's food shortage problems have also increased. That is why high-quality compost production by the interaction of many organisms at a low cost has been introduced as an important alternative fertilizer production method. However, it may be noted that many microbes cited for composting are difficult to isolate and are characterized by conventional cultivation methods (Atkinson et al., 1996; Gautam et al., 2009b).

Composts that are prepared from municipal refuse are available but these mainly have low nitrogen and phosphorous content thus poor sources of nutrients for plant growth (Kumar, 2013). So they need to be suitably amended and converted into nutrient-enriched organic manure using microbial inoculants. The amendments not only influence soil fertility, but may also enhance the composition and activity of soil microorganisms.

From municipal waste compost *Chaetomium thermophilum* fungus is isolated, which produces extracellular enzymes and are essential for the formation of polyaromatic humic substances with phenoloxidase and peroxidase. So far, *Bacillus licheniformis*, *Trichoderma viride,* and complex microorganisms, such as *Trichoderma* sp., *Candida rugopelliculosa*, *Bacillus casei,* and *Lactobacillus buchneri,* have been reported, which accelerate humification of organic wastes in the composting process and are significant for compost maturing (Gautam et al., 2010a, 2010b).

Most of the developed countries collect green waste separately from other wastes. After which, it is mechanically shredded, composted either alone or with other organic wastes, (Fig. 21.4) and used as garden mulch, organic soil amendment, or garden compost. In some countries like Australia, for field-landscaping purposes, a substitute of natural top soil, composted

FIGURE 21.4 General flow process of composting.

material is mainly used as "manufactured soil." Sometimes, inorganic additives, such as sand, subsoil, and fly ash are blended with the composted material, which is considerably cheaper than excavated natural topsoil (Albiach et al., 2001).

7 CELLULASE IN WASTEWATER AND SLUDGE TREATMENT

Since the late twentieth century, statistics showed that Western countries have been experiencing an increase in excess sludge production annually. The excessive sludge for treatment increased from 9.4 million tons in 2005 to 10 million tons in 2007 in European Union member countries whereas excess sludge production singly rose from 7.6 million tons in 2005 to 8.2 million tons in 2010 (Ginestet, 2007; Laturnus et al., 2007) in the United States.

Wastewater treatment works effluents are used to produce cleaner wastewater with the generation of a huge volume of sludge. Thus sludge treatment and disposal have become a challenge in the field of environmental engineering.

The techniques compromising wastewater treatment have gradually developed from the simple sewage farms to more sophisticated processes, such as the activated sludge process. This technique has altered the production of an increasing volume of sludge and also with the improvements of enactments of the increased removal of carbon and nutrients from water.

Sludge production is reduced in wastewater treatment by using physical, chemical, and biological methods resulting in the uncoupling and maintenance of metabolism by enhancing lysis-cryptic growth and the action on sludge bacteria, which reduce the amount of sludge for disposal (Mahmood and Elliott, 2006; Wei et al., 2003). Practically, minimization of excess sludge production during wastewater treatment rather than treating the sludge after its generation solves the problem. The introduction of physical and chemical methods as a new technology can bring additional high cost, secondary pollution, and energy consumption (Jin-Song, 2011). The introduction of new biological methods can be problematic with time-consuming techniques and caustic reaction conditions, which ultimately lead to energy consumption, imposing additional cost, and increasing environmental pollution (Wei et al., 2003; Chen et al., 2002; Egemen et al., 2001; Saby et al., 2002). For this purpose, alternatives for sludge treatments, such as lysis cryptic growth, uncoupling and maintenance metabolism, and bacterial predation (Guo et al., 2007; He et al., 2006; Li et al., 2008; Liang et al., 2006; Wei and Liu, 2006) are emphasized nowadays.

The most commonly used method for wastewater treatment worldwide is activated sludge process because of its improved technology, efficient performance, and low cost.

7.1 Action of Enzyme in Sludge Hydrolysis

Bacteria tend to accumulate and form sludge flocs, consisting of microbial, prokaryotic (bacteria, archaea), and eukaryotic (algae, fungi) microorganisms kept together by extracellular polymeric substances (EPS) in the activated sludge process. About 60–70% of the organic fraction is included in the sludge (Qiang, 2003).

Microbial cells undergo lysis or death and release the cell contents (substrates and nutrients) into the medium by providing a substrate that is subsequently used in microbial metabolism. In lysis-cryptic growth, which was first introduced by Ryan (Guo et al., 2007) as a product of respiration, a certain amount of carbon and metabolism are released, which reduce the final production of biomass. This involves two stages: lysis and biodegradation. The first step is cell fractionation; cell lysis considers the cell destruction of microbial cells, which is catalyzed by a hydrolytic enzyme (mainly protease). The biomass grows on an organic lysate, which is much more different from that on the original substrate, and is therefore termed as cryptic (Guo et al., 2007). The insoluble, large organic molecules in activated sludge flocs can be broken down into simpler carbohydrate molecules by the action of hydrolytic enzymes in the hydrolysis process, which causes the breakdown of proteins into peptides and amino acids, which eventually can turn into low-molecular weight organic acids, ammonia, and carbon dioxide.

7.2 Influencing Factors and Location of Hydrolytic Enzymes

Due to the selectivity of cell membranes, some eukaryotic ones in the activated sludge floc can absorb only low-molecular weight (<1000) compounds (Cadoret et al., 2002). Therefore, hydrolysis is needed for most of the substrates subjected to a metabolism process of living matter in the activated sludge floc, by inducing hydrolytic enzymes.

Frølund et al. (1996) found that the *exo*-enzymes were immobilized in the sludge resulting from EPS matrix adsorption, where a very small fraction of the *exo*-enzymes is released into the water. Bihan and Lessard (2000) compared the changes in the enzymatic activity using an activated sludge mixed liquor indicating that a major fraction of the total enzyme activity is associated within the flocs.

Therefore, it can be concluded that

- Due to bound *exo*-enzymes to EPS, the retention time of hydrolase is not lower than the retention time of sludge;
- A good environment for enzyme stability can be created by EPS; and
- Hydrolysis sites for complex macromolecules can be provided by EPS.

7.3 Cellulase as a Key of Sludge Hydrolysis

Due to high-molecular weight linear polymers mainly polymerized by *p*-β-glucose monomers through β-1- and β-4-glycosidic bonds cellulose, which is polysaccharide in nature, do not dissolve in water and in common organic solvents.

In any urban sewage treatment plant, especially in the paper and textile industries, the activated sludge contains large amounts of cellulose and other organic substances. An important development is the use of cellulase to hydrolyze cellulose in sludge-floc treatment, which efficiently enhances the hydrolysis (Ayol, 2005; Ayol and Dentel, 2005; Parmar et al., 2001) step. Studies have shown that sludge-floc hydrolysis heavily depends on *Aspergillus*, *Penicillium*, *Rhizopus*, and *Myrothecium*, and all of which have the ability to produce cellulase (Hageskal et al., 2009). The mixtures of protease, lipase, and endoglycanases can enhance the solubilization of municipal sludge. Mixed fungi can accelerate the utilization of substrate through the combination of enzymes and the symbiotic association of a fungi mixture can increase colonization of the substrate (Molla et al., 2001; More et al., 2010). The study of the mixed fungal culture of *Aspergillus niger* and *Penicillium corylophilum* degraded sludge more efficiently (92% of COD) compared with the control after 6 days of sludge-fungal treatment (4% w/w of TSS) (Alam et al., 2003a, 2003b; Fakhru'l-Razi et al., 2002; Mannan et al., 2007). Thus, for the minimization of sludge, technologies are usually adapted by adding bacteria with hydrolytic enzyme secretory function, commercial enzymes, or antibiotics, among others but it is relatively expensive. So, a low-cost and efficient solution for sludge minimization in water treatment facilities is needed.

Culture microorganisms with hydrolytic enzyme secretory function can be chosen like filamentous fungi, present in sewage sludge either as spores or vegetative cell having an exceptionally high capacity to express and secrete proteins, enzymes, organic acids, and other metabolites, and can produce secondary metabolites in large quantities. Also, the degradation of the refractory organic substrate in sludge can be enhanced by some enzymes excreted by these fungi (Wawrzynczyk et al., 2003). A similar conclusion was given by Alam et al. (Fakhru'l-Razi et al., 2002), using bioconversion in liquid state, which reduced the amount of organic materials. Yan et al. (More et al., 2010) showed that the sludge treated with microfungi resulted with the amount of dry matter being reduced by approximately 10–50% (typically by approximately 20–30%) compared to the untreated sludge, which can be greater by altering the control parameters. Gutierrez-Correa and Tengerdy (Parmar et al., 2001; Alam et al., 2003a) assumed that a mixed culture leads to higher enzyme production with respect to little increase in their cell biomass. The white rot fungus *Phanerochaete chrysosporium* degrades a variety of persistent environmental pollutants, which was reported by Cameron et al. (Lacina et al., 2003). Mannan et al. (Wawrzynczyk et al., 2003; Mannan et al., 2005) found that *P. corylophilum* is suitable for the biodegradation of domestic activated sludge. About 87% removal of the COD in treated sludge, with a 98% removal of suspended solids after 6 days with filamentous fungi *Mucor hiemal* was also reported (Fakhru'l-Razi and Molla, 2007; Molla and Fakhru'l-Razi, 2012). Theoretically, filamentous fungi have a potency to degrade domestic activated sludge because of their extracellular enzymes; they also enhance the biodegradation of sludge flock as well as cryptic growth.

8 CELLULASE IN AGRICULTURAL WASTE MANAGEMENT

Cellulose and hemicellulose comprise the major part of all green plants and this is the main reason for using such terms as "cellulosic wastes" or simply "cellulosics" for those materials that are produced especially as agricultural crop residues (residual stalks, straw, leaves, roots, husks, shells, etc.), crop processing wastes, fruit and vegetable wastes, animal waste (manures), and so on (Ryu and Mandels, 1980; Wood, 1992). The bioconversion of agricultural waste with cellulase into valuable by-products is shown in Fig. 21.5 and discussed in the following section.

8.1 Bioconversion of Banana Agro Waste

Saccharification, that is, bioconversion of banana-agro waste (pseudostem and -leaves) using cellulase enzyme releases sugar at different incubation periods. Bacterial strains used for the production of enzyme for this purpose belong to the

FIGURE 21.5 Conversion of agricultural wastes into various economic resources .

genus *Bacillus, Klebsiella,* and *Pseudomonas*. However, the strain with most potential is *Bacillus pumilus* due to its ability to form the highest zone of about 17 mm. Actually, cellulolytic enzyme complex is incubated with agro waste to release sugars (Kanmani et al., 2011).

8.2 Bioconversion of Rice Straw

Cellulase showed good saccharification ability on acid-pretreated rice straw. After acid or alkali pretreatments dried solids obtained from rice straw is incubated with acetate buffer (0.05 M, pH 5.6) and crude concentrated *Sporotrichum* sp. LAR5 cellulase (1 mL, 7.88 IU) to extract sugar (Bajaj et al., 2014).

8.3 Bioconversion of Waste Leaves and Bamboo

Bioconversion of agricultural waste (leaves of jamun, mango, neem, eucalyptus, poplar, asoka, wild grass, and bamboo) to ethanol have been carried out by simultaneous saccharification and fermentation (SSF) using recombinant cellulase from *C. thermocellum.* The use of recombinant cellulase for bioethanol production reduces the enzyme cost. The agricultural wastes having considerable disposal problem can be used for ethanol production (Mutreja et al., 2011).

8.4 Bioconversion of Sorghum Straw

An alkali-pretreated sorghum straw, a lignocellulosic substrate, has been hydrolyzed using native cellulose produced by *Trichoderma reesei* (NCIM 992) to release sugar. Enzymatic saccharification of the acid-pretreated sorghum straw into glucose has been optimized using the enzyme supernatant of *T. reesei* (NCIM 992). The parameters, such as optimum cellulase loading, temperature, saccharification time, and substrate-to-liquid ratio, play a crucial role in the enzymatic hydrolysis of lignocelluloses to get satisfactory yield of monomeric sugars (Rodhe et al., 2011).

8.5 Bioconversion of Corn Cob

Corn cob is a major component of agricultural waste in many parts of the world. It is composed mainly of cellulose, which can be converted to fuel energy in the form of bioethanol as an effective and efficient waste management method. Alkali-pretreated corn cobs have been hydrolyzed with the partially purified cellulases of *A. niger* and *Penicillium decumbens* and the product of hydrolysis has been fermented using the yeast *Saccharomyces cerevisiae* to ethanol. Alkali-pretreated corn cob, hydrolyzed with cellulases of *A. niger*, is a suitable feedstock for bioethanol production (Saliu and Sani, 2012).

8.6 Bioconversion of Cotton Wastes

At present, there are no solutions for the permanent disposal of cotton waste; it accumulates in lagoons outside the production facility. An eventual means of permanent disposal will be needed when the lagoons become filled. Waste cotton products can be degraded by cellulose. Enzymatic degradation of cotton waste has been investigated for safe disposal (Luther Mitchell Swift, 2008).

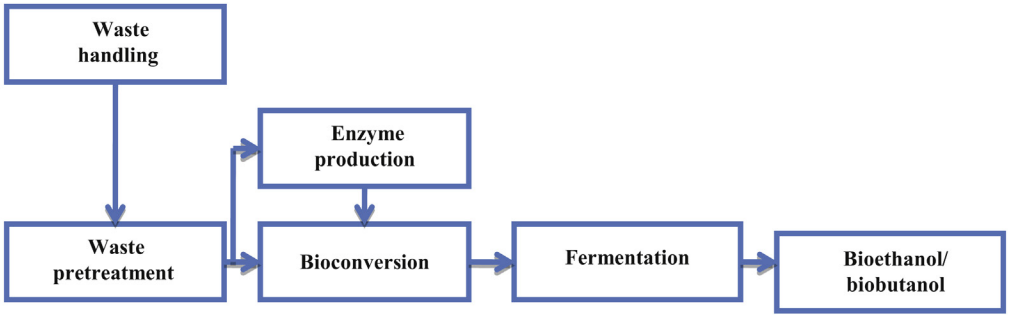

FIGURE 21.6 Flow diagram for bioethanol/biobutanol production from vegetable wastes.

8.7 Bioconversion of Sawdust

Sawdust, a voluminous waste generated during timber and wood processing, can be treated by digestive cellulase. Sawdust degrades slowly in nature due to its lignin content, and its low bulk density makes the traditional disposal of this material an economic and environmental challenge. If sawdust is not pressed into wood products for commercial uses, it can be used as a combustible fuel (Luther Mitchell Swift, 2008).

8.8 Bioconversion of Vegetable and Fruit Wastes

Vegetable and fruit wastes, such as potato (peel, mesh), tomato (solid waste), onion (onion tops peelings and whole bulbs), pea (peel, shell, and solid waste), sugar beet (pulp, silage, and leaves), carrot residues, apple pomace, and orange peel, are biodegradable materials generated in large quantities, much of which is dumped on land to rot in the open air. It not only emits a foul odor, but also creates a big nuisance by attracting birds, rats, and pigs – vectors of various diseases. Vegetable and fruit wastes pose an environmental threat and cause pollution. Generation of renewable energy by bioconversion of vegetable and fruit wastes gains much importance as it has proved to be a proficient means of utilizing the perishable vegetable residues.

Use of vegetable and fruit wastes for biogas production solves the problem of residual disposal and indoor pollution and also reduces dependency on fuel wood. These wastes, being rich in polysaccharides (cellulose, hemicellulose, and lignin), can be subjected to solid-state fermentation for the production of ethanol and butanol, which has several uses (Jørgensen et al., 2007; Laufenberg et al., 2003) such as a solvent in many industries and also as a liquid fuel supplement. Vegetable and fruit wastes can be a potential substrate for bioethanol and biobutanol production due to their high cellulose and starch content, and noncompetitiveness with our food chain (Tang et al., 2008). Biofuel production from vegetable and fruit wastes consists of biomass pretreatment, saccharification, and fermentation as shown in Fig. 21.6.

C. thermocellum is a potential microorganism for ethanol production (Singh et al., 2012). *C. thermocellum* ATCC 27405 (and its improved cellulase-producing mutant, AS-39) is an anaerobic thermophile, which produces *endo*-β-glucanase and *exo*-β-glucanase (components of cellulase enzyme), when grown on cellobiose or cellulose as major carbon source (Garcia-Martinez et al., 1980).

9 CELLULASE IN INDUSTRIAL WASTE MANAGEMENT

Industrial wastes are generated from different types of chemical, leather, jute processing, sugar, fertilizer, food processing, and other industries among which lignocellulosic biomass represents the major share of wastes and which represents the most important energy resource. These waste products can potentially be bioconverted into value-added products through the action of enzymes. These are discussed here in detail.

9.1 Enzymatic Biodegradation of Cellulose Contaminated with Radioactive Material

Cellulases used in denim production and in detergent formulations are able to digest cellulose-containing sorbents and other cellulose-based wastes contaminated either with crude oil or with uranium. A commercially available cellulase was able to reduce the volume of cellulose substrates contaminated with lanthanide surrogates and PuO_2. Residual radioactivity remained primarily with the solid residue following digestion (under graded cellulose plus filter). This indicates that enzyme digestion of low level (LLW) and transuranic (TRU) wastes to reduce their volume is a promising technology (Heintz et al., 1999).

9.2 Enzymatic Saccharification of Pretreated Hemp Biomass

A large amount of cellulosic waste is produced from the fiber industries. This waste can be further utilized for bioenergy production, thus adding value to the material. Hemp (*Cannabis sativa*) hurd is easily available due to its extensive application in the fiber industry. Hemp is an annual herbaceous crop that exhibits both bast fiber and a woody core (Sipos et al., 2010), the former of which finds a host of applications in industry. The remaining woody core is typically considered a waste product, making it an ideal candidate source of cheap, readily available cellulose for the production of fermentable sugars to produce ethanol (Rehman et al., 2013). Cellulase from *T. reesei* was immobilized on an activated magnetic support by covalent binding and used to hydrolyze microcrystalline cellulose and hemp hurds (Abraham et al., 2014).

9.3 Enzymatic Removal of Toners and Inks from Office Waste Papers

Office waste paper is one of the fastest growing segments of the recycled fiber industry. However, the toners used in xerographic and laser printers present a special challenge. They consist of thermoplastic polymers of styrene and butadiene, acrylic or polyester along with carbon black or other pigments. Office waste papers (100% toner printed) were treated with a commercial-cellulase preparation in a pilot-scale cellulase treatment in the presence of a surfactant, and mechanical agitation can increase the efficiency of enzymatic deinking. This effect is probably due to increased flotation efficiency resulting from greater detachment of toner particles from fiber surfaces, but it may also result from increased washing efficiency as a result of improved drainage properties. Cellulases are employed in the removal of ink coating and toners from paper. Biocharacterization of pulp fibers is another application where microbial cellulases are employed. The use of enzymes for deinking has been reviewed and investigated (Welt and Dinus, 1995).

9.4 Enzymatic Degradation of Textile Wastes

Textile waste samples include cotton sludge, finished denim strips, and various bits of cotton residue from different steps along the cotton machining process. Cellulase can be used to degrade the voluminous waste streams generated by the cotton textiles industry; however, the temperature and pH limits the scope. Under regulated conditions, cellulase is not significantly affected by the presence of hydrocarbons, low concentrations of heavy metals, lanthanides, and actinides, thereby offering an alternative to landfilling or long-term storage of contaminated cellulosic wastes.

10 BIOREMEDIATION OF LIGNOCELLULOSIC WASTES USING CELLULASE

Lignocellulosic biomass is composed of structural carbohydrates cellulose and hemicellulose and heterogeneous phenolic polymer lignin as its primary components (Martinez et al., 2009). However, their contents vary, depending on the species, variety, climate, soil fertility, and fertilization practice (Pauly and Keegstra, 2008). Lignocellulose is the main source of renewable organic matter. The chemical properties of its components make it a material of great biotechnological value. Therefore, the concept of lignocellulose biorefinery has received growing attention due to the potential of conversion of this material into many high added value products (Demirbas, 2007; Ragauskas et al., 2006). The wastes generated from forests, agricultural fields, and agro industries contain a large amount of unutilized or underutilized cellulose, causing environmental pollution. Nowadays, these so-called wastes are judiciously utilized to produce valuable products.

10.1 Sugars and Bioethanol

Consumption of energy originating from fossil resources has aggravated the problem of atmospheric pollution by the release of greenhouse gases. Besides, due to the high cost of petroleum and the eminent depletion of these resources in a few decades, lignocellulosic biomass has aroused great interest in the last years. Ethanol, the main form of bioenergy, is the best alternative to the use of fossil fuels (Wang et al., 2011). New technologies have been developed for the efficient harvesting of biofuels (e.g., bioethanol and biodiesel) from lignocellulosic biomass (Hamelinck et al., 2005; Prasad et al., 2007). *Prosopis juliflora* (Mesquite) is a raw material for the long-term sustainable production of cellulosics ethanol. Acid pretreatment, delignification, and enzymatic hydrolysis has been utilized to produce sugar, to be fermented to ethanol (Gupta et al., 2009).

Bioethanol, one of the liquid biofuels, receives the most interest due to its simplicity. The so-called first-generation bioethanol made from starch and sugar is now considered less desirable due to its alleged influence on food prices. Cellulosic bioethanol, also known as second-generation bioethanol, has become a more attractive alternative. It can be produced from

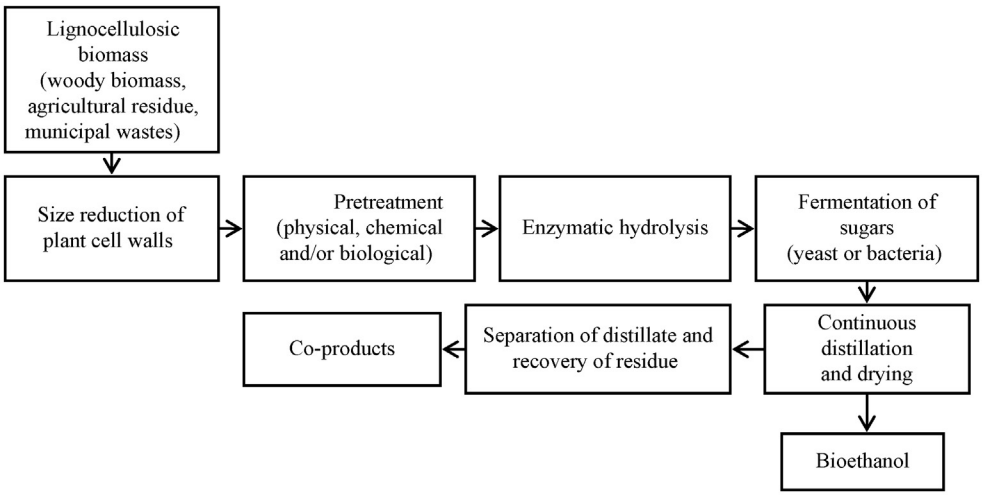

FIGURE 21.7 Simplified flow sheet for ethanol production from lignocellulosic biomass. *From Kristensen (2008) and Limayem and Ricke (2012) ©2012, with permission from Elsevier.*

all kinds of plant materials, ranging from corn stover and wheat straw to forest residues. Furthermore, cellulosic ethanol has the ability to produce large quantities of fuel with more significant reductions in greenhouse gas emissions (Himmel et al., 2007). The main process steps involved in producing cellulosic bioethanol are illustrated in Fig. 21.7.

After a preliminary size reduction of plant cell walls to 10–30 mm through mechanical methods such as chopping, pretreatment is needed to deconstruct lignin carbohydrate complexes for efficient enzymatic hydrolysis of cellulose (Kumar et al., 2009). The heterogeneous characteristic of biomass particles, surface area, and the presence of hemicellulose–lignin complexes covering cellulose are responsible for the resistance of lignocellulosic biomass toward hydrolysis (Chang and Holtzapple, 2000). The objective of pretreatment of lignocellulosic material is to minimize or remove the constraints of hydrolysis, improving enzymatic hydrolysis rate, thus increasing the yield of fermentative sugars (Fig. 21.8) (Martins et al., 2011). After hydrolysis, sugars are fermented into ethanol. The two steps of enzymatic hydrolysis and fermentation

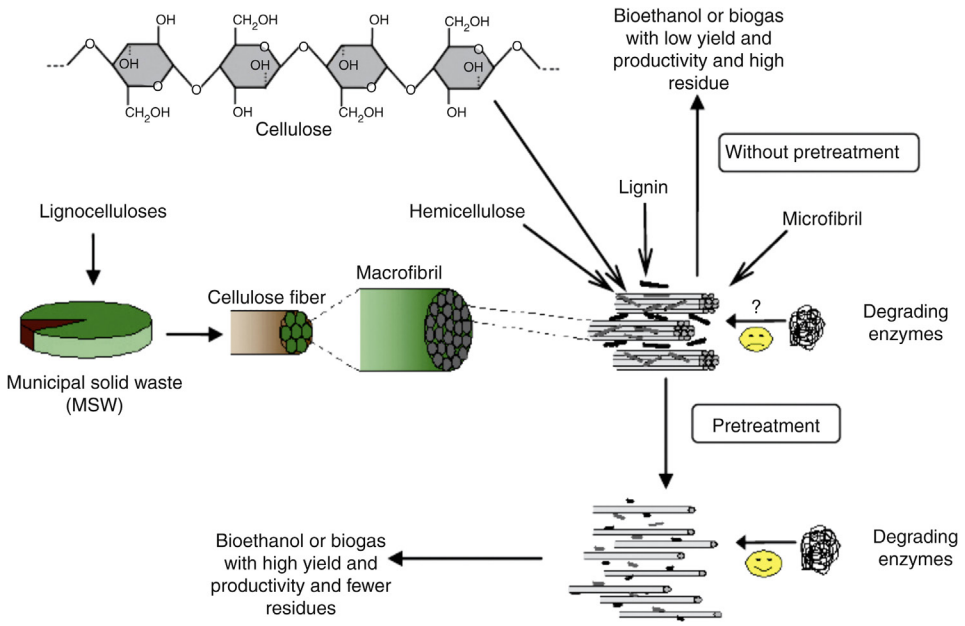

FIGURE 21.8 Effect of pretreatment on accessibility of degrading enzymes. *Reproduced with permission from Taherzadeh and Karimi (2008).*

may be combined into a single processing step known as simultaneous saccharification and fermentation (SSF). Finally, the generated ethanol must be isolated through distillation.

Biological pretreatment by solid fermentation employs microorganisms that degrade lignocellulosic biomass. Bacteria and fungi have been utilized, but white-rot fungi are the predominant species in lignocellulose degradation for the purpose of biofuel production, due to their abundant ligninolytic enzymes (Dashtban et al., 2010). Compared with physical and chemical pretreatments, biological pretreatment can be performed at mild conditions without special requirements for equipment (Keller et al., 2003), and it is an energy-saving and environmentally friendly technique. Tables 21.3 and 21.4 show the composition of some lignocellulosic biomass and ethanol yield and annual total tonnages of biomass for biofuel in the United States.

10.2 Improved Animal Feeds

Applications of cellulases and hemicellulases in the feed industry have received considerable attention because of their potential to improve feed value and performance of animals (Dhiman et al., 2002). Pretreatment of agricultural silage and grain feed by cellulases or xylanases can improve its nutritional value (Godfrey and West, 1996).

Some of the proposed methods for conversion of agricultural wastes into animal feed are presented in Table 21.5.

TABLE 21.3 Composition of Some Lignocellulosic Biomass (Based on Dry Biomass) and Potential Ethanol Yield

Biomass	Residue/Crop Ratio	Dry Matter (%)	Lignin (%)	Carbohydrates (%)	Ethanol Yield L/kg of Dry Biomass
Barley	1.2	88.7	2.9	67.10	0.41
Barley straw		81.0	9.00	70.00	0.31
Corn	1.0	86.2	0.60	73.70	0.46
Corn stover		78.5	18.69	58.29	0.29
Oat	1.3	89.1	4.00	65.60	0.41
Oat straw		90.1	13.75	59.10	0.26
Rice	1.4	88.6		87.50	0.48
Rice straw		88.0	7.13	49.33	0.28
Sorghum	1.3	89.0	1.40	71.60	0.44
Sorghum straw		88.0	15.0	61.00	0.27
Wheat	1.3	89.1		35.85	0.40
Wheat straw		90.1	16.0	54.00	0.29

From Seungdo Kim et al. (Kim and Dale, 2004) ©2004, with permission from Elsevier.

TABLE 21.4 Annual Total Tonnages of Biomass for Biofuel in the United States (US Department of Energy Biomass Program, 2009)

Biomass	Million Dry Tons/Year
Agricultural residues	428
Forest resources	370
Energy crops	377
Grains and corn	87
Municipal and industrial wastes	58
Others (i.e., oilseeds)	48
Total	1368

From Alya Limayem et al. (Limayem and Ricke, 2012) ©2012, with permission from Elsevier.

TABLE 21.5 Methods for Conversion of Cellulosic Agricultural Wastes into Animal Feed

Treatment	Microorganisms	Substrate	References
Ensiling	Mixed anaerobes	Waste lage	Anthony (1971)
Dilute alkali	None	Straw	Rexen (1975)
Aerobic mesophiles °C	*Cellulomonas*	Bagasse	Dunlap (1975)
Mold growth 25°C	*T. viride*	Waste paper	Mandels et al. (1974)
Aerobic thermophiles 55°C	*Thermoactinomyces*	Fermented livestock wastes	Bellamy (1974)

10.3 Compost Product

Direct application of raw organic wastes is inappropriate for land use due to their unknown composition for having pathogens, toxic compounds, weed seeds, heavy metals, and foul odors. Composting is considered the most appropriate option for addressing the constraints associated with organic solid waste materials for agricultural use (Wolkowski, 2003). Largely accessible organic wastes (cow dung, kitchen waste, and yard waste) can be turned into valuable compost product using enzymes like cellulase for raising crops organically on one hand, and get them disposed of safely on the other end (Ahmad et al., 2007).

10.4 Organic Acids

Several organic acids, including lactic, citric, acetic, and succinic acids, may be produced by cellulose conversion. Lactic acid may be produced from lignocellulose materials by sequential steps namely, chemical processing (in order to make the cellulose more accessible to the enzymes), enzymatic saccharification (for obtaining solutions containing glucose as main sugar), and finally, hydrolysate fermentation by microorganisms, especially *Lactobacillus* species (Mussatto et al., 2008). The conventional process for cellulosic biomass conversion to acetic acid includes also an initial stage of acid or enzymatic hydrolysis of the substrate, followed by yeast fermentation and oxidation to acetic acid by *Acetobactor* sp. (Ravinder et al., 2001). Almost the entire production of this acid has been obtained using crops and crop residues as substrates and *A. niger* as production strain. When comparing sugarcane bagasse, coffee husk, and cassava bagasse as solid substrate for citric acid production by *A. niger*, cassava bagasse showed the highest production results (Vandenberghe et al., 2000).

10.5 Flavors

Natural flavors are chemical substances with aroma properties that are produced from feedstock of plant or animal origin by means of physical, enzymatic, or microbiological processing (Rodríguez-Couto, 2008). Flavor synthesis by biotechnological processes plays an increasing role in the food, feed, cosmetic, chemical, and pharmaceutical industries. SSF has been used for the production of flavor compounds by cultivating yeasts and fungi. The production of flavor compounds is related to the low oxygen availability in the medium, which results in the production of odor compounds including alcohols, aldehydes, and ketones (Feron et al., 1996; Medeiros et al., 2001).

10.6 Bioactive Compounds

Several bioactive compounds may be produced by SSF from different lignocellulose wastes. Some examples include (1) the production of gibberellic acid by *Giberella fujikuroi* and *Fusarium moniliforme* from corn cobs, (2) the production of tetracycline from cellulosic substrates, (3) production of oxytetracycline by *Streptomyces rimosus* from corn cobs, (4) the production of destrucxins A and B (cyclodepsipeptides) by *Metarhizium anisopliae* from rice husk, and (5) production of ellagic acid by *A. niger* from pomegranate peel and creosote bush leaves (Aguilar et al., 2008; Pandey et al., 2000).

11 CONCLUSIONS

The bioconversion of cellulosic materials is now the focus of intensive research as a contribution to the development of large-scale conversion processes beneficial to humanity. So, one of the most important biotechnological applications is the green conversion of all lignocellulosic wastes into products of commercial interest such as compost, bioethanol, glucose,

animal feeds, and single-cell products. The key element in this bioconversion process of lignocellulosic wastes to useful products is the hydrolytic enzymes, mainly cellulases. Global sales of industrial enzymes have already reached a value of approximately $1.6 billion in the market of which cellulase and allied enzymes occupy a significant position. For their immense industrial applicability and relatively low cost of production, microbial cellulases are preferred. In fact the demand for these cellulase enzymes is increasing daily worldwide for their use in sludge-floc degradation, waste management in food processing, pharmaceuticals, pulp and paper, and other industries.

Abundant research works are resulting into improved scientific knowledge related to cellulase enzyme for the production of valuable by-products, such as bio-alcohols, compost, methane gas, organic acid, animal feed, and flavor from bioconversion of cellulosic biomass that will certainly fetch a great prospect in the field of industrial green chemistry. With the success of meeting the growing demands of cellulase, it will be possible to meet the shortages of food and animal feeds, solve modern waste disposal problems, and diminish our dependence on fossil fuels by providing a convenient and renewable source of energy in the form of bioethanol. It is opening new ventures for the utilization of various agro-wastes and organic pollutants as a source of renewable energy instead of dumping them and cause environmental degradation.

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