Chapter 6
Cellulase: A Catalytic Powerhouse for Lignocellulosic Waste Valorisation

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Abstract Cellulose is the world's most ubiquitous organic compound and has an abundant potential to be transformed into a renewable source of energy and other industrial products. To break down this complex polymer, cellulase has been conventionally used as a biocatalyst. Other than numerous industrial uses, including detergent, paper and pulp, textile, beverages, feed, baking, and biofuel, the application of cellulase for waste valorisation is also a central thrust area. The cellulolytic action of this enzyme has been explored for utilisation of widely available lignocellulosic waste. This reaction has been critical for the cellulose conversion to glucose and then to biofuel. Apart from biofuel, cellulase has also been employed to convert green waste into value-added products such as prebiotic oligosaccharides, organic acids, and biopolymers that can be integrated into the circular economy. Numerous factors affect the catalytic actions of cellulase, like pretreatment of lignocellulosic biomass, structural and compositional variation, working temperature, and pH. This chapter encompasses a detailed insight into the biocatalytic mechanism, applications, and limitations of cellulase as a potent enzyme for waste valorisation.

Keywords Cellulase · Waste valorisation · Lignocellulosic biomass · Circular economy · Biorefinery

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Abbreviations

3Rs	Reduce reuse and recycle
ATCC	American type culture collection
BSG	Brewer's spent grain
CBG	Compressed biogas
CMCase	Carboxymethyl cellulase
CNCs	Cellulose nanocrystals
CNF	Cellulose nanofibrils
CO_2	Carbon dioxide
Co^{60}	Cobalt-60
FPA	Filter paper assay
FPU	Filter paper units
GHG	Greenhouse gas
IFPU	International unit of filter paper activity
IL	Ionic liquid
IU	International enzyme unit
LCA	Life cycle assessment
MNPs	Magnetic nano-particles
MPa	Megapascal
PHA	Polyhydroxyalkanoates
PLA	Poly-lactic acid
RSM	Response surface methodology
SDS	Sodium dodecyl sulphate
SHF	Separate hydrolysis and fermentation
SmF	Submerged fermentation
SMS	Spent mushroom substance
SmSF	Simultaneous Saccharification and Fermentation
SSCF	Saccharification and co-fermentation
SSF	Solid-state fermentation
TCA	Tricarboxylic acid
TEA	Techno-economic analysis
U/g	Enzyme unit per gram
U/mL	Enzyme unit per millilitre
USD	United States dollar

6.1 Introduction

The focus of waste treatment has now shifted to its valorisation, as it promises better management of available resources. The valorisation method, if assisted by biological techniques, becomes a better environment-friendly solution. The utilisation of biological methods for waste valorisation has spawned the concept of a bio-based

circular economy. Cellulase is one such biocatalytic agent which is categorised as endoglucanase (E.C.3.2.1.4), exoglucanase, or cellobiohydrolase (E.C.3.2.1.91), and β-glucosidase (E.C.3.2.1.21).

The source of cellulase can vary from natural to commercial production (Bhardwaj et al. 2021a). The commonly reported bacteria, streptomyces, and fungi for cellulase production are Clostridia sp., *Bacillus licheniformis, Streptomyces argenteolus*, *S. griseorubens, S. lividans, Aspergillus oryzae, Candida cylindracea, Trichoderma reesei, etc.* (Hamdi et al. 2020; López-Mondéjar et al. 2019; Saldarriaga-Hernández et al. 2020; Kumar et al. 2018, 2020a). The genetically developed producers include *E. coli* and *Pichia pastoris*.

The mechanistic insight of cellulase explains the hydrolysis process. Endoglucanase is responsible for the breakdown of β-1,4 glycosidic bond, which exposes new chain ends. The ends so produced are broken by exoglucanase/cellobiohydrolase to produce oligosaccharides, whereas β-glucosidase causes lysis of these oligosaccharides into their monomer units. For a detailed review of the mechanism of action and structure of cellulase, an article by Rabinovich et al. can be referred (Rabinovich et al. 2002). Most of the cellulases have been found to be a multidomain proteins containing a catalytic domain, a cellulose-binding domain and an interdomain linker.

Earlier reports suggest the use of cellulase in waste management such as deinking of paper, degradation of voluminous cotton sludge, and bioconversion of sawdust, vegetable and fruits waste, rice straw, leaves and bamboo, and sawdust. Thus, cellulase-mediated waste valorisation becomes a potent tool for the proficient utilisation of agro-residues, food, paper, and textile industry waste (Khan et al. 2016; Vermelho et al. 2012). The cellulose content of municipal solid waste has also been utilised for biogas production (Demirbas et al. 2016). According to a report, about 10–50 billion tons of dry lignocellulosic waste are produced every year across the globe (Motaung and Linganiso 2018). This waste has been reported to contain about 45% cellulose, 10% hemicellulose, and 10–15% lignin. The agro-waste mainly includes sugarcane bagasse (grown in China, India, Brazil, Thailand, South Africa, and Australia), maize stalks (major crop of countries like South Africa and the United States), rice husk (grown worldwide), and sorghum stalks (tropical and subtropical regions of Asia and Africa). According to Khan et al., lignocellulosic material consists of 30% solid content composed of about 65% holocellulose and 20% lignin (Khan et al. 2016; Kumar and Verma 2021a). The meticulous design of the valorisation method can lead to the recycling of by-products with economic advantage. With the advancement of current technologies, diverse applications of these processes have been explored. These include fossil-fuel alternatives such as bioethanol, biopropanol, aviation fuel, and biogas (Kumar and Verma 2021b). Other platform chemicals, including succinic acid, butyric acid, and enzymes are also being produced by using enzymatic hydrolysis of food and agro-industrial waste. This further extends to the industrial production of innumerable polymers with various commercial applications such as polyhydroxy butyrate, poly-lactic acid, xanthan, pullan, and poly-hydroxy valerate. The application of cellulase for the valorisation of waste from different industries has been summarised in Table 6.1.

Table 6.1 Application of cellulase in the waste valorisation

Industrial waste as substrate	Source of cellulase	Value-added product formed	Method used	Reference
Lignocellulosic biomass	*Bacillus. tequilensis* G9	Bioethanol	Co-culturing of *Bacillus. tequilensis* G9 with *Saccharomyces cerevisiae*	Dar et al. (2019)
Forest waste (spruce and birch)	Obtained from NZYTech Lda.	Prebiotics	Pretreated residues were enzymatically digested by four cellulase	Karnaouri et al. (2019)
Paper sludge	Obtained from Novozymes and Sigma-Aldrich	Bioethanol production	Supplementation of β-glucosidase with a commercial cocktail of enzyme	Gomes et al. (2016)
Lignocellulosic biomass	yeast *Yarrowia lipolytica*	Lipid (ricinoleic acid) production	Consolidated Bioprocessing	Guo et al. (2018)
Sugarcane bagasse	Bacterial consortium from sugarcane bagasse and thermophilic sludge	Methane, hydrogen, and other organic acids	Bioaugmentation of cellulase producing bacteria obtained from lignocellulosic biomass with sludge	Soares et al. (2019)
Spent mushroom and food waste	*A. niger*	Lactic acid	Cellulase produced by *A. niger* led to the breakdown of waste to produce hexoses and pentoses. These were then utilised by *Enterococcus mundtii* CGMCC 22,227	Ma et al. (2021)
Textile waste (cotton-polyester 50:50 blend)	Commercial cellulase and β-glucosidase	Bioplastics, biosurfactants, and other biochemicals	Pretreatment, enzymatic hydrolysis, and recovery step include the use of activated carbon, filtration. Ion exchange chromatography and solvent evaporation	Subramanian et al. (2020)
Tapioca flour commercial waste	NA	Bioethanol gel	Pretreatment, enzymatic hydrolysis, and fermentation	Amalia et al. (2021)
Pomegranate peel	Commercial cellulase and pectinase (Novozyme)	Antimicrobial property for application in therapeutics	High-pressure extraction at 300 and 600 MPa	Alexandre et al. (2019)

(continued)

Table 6.1 (continued)

Industrial waste as substrate	Source of cellulase	Value-added product formed	Method used	Reference
Sugarcane straw	Commercial enzyme (Celluclast and Novozyme 188)	Bioethanol	Steam explosion pretreatment followed by enzymatic hydrolysis	Oliveira et al. (2013)
Sugarbeet	Accellerase (Merck-Sigma Aldrich)	Pectin, phenolic compounds, and succinic acids	Fractionation is followed by enzymatic hydrolysis. The hydrolysate was fermented by *Actinobacillus succinogenes* to produce succinic acid	Alexandri et al. (2019)

This chapter encompasses applications of cellulases in the valorisation of various waste belonging to food, textile, agricultural, and other industrial sectors. The current state of the art pushes toward a bio-based circular economy where the waste stream is maximally recycled to other production input stream.

6.2 Recent Advances in Cellulase Production and Purification

The potential of abundant natural polymer cellulose in nature has been critically known in biofuel production such as bioethanol and biobutanol. However, the major constraint is in the conversion of cellulose to fermentable sugars (saccharification) that can be overcome by treating the biomass with the cellulase enzyme. The cellulolytic enzyme can be obtained from plant, animal, and microbial sources (Zhang and Zhang 2013), among which microbial sources can be used to scale up production.

6.2.1 Microbial Production of Cellulase

Most cellulolytic microorganisms were isolated from the decaying wood and used to produce the cellulase enzyme at a large scale by either solid-state fermentations or submerged fermentations.

6.2.1.1 Solid-State Fermentation (SSF)

The type of fermentation occurs almost without free water; however, the substrate moisture must be present to assist the microorganism's growth. Hence, SSF imitates the natural habitat of maximum number of cellulolytic filamentous fungi. The critical factors that play a crucial role in the extracellular release of cellulase in SSF are moisture content, harvest time, spore count, particle size, type of substrate, incubation time, temperature, and initial pH values that render the adaptation cellulase excretion. The SSF begins with inoculating an optimised number of pure spores of filamentous fungus into the sterile solid medium under the necessary optimised conditions. The cellulase production using *Trichoderma reesei* ZU-02 through SSF by reusing solid substrates in at least three batches yielded the optimum cellulase production (158 IFPU/g koji) was obtained in the second batch of fermentation (Xia and Cen 1999). The comparative investigation of cellulase production in SSF by the two mutant strains of *Trichoderma reesei* showed to be critically varied with the physicochemical parameters like moisture content and the temperature in which *T. reesei* QM9414 strain indicated that only moisture content (70% optimum) had a major influence on the production of cellulase with maximum FPA (filter paper assay) activity (1.1635 U/g). Whereas, temperature (25°C optimum) and moisture (55% optimum) were found to be important in the extracellular cellulase production with maximum FPA activity (2.314 U/g) by *T. reesei* MCG77 strain (Latifian et al. 2007). In addition, Kim et al. had investigated the improvement of cellulase production by *Aspergillus sp.* SU-M15 with the repeated sequential treatment of mutagens Co^{60} γ-rays, N-methyl-N′-nitro-N-nitrosoguanidine, and ultraviolet irradiation to spores of *Aspergillus sp.* The production in wheat bran medium was found to be higher (82.5 U/g) than husk and sawdust by SSF (Vu et al. 2011). Sometimes, a mixture of substrates induces the extracellular cellulase production rather than the individual substrate, as investigated by Barnabe et al., who found that the mixed sludge of paper and pulp that influenced the lignocellulolytic secretions to 7.3 IU/mL from 1.5 IU/mL by *Trichoderma reesei* RUT C-30 (Lai et al. 2017). However, the cost of cellulase production is considerably reduced by utilising the waste residues or low-cost substrates from the municipal corporation, horticulture, kitchen, industrial, or open terrestrial sources as the substrates for SSF by optimising the required physicochemical environment (Bansal et al. 2012; Bharti et al. 2018; Xin and Geng 2010). The purification of extracellular cellulase enzyme can be done by extraction with the circulation of buffer and collection as a crude cellulase preparation that can be used for the saccharification of lignocellulosic materials and other applications.

6.2.1.2 Submerged Fermentation (SmF)

The type of fermentation that occurs in the presence of excess water is known as submerged fermentation (Kumar et al. 2020b). In most industrial-scale enzyme

operations, the production by SmF is of priority because of ease of monitoring and operating. Most of the commercially available cellulases are produced by filamentous fungi; however, some bacteria (Ariffin et al. 2006; Ekperigin 2007; Irfan et al. 2012) and actinomycetes (Das et al. 2014; George et al. 2001; Van Zyl 1985) can also have the ability to produce but in low titre. The aerobic production by SmF depends on several factors like cellulosic substrate, nutrient availability, pH of the medium, inducer concentration, rate of oxygen supply, and fermentation temperature. The media formulation is necessary to result in optimal growth of biomass and cellulase production. The generalised medium used for the growth of filamentous fungi and cellulase enzyme production is Mandel's medium, Vogel's medium. However, to increase cellulase production, these medium needs to be optimised with respect to cellulolytic fungus. The cellulase production can be increased by genetically engineering the cellulolytic strains by either random mutation or site-directed mutagenesis. Separation of extracellular enzymes from the fermented solid biomass is carried out by centrifugation (10,000 × g, 10 min, 4°C) that can be used directly as a crude enzyme for the hydrolysis of cellulosic material (Lai et al. 2017). The cellulase production by SSF and SmF using various substrates has been listed in Table 6.2.

6.3 Immobilisation and Molecular Approaches for Enhanced Cellulolytic Action

The objectives of immobilisation of cellulase enzyme are reusability of the same enzyme for several cycles with excellent efficiency and stability that can substantially reduce the overall cost of the product. The materials used to immobilise the enzymes are both organic and inorganic, including polysaccharides, proteins, polymers, activated carbon, and metals (Datta et al. 2013; Nawaz et al. 2016; Safarik et al. 2016). There are various strategies to immobilise the enzymes, as represented in Fig. 6.1.

Recent reports suggest several methods for cellulase immobilisation for the valorisation of agro-residues, as shown in the above figure (Fig. 6.1). There have been studies for the comparison of conversion efficiency of free and immobilised enzymes for bioethanol production using lignocellulosic residues. The use of nano-structured support for the immobilisation of cellulase has been widely investigated (Ahmad and Khare 2018; Brindhadevi et al. 2020; Han et al. 2018; Husain 2016; Sillu and Agnihotri 2019). These nano-carriers impart reusability and stability of the enzyme and exhibit a larger surface area for enzyme attachment. This facilitates overcoming the mass transfer limitations by increasing the amount of enzyme that can be employed for catalytic conversion. The use of Magnetic Nano-Particles (MNPs) further improvises the process by allowing easy withdrawal of enzymes from the solution utilising an external magnetic field. In another study, the immobilisation was done on MNPs produced using green synthesis. Immobilisation

Table 6.2 Examples of cellulase production using different substrates

Substrate	Microorganism	Fermentation process	Activity			Reference
			FPU	CMCase assay	β-glucosidase	
Wheat bran	*Trichoderma reesei* RUT C30	SSF	1.14 U/mL	14.98 U/mL	0.22 U/mL	Sukumaran et al. (2009)
Enriched Mandel's cellulose medium	*Trichoderma spp.*	SmF	0.396 U/mL	0.488 U/mL	–	Chand et al. (2005)
Solka Floc	*Acremonium cellulolyticus* strain CF2612	SmF	17.8 U/mL	–	40.3 U/mL	Fang et al. (2009)
Wheat bran	*Fomitopsis sp.* RCK2010	SSF	3.49 U/g of substrate	71.6 U/g of substrate	53.6 U/g of substrate	Deswal et al. (2011)
Rice straw	*Melanocarpus sp.* MTCC 3922	SSF	25.3 U/mL	42.3 U/mL	74.1 U/mL	Jatinder et al. (2006)
Pretreated sugarcane bagasse	*Penicillium echinulatum* 9A02S1	SSF	32.89 U gdm^{-1}	282.36 U gdm^{-1}	58.95 U gdm^{-1}	Camassola and Dillon (2007)
Pretreated willow	*Trichoderma reesei* Rut C30	SmF	0.62 FPU/mL	132 U/mL	0.07 U/mL	Kovács et al. (2008)
Sugarcane bagasse	*Trichoderma atroviride* 676	SmF	0.25 U/mL	1.9 U/mL	0.17	Grigorevski-Lima et al. (2013)
Wheat bran	*Penicillium citrinum*	SSF	1.72 U/mL	1.89 U/mL	–	Dutta et al. (2008)

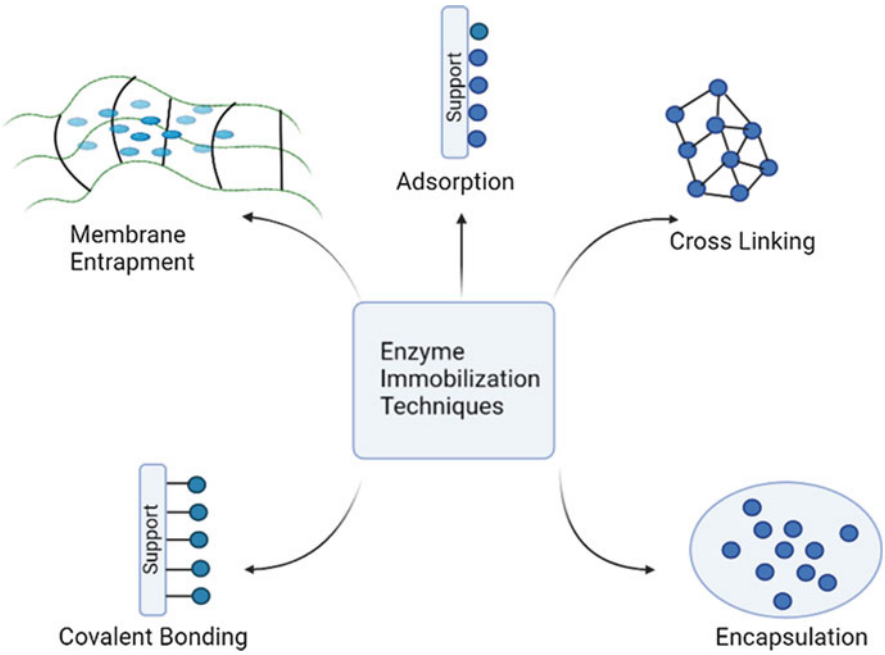

Fig. 6.1 Various methods to adopt immobilisation of enzymes

was secured by the covalent linking of cellulase to MNPs using glutaraldehyde. The results showed higher conversion efficiency of free enzymes (78%) than the covalently bonded enzymes (72%) in the first cycle, owing to the mass transfer limitation. However, subsequent cycles showed stable conversion efficiency, which ultimately is responsible for improved process economics (Ingle et al. 2017). Thus, immobilisation of cellulase contributes to its reusability, stability, and storage.

Cellulase can also be covalently immobilised using glutaraldehyde as a linker. In a significant work, it was immobilised on biochar prepared using sugarcane bagasse and coated with chitosan in different concentrations. This immobilisation process was successful in achieving 90% residual activity of cellulase even after 10 cycles of conversion (Mo et al. 2020). A detailed analysis of the pros and cons of selecting suitable micro- and nano-carriers with different methods of immobilisation is available elsewhere (Rajnish et al. 2021).

6.4 Role of Cellulase in Biorefinery Application

Cellulases are potential candidates for the bioconversion of agro-industrial wastes into several beneficial products. Such wastes are otherwise burnt or used in landfills (Singh et al. 2021). A zero-waste integrated biorefinery would involve cellulases in

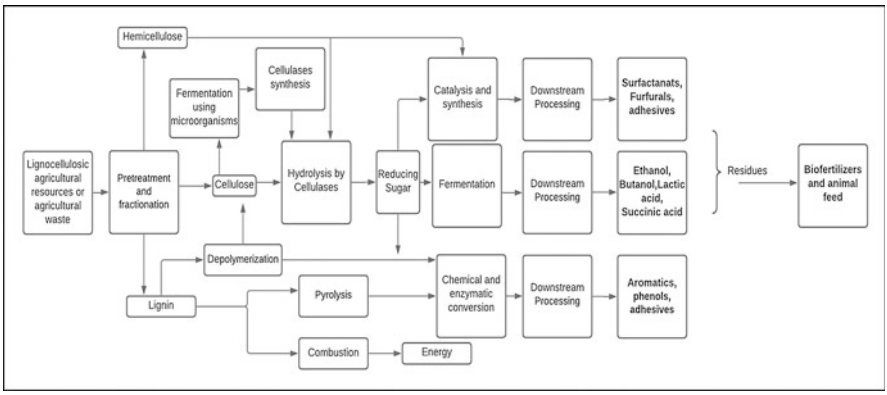

Fig. 6.2 Application of cellulases in a lignocellulosic biorefinery

the sequential production of sugar, organic acids, surfactants, furfurals, and biofuels such as bioethanol, biobutanol, biohydrogen, and compressed biogas (CBG) using agro-industrial residues, and the remaining organic matter can be used as compost or animal feed (Carrillo-Nieves et al. 2020; Kumar and Verma 2021a, b). Commercial enzyme manufacturing companies strive to manufacture enzyme cocktails for industrial biorefineries (Chandel et al. 2012). The biorefinery scheme utilising cellulases is depicted in Fig. 6.2.

6.4.1 Consolidated Bioprocessing for Biorefinery

The integrated approach to biomass conversion is known as consolidated bioprocessing. In this, a single microbe's amalgamation of saccharification and fermentation capabilities reduces environmental losses and expenses in lignocellulosic biorefineries (Bhardwaj et al. 2021a; b). The cellulase production, enzymatic hydrolysis, and bioconversion are combined (Cunha et al. 2020). The method involves hydrolysis of the structural carbohydrates to oligomers using the synthesised enzymes. The oligomers are depolymerised. The residual sugar is then fermented into various valuable products (Daniel et al. 2012). 10–30% of the pretreated liquor contributes as the carbon source for the production of enzymes and propagation of seeds. The enzyme loading is reduced to 10 mg protein/g cellulose (Scarlata et al. 2015). These modifications make the process economical and efficient. In a recent study on the biorefinery approach, consolidated bioprocessing using engineered *Trichoderma reesei* and *Saccharomyces cerevisiae* led to the highest production of 0.5 g/L glucaric acid from steam-treated corn stover in 7 days (Li et al. 2021). In another study, consolidated bioprocessing was used to produce bioethanol using surfactant (Tween 20)-assisted ionic liquid (1-ethyl-3-methylimidazolium methane sulphonate) from pretreated biomass of *Parthenium*

hysterophorus L. The optimisation of pretreated conditions like biomass loading, pretreatment temperature, and time could increase the sugar yield by 40% during saccharification with IL-stable cellulase and xylanase enzymes produced from *Aspergillus aculeatus* PN14 (Nargotra et al. 2019). A novel consolidated bioprocessing method was developed for hyperproduction of butyric acid from delignified rice straw using co-fermentation of *Clostridium thermocellum* ATCC 27405 and *C. thermobutyricum* ATCC 49875. The butyric acid yield was increased up to 33.9 g/L by fed-batch fermentation with a selectivity of 78% (Chi et al. 2018). Therefore, consolidated bioprocessing combines many processes and reduces the overall cost of production by reducing energy requirements and contamination risk as well as maximising cost-effectiveness.

6.4.2 Cellulases in Agro-industrial Waste Biorefinery

Enzymatic pretreatment is an indispensable step of any lignocellulosic biorefinery. Using cellulolytic hydrolysis, the waste from agricultural practices and industries can be converted into several valuable products (Dragone et al. 2020). A cellulolytic enzyme derived from *Bacillus pumilus* is used for sugar production from banana-agro waste in the form of pseudostem and leaves (Kanmani et al. 2011). Cellulases synthesised by *Sporotrichum* sp. LAR5 with 7.88 IU was used to extract reducing sugar from acid-pretreated rice straw (Bajaj et al. 2014). In another instance, cellulases derived from *Penicillium decumbens* were used to hydrolysis corn cobs to produce reducing sugar used for ethanol production (Saliu and Sani 2012). The use of cellulases enzyme for the valorisation of various agricultural and industrial wastes is depicted in Table 6.3.

Cotton waste from textile industries is decomposed using cellulases for safe disposal into the environment. Waste generated from textile industries includes cotton and denim residues from the cotton machining process. These waste streams are treated with cellulase. Such industrial residues are subjected to enzymatic hydrolysis for recycling and production of valuable products such as glucose syrup (Subramanian et al. 2020). Fruit and vegetable wastes are rich in disaccharides and polysaccharides. They are also non-competitive with our food chain. Cellulases can hydrolyse the fruit and vegetable waste for further bioconversion into numerous commercial products (Srivastava et al. 2021). Hemp Hurd is a by-product of the fibre industry. Immobilised cellulase on an activated magnetic support was used to hydrolyse hemp hurds (Abraham et al. 2016). In another study, Liu et al. (2019) investigated the hydrolysis of apple pomace by pectinase and cellulase and further optimised the cellulase hydrolysis parameters by RSM after pectin hydrolysis. They obtained a maximum fermentable sugar production yield of 67.54 ± 1.45%, which provided the highest lipid production of 25.8 g L^{-1} after fermentation by a genetically modified strain *Yarrowia lipolytica* po1f (pex10−mfe− leu+) (Liu et al. 2019).

Table 6.3 Application of cellulases for treating different agro-industrial biorefinery

Agro-industrial waste	Enzyme source	Processing parameters	Observations	References
Paper sludge	Recombinant cellulase cocktail, Opt CelMix similar to Cellic CTec2 and Celluclast	0.015 g/g cellulase cocktail and 10% (w/v) biomass loaded	80% glucan conversion. High glucose yields using low enzyme loading	Malgas et al. (2020)
Newspaper waste	Celluclast 1.5 L and Novozyme 188	Pretreatment using SDS and Tween-80. 15% solid content and enzyme loading was 15 FPU/g waste	29.07 g/L reducing sugar produced after 72 h at 50 °C. 0.42 g/g ethanol produced by co-culture of *Saccharomyces cerevisiae* and *Pichia stipitis*	Xin et al. (2010)
Citrus peel waste	Indigenously from *Aspergillus niger*	30 IU g^{-1} $_{Drm}$ cellulase, processing at 116 °C for 10 min	11.18 mg/ L reducing sugar. 30.7 g/ L ethanol and (339–356 mL/gVS methane)	Patsalou et al. (2019)
Corn stover	Commercial acidic cellulase	Steam-exploded 20 FPU/g dry stover	103 g/L reducing sugar	Lu et al. (2010)
Sugarcane bagasse	*Trichoderma reesei* and *Aspergillus niger*	2.0 IU/g cellulases	0.5 g reducing sugar obtained per dry substrate	Rabelo et al. (2008)
Wastewater sludge	Indigenously from *Trichoderma harzianum*	Bubble column bioreactor and membrane reactor for a system with 30 kDa membrane	73.5% cellulase recovery and 81.37% reduction in chemical oxygen demand	Libardi et al. (2019)
Rice husk	Celluclast	Lime pretreated biomass, 48 FPU/g	0.2 g/g reducing sugar	Saha and Cotta (2008)
Textile residues	Indigenously from *Aspergillus niger* CKB	Alkali pretreated biomass, 0.43 ± 0.01 FPU g enzyme loading	70.2% reducing sugar recovered	Hu et al. (2018)
Sorghum waste	Spezyme-*CP* and Novozyme	10% pretreated (by ammonia) fibres were hydrolysed using 60 FPU Spezyme-CP and 64 CBU Novozyme 188/g glucan	84% digested for a pretreated sample and 38% cellulose digested for untreated biomass 0.24 g ethanol per g dry biomass was obtained for the pretreated sample	Salvi et al. 2010

6.4.3 *Challenges of Cellulase-Based Biorefinery*

Driving down the cost of enzyme production poses the main challenge for agro-industrial biorefineries. Mass bio-prospecting programme for the discovery of efficient cellulase enzyme synthesising microbes can make the production economical in the long run (Jayasekara and Ratnayake 2019). Integrated processing techniques would also be helpful in improving cellulase production and ethanol productivity in a simplified manner (Kuhad et al. 2016). Instead of single enzymes, concoctions and cellulolytic enzyme cocktails should be explored for their applications in hydrolysis (Victoria et al. 2017). In-house enzyme production is an economical option to produce ethanol for biorefineries (Cunha et al. 2017). This would ensure waste to wealth conversion in a sustainable manner.

6.5 Role of Cellulase in Value-Added Product Formation

Lignocellulosic biomass can be transformed into value-added chemicals through various existing chemical, thermochemical, and biological routes. However, the biological method of lignocellulosic biomass conversion using enzymes is a feasible and environment-friendly, and sustainable process. Many bacterial and fungal species have the ability to breakdown the lignocellulosic biomass into simple sugars such as glucose and xylose by secreting enzymes (cellulase and hemicellulase), which can further be utilised to produce several value-added products like acids (lactic acid, citric acids, succinic acid, gluconic acids), butanol, xylitol, microbial polysaccharides, and other fine chemicals (Ning et al. 2021). In combination with other enzymes (utilised pectinase and xylanase), cellulase improves the hydrolysis efficiency and reduces the overall cost associated with the process (Bhardwaj and Verma, 2021). Some of the important value-added products derived from lignocellulosic biomass are mentioned in Table 6.4.

6.5.1 *Lactic Acid*

Lactic acid is used as a green solvent and building block for polylactic acid (PLA), a bio-degradable plastic alternative to petroleum-based plastic. Lactic acid can be produced from simple carbohydrates derived from the hydrolysis of agricultural lignocellulosic biomass. However, these recalcitrant lignocellulosic materials should be pretreated first to ease the cellulosic hydrolysis process governed by the cellulase enzymes. Various pretreatment such as physical (milling, grinding, shredding), chemical (acidic, alkali, ionic liquids), and thermal pretreatments can enhance the cellulosic hydrolysis process based on the nature of the lignocellulosic biomass. Yadav et al. (2021) inspected the role of imidazolium ionic liquid in the pretreatment

Table 6.4 List of some value-added chemicals produced from lignocellulosic biomass

Product	Substrate used	Microorganism	Concentration (g/L)	Yield	Reference
Lactic acid	Rice straw	*L. plantarum* SKL-22	36.75	–	Yadav et al. (2021)
Citric acid	Corn stover biomass	*Aspergillus niger* SIIM M288	136.3	74.9% of cellulose	Hou and Bao (2018)
Succinic acid	Fruit and Vegetable waste	*Yarrowia lipolytica*	43.1	0.46 gg^{-1}	Li et al. (2018)
Butyric acid	Paper mill sludge	*Clostridium tyrobutyricum*	7.0	–	Liu et al. (2018)
Gluconic acid	Microcrystalline cellulose	–	9.60	0.27 gg^{-1}	Ruales-Salcedo et al. (2020)
PHA	Brewer's spent grain	*Burkholderia cepacia*	–	9.0 ± 0.44 mg PHA/g dry BSG	Llimós et al. (2020).

of rice straw and further saccharified with commercial cellulase enzyme for lactic acid production by *Lactobacillus plantarum* SKL-22 strain. They observed that the media optimisation using response surface methodology could enhance the lactic acid yield by 1.11%, with a final yield of 36.75% using rice straw in a single pot bioprocess.

In many bioprocesses, utilised cellulase enzymes are used repeatedly to saccharify lignocellulosic biomass to simple carbohydrates, which could be further used to produce value-added chemicals. This could be a holistic approach for upcoming biorefineries in the future and sustainable product formation. Grewal et al. (2018) explored the one-pot bioprocess of lactic acid production from ionic pretreated lignocellulosic waste saccharified by nano-immobilised cellulase enzyme. In this process, the simultaneous saccharification and co-fermentation (SSCF) process was performed by *Lactobacillus brevis*, which provided a lactic acid yield of 0.22, 0.52, and 0.49 g/g with sugars derived from ionic pretreated cottonseed cake, sugarcane bagasse, and wheat straw, respectively. To reduce a large amount of cellulase requirement in lignocellulosic-based lactic acid production, Chen et al. (2020) established the integration of fed-batch simultaneous saccharification process with membrane separation, in which residual cellulase enzyme present in the waste stream and solid-residue of corn stover were recycled and reused for successive fermentation. Around six rounds of operation were carried out, which increased the lactic acid yield (0.389 g/g) by 1.2 times higher than the conventional process and reduced the cellulase consumption, wastewater discharge and nutrient consumption by 47%, 73.7% and 86.1%, respectively.

In addition to lignocellulosic biomass, other agricultural wastes such as food waste and fungal biomass have also been utilised to produce lactic acid with the assistance of the cellulase enzyme. Ma et al. (2021) studied the co-fermentation of spent mushroom substance (SMS) and food waste to produce lactic acid. They reported that 92.62% of sugar was released from pretreated SMS by *Aspergillus niger* cellulase, which could increase the lactic acid concentration by 22.97% (Ma et al. 2021).

In another study, Abdel Rahman et al. (2019) isolated a thermo-alkaliphilic lactic acid bacterium, *Enterococcus faecium* FW26, which could produce lactic acid from mixed food waste and banana peels with a lactic acid yield of 84% g/g-consumed sugars. *Enterococcus faecium* FW26 secreted the amylase and cellulase enzyme and grew on a mixed substrate of food waste and banana peels without any treatment. In addition, the isolated strain was able to survive and facilitate fermentation under the stress of thermo-alkaline conditions, which might reduce the chances of contamination.

6.5.2 Succinic Acid

Succinic acid is a dicarboxylic acid and an intermediate compound of the tricarboxylic acid (TCA) cycle. It has various uses in the food industry as a flavouring agent,

acidulant, and antimicrobial agent and is also used as a solvent additive, surfactant, foaming agent, detergent extender, coating resins, and ion chelator (Kumar et al. 2019). Glucose, maltose fructose, sucrose, and glycerol have been the most frequently used carbon sources for succinic acid production by fermentation using strains of bacteria such as *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Mannheimia succiniciproducens,* and recombinant *Escherichia coli* (Karthik and Rathinamoorthy 2017). However, utilisation of cheaper carbon sources such as lignocellulosic biomass with the help of cellulase enzyme could improve the overall process economics. Some microorganisms can utilise partially hydrolysed cellulose and produce valuable products. Jiang et al. (2013) performed the incomplete hydrolysis of cellulose for succinic acid production by *Actinobacillus succinogens*. A succinic acid concentration of 20 g/L with a yield of 64.7% was achieved from 18 g/L of cellobiose and 17 g/L of other sugars obtained from sugarcane bagasse hydrolysate. In another study, Li et al. (2018) hydrolysed the fruit and vegetable waste with a cocktail of enzymes (2% glucoamylase, 2% hemicellulase, 1% cellulase, and 0.25% pectinase) and obtained the final glucose titre of 56. 7 g L^{-1} and a yield of 0.46 g g^{-1}. The obtained hydrolysate was further utilised to produce succinic acid by *Y. lipolytica* PSA02004 with a titre and yield of 43.1 g L^{-1} and 0.46 g g^{-1}, respectively. They improved succinic acid productivity (0.69 g L^{-1} h^{-1}) and concentration (140.6 g L^{-1}) using in situ fibrous bed bioreactor and fed-batch fermentation. Therefore, cellulase facilitates the utilisation of agro-waste for the production of value-added chemicals through biochemical pathways with good efficiency and cost-effectiveness.

6.5.3 Gluconic Acid

Gluconic acid, a non-toxic, non-corrosive, and weak organic acid is industrially produced through the fermentation of glucose by *A. niger* or *Gluconobacter suboxydans*. The gluconic acid production was also reported by various strains of *Pseudomonas*, *Acetobacter*, and *Zymomonas*. Gluconic acid has many applications in the food industry as a flavouring agent, in dietary supplements as different salts (sodium, calcium, and iron gluconate), and in the pharmaceutical and textile industries. Microbial fermentation is the most suitable and chosen method for gluconic acid production due to being less expensive and more efficient than other methods. Large-scale gluconic acid production is mainly affected by three crucial factors: cheaper substrate, oxygen concentration, and pH. Using the lignocellulosic biomass can solve the problem of the cheaper substrate if a cost-effective enzyme production method is developed. Yu et al. (2021) explored the co-immobilisation of multienzymes (cellulase, catalase, and glucose oxidase) on reversibly soluble polymer Eudragit L-100 in a cascade reaction for the direct transformation of corn straw to gluconic acid. The gluconic acid yield was achieved at 0.28 mg/mg, and the rate of conversion of cellulose in corn straw to gluconic acid was obtained at 61.41%. Similarly, Zhou et al. (2019) studied an integrated process of co-production of

gluconic acid with xylooligosaccharides from sugarcane bagasse. After the xylooligosaccharides production from the prehydrolysis, 88.6% of cellulose was converted into simple sugars in fed-batch enzymatic hydrolysis using cellulase (C2730, Celluclast®). The gluconic acid yield of 96.3% was produced by fermentation of sugars using *Gluconobacter oxydans* ATCC 621H. Therefore, approximately 105 g of xylooligosaccharides and 340 g of gluconic acid were co-produced in the integrated process from 1 kg of dried sugarcane bagasse (Zhou and Xu 2019).

6.5.4 Citric Acid

Citric acid is another crucial organic acid used as a preservative and acidulant in the food industries and also used as a starting material in the pharmaceutical and cosmetic industries. It is mainly produced through microbial fermentation from using carbohydrates (mainly starch) or agro-industrial waste. However, due to the restricted supply of food starch, potential alternative sources like lignocellulosic biomass are also being explored to meet future demand. Cellulase plays a vital role in the conversion of these recalcitrant compounds into simple sugars. Citric acid is produced on large scale through fermentation by *A. niger* due to its high citric acid productivity and tolerance to low pH. It is mainly produced through submerged fermentation. However, many researchers have explored the use of agro-residues and lignocellulosic biomass as the starting material for citric acid production. Hou et al. (2018) studied the simultaneous saccharification and fermentation (SmSF) of dilute acid-pretreated corn stover for citric acid production by *A. niger*. In SmSF, pretreated corn stover was hydrolysed by cellulase enzyme, Cellic CTec 2.0, from Novozymes. The highest citric acid concentration (136.3 g/L) was obtained with an overall yield of 74.9% of cellulose in the aerobic SmSF process. This study compared the citric acid production from SmSF with separate hydrolysis and fermentation (SHF) and concluded that the cellulosic citric acid production by SmSF could be highly comparable to starch-based citric acid production.

6.5.5 Microbial Polymers

6.5.5.1 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) are the most potential, degradable, and environment-friendly substitute for petrochemical-based plastics. The main constraint that prevents PHA from commercialisation is the cost of production. Therefore, researchers are focusing on cheap substrates for sustainable PHA production. In this regard, lignocellulosic waste can be used as a possible substrate as it is cheap, readily available, and most importantly, it does not compete with the food requirements of humans. Nowadays, a wide range of lignocellulosic waste generated from industries,

forestry, agriculture, marine biomass, etc. has gained great consideration for PHA production (Obruca et al. 2015).

PHA production using lignocellulosic biomass at the industrial level includes mainly three steps, especially pretreatment, enzymatic/acidic hydrolysis, and fermentation. Many pretreatment techniques are used before the enzymatic hydrolysis of cellulose which includes physical processes for size reduction, chemical treatment (with hydrogen peroxide, acid, alkali, or sodium chlorite) for delignification, physicochemical processes (like a steam explosion, ammonia fibre expansion, etc.) or a biological pretreatment using microbial enzymes. Further, enzymatic hydrolysis is performed for the degradation of complex carbohydrates into simple sugar, which serves as a carbon source for microbes in the successive fermentation step. Later fermentation is carried out using PHA-producing microbes (e.g., *Bacillus sonorensis, Halomonas hydrothermalis, Pseudomonas putida, Bacillus cereus*, etc.) and PHA is recovered. It was suggested that the average sugar and PHA yields were higher in the case of enzymatic hydrolysis as compared to acid-based hydrolysis and cellulase plays an important role in enzymatic hydrolysis (Al-Battashi et al. 2019).

Production of PHA without pretreatment steps is also used to decrease the cost of the process. Israni and Shivakumar (2020) used cellulase, lipase, and amylase positive *Bacillus megaterium* strain Ti3 for the production of PHA without pretreatment step. PHA was produced using 16 different lignocellulosic substrates by submerged fermentation at 30 °C for 48 h at 120 rpm. They demonstrated that ragi husk and sesame oil cake have the most influential factors on the basis of Taguchi orthogonal array. Similarly, Heng et al. (2017) demonstrated a three-step process for PHA production using *Burkholderia cepacia* USM, *Cupriavidus necator* NSDG-GG, and a genetically modified strain of *Cupriavidus necator* H16. They used rice husk as raw material and performed alkaline pretreatment followed by enzymatic hydrolysis and fermentation. For enzymatic hydrolysis, they used a cellulase-based enzymes Celluclast 1.5 L and Novozyme-188 at 50 °C 160 rpm for 72 h. They obtained the highest sugar yield of 87% after enzymatic hydrolysis and obtained 7.8 g/L of dry cell weight and 50% PHA content using a 5 L fermentor.

Llimós et al. (2020) performed two-stage valorisation using Brewer's spent grain (BSG) as a lignocellulosic substrate for PHA production. First, they produced lignocellulolytic enzymes by three fungal strains (*Trichoderma reesei*, *Aspergillus niger* and *Thermoascus aurantiacus*) through SSF of dried BSG. Then these enzyme were used for the hydrolysis of BSG and production of PHA. The PHA was produced by fermentation of sugar rich BSG hydrolysate at 30 °C and 120 rpm using *Burkholderia cepacia* and *Cupriavidus necator*. *C. necator* cells were harvested after 48 h and *B. cepacia* cells were harvested after 72 h. The maximum yield of PHA they obtained was 9.0 ± 0.44 mg PHA/g dry BSG, and the result showed that BSG could be utilised as a suitable raw material for the production of value-added products (Llimós et al. 2020).

PHA is a potential material with a broad range of uses which includes its use as bioplastics, drug carrier, and synthesis precursor, packaging materials, biofuels, implant materials, cosmetic ingredients, disposal cups, and bottles, etc. Presently,

PHA is not well commercialised as compared to fuel-based plastics and products. The main hurdles are the high cost of production and relatively poor characteristics. Still, researchers are searching for the solution to this problem as PHA offers an eco-friendly and sustainable alternative to petrochemical-based products and has a promising future (Wang and Chen 2017).

6.5.5.2 Nanocellulose

In the last few years, remarkable development has been noticed in nanotechnology, especially in nanocellulose production. Nanocellulose produced from lignocellulosic waste is a potential alternative for obtaining various value-added products that can meet the demand of the market and environmental issues (Jaiswal et al. 2021). According to various literature, lignocellulosic biomass like rice husks, wheat straw, cotton, sugarcane bagasse, flax fibres, wood bark, softwood pulp, corncob, hardwood, etc. can be used for the production of nanocellulose (Michelin et al. 2020).

Currently, acid hydrolysis is the most regular method for the production of nanocellulose. However, nowadays production of nanocellulose using enzymatic hydrolysis is gaining significant importance as toxic residues are not produced. Moreover, during enzymatic hydrolysis, mild conditions are used, which makes it a low energy-intensive process (Karim et al. 2017). The process of nanocellulose production by enzymatic hydrolysis contains several steps. The first or pretreatment step consists of delignification where lignin is removed using various mechanical processes (chipping and milling) and chemical processes (alkaline pretreatment and bleaching pretreatment). The second step is enzymatic hydrolysis which is generally carried out at the pH range from 4 to 7 and temperature range from 45 to 50 °C. The last step includes homogenisation, where the enzymatically treated material is homogenised by washing or mechanical homogenisation (Ribeiro et al. 2019).

de Aguiar et al. performed enzymatic hydrolysis of sugarcane bagasse and straw at 50 °C and 200 rpm. The cellulose nanocrystals (CNC) produced by both bagasse and straw showed a crystallinity index of approximately 70% and high thermal stability (de Aguiar et al. 2020). In another study, Yarbrough et al. (2017) reported that the synergistic effect of two or more enzymes shows a better effect than an individual enzyme. They demonstrated that the *Caldicellulosiruptor bescii* with multifunctional enzymes is better in cellulose and nanocellulose production than the classical system of "free enzyme" produced by *Trichoderma reesei*.

The application of only one technique is not as effective as the combination of two or more techniques. For example., high-quality nanocellulose is produced using a coupled ball milling process and enzymatic hydrolysis, which can be further improved when combined with any other technique (Teo and Wahab 2020). Zhang et al. (2020) designed a new approach using poplar wood as lignocellulosic biomass to produce nanocellulose. They used steam explosion and delignification for the pretreatment of poplar wood. Further, the enzymatic hydrolysis was performed using cellulase enzyme followed by ultrasonic treatment. The optimum

condition determined by them for enzymatic hydrolysis was 200 U/g at 50°C for 12h, giving approximately 13.2% yield of nanocellulose. The crystallinity of the composite produced in this study was 61.98% which was higher than the crystallinity of poplar cellulose, and the structure of the composite was not damaged during the preparation steps. Squinca et al. (2020) performed enzymatic hydrolysis using a cellulolytic enzyme complex with the high specific activity of endoglucanase produced by the fungus *Aspergillus niger*. They pretreated the cellulose pulp of eucalyptus by ball milling, followed by enzymatic hydrolysis and sonication, which yielded 24.6% of cellulose nanocrystals (CNCs) by the enzymatic hydrolysis of 96 h. CNC's length, diameter, and crystallinity were 294.0 nm, 24.0 nm, and 78.3%, respectively.

Apart from nanocellulose isolation, cellulases can also be used for the pretreatment of lignocellulosic waste and other pretreatment techniques. Zeng et al. (2020) used endocellulase for enzymatic pretreatment of the bleached pulp of softwood kraft before wet ball milling. Further, they used mechanical grinding for the production of cellulose nanofibrils (CNF).

Nanocelluloses have a wide range of applications in different industries it can be used as material for food packaging in food industries, electronic displays in the electronic industry, paper and coating material in the paper and pulp industry, a supporting material in the medical and tissue industry, absorbent material in the water treatment industry, etc. (Zaki et al. 2021). The enzymatic hydrolysis is a time-consuming process for the production of nanocellulose. However, it has gained the attention of companies and researchers as it has shown better saccharification efficiency and penetration. Despite the challenges, enzymatic hydrolysis for nanocellulose production has great potential because of its unique characteristic and eco-friendly nature (Teo and Wahab 2020).

6.6 Role of Cellulase in a Circular Economy

The application of cellulases for the efficient valorisation of agro-industrial waste has tremendous potential for generating biofuels and biochemicals with low carbon and water footprints that can be recycled back to the earth within the scope of a circular economy (Astolfi et al. 2019). This conceptualises the 3Rs of a circular economic system. In situ resource utilisation, enzyme recycling, and sequential zero-waste biorefinery approaches are commercially viable solutions for sustainable development.

6.6.1 Valorisation via Carbon-Neutral Technologies

Fossil-based resources are now being replaced by alternative renewable feedstocks for the synthesis of cellulases and bioconversion for biofuels, commercially

important chemicals, and biomaterials to envisage a decarbonisation system (Adsul et al. 2020). Millions of tonnes of lignocellulosic waste are generated per annum globally. Their valorisation in an economical and eco-friendly manner forms the core of a bio-based circular economy (Sheldon 2020). In the current scenario, the entire focus lies in the thoughtful conversion of the so-called wastes into valuable products such as reducing sugars, biochemicals, and biofuels, primarily regulated by cellulase enzymes.

6.6.2 *Life Cycle Assessment of Cellulase-Based Biorefinery*

The environmental and economic effects of a process analysed by the life cycle assessment (LCA) technique include all inputs and outputs of material and energy flow at different life cycle stages (Russell et al. 2005; Kumar and Verma 2021c). LCA technology in the assessment of cellulase production and utilisation is limited by rigid system boundaries, data accuracy and availability, variations in statistical techniques, variations in product utility, its selectivity, and local environmental conditions (Borrion et al. 2012). System boundaries of the cellulase manufacturing process focus on the greenhouse gas emissions related to the cradle-to-gate activities for cellulase production (Fig. 6.3). In a case study on cellulase production from corn feedstock, Aspen-Plus and GREET 1.8c.25 were used for estimation of emissions and financial matrices. 258 g CO_2 eq. L^{-1} of bioethanol was estimated for onsite production and 403 g CO_2 eq. L^{-1} for offsite production. The cost estimation was made on the basis of bioethanol plant size and cellulase loading. An additional input like return on investment is used for offsite data. The final production cost was 0.46 USD gal^{-1} of bioethanol for cellulase for on-location production (Hong et al. 2013).

Cellulase production from coffee husk was assessed on the basis of data taken from EcoInvent v3.4 (SimaPro v8.5 software) database. The results showed that the downstream process significantly impacts the environment and economic value (Catalán et al. 2019). In another study, an integrated ethanol production process using spruce loggings was compared to an offsite production scenario. The greenhouse gas emissions were lower in the onsite process as compared with that of the offsite process. The ethanol selling rate was 0.568 euro per litre for the integrated cases and 0.581 euro per litre for the off-location case (Olofsson et al. 2017).

6.6.3 *Increased Circularity via Onsite Application*

The change in the substrate from enzyme production to hydrolysis and offsite enzyme application often alters the enzymatic efficiency. Researchers have found that the microorganisms grown on various carbon sources under different physico-chemical environments produce different isoforms of cellulase, which show behavioural diversity while interacting with the same substrate (Siqueira et al.

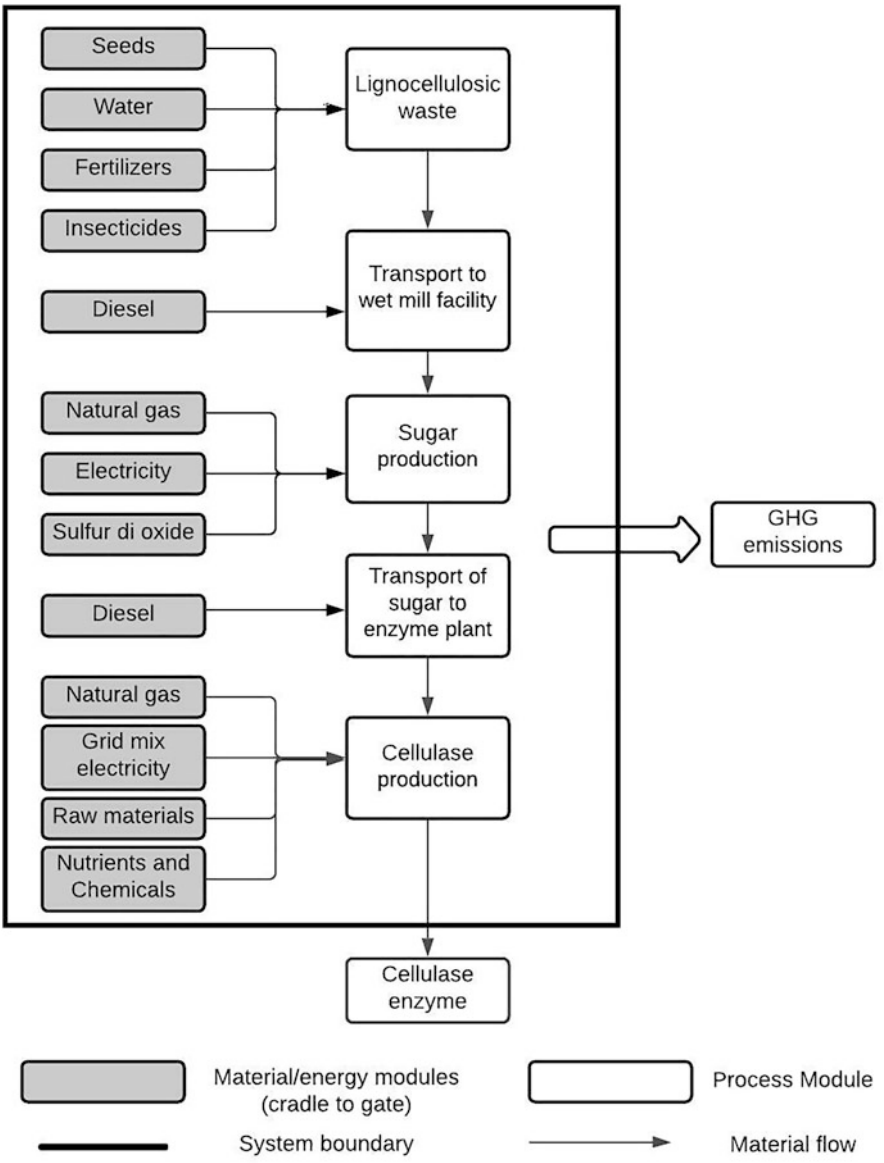

Fig. 6.3 System boundaries for cellulase application in biorefinery

2020; Zhao et al. 2018). The techno-economic feasibility was assessed for the onsite and offsite cellulase synthesis and utilisation for ethanol generation using corn stover as feedstock. The biomass channelised from the ethanol production stream improved the value of onsite cellulase production (Kazi et al. 2010). The comparative routes for onsite, offsite, and integrated biorefinery are shown in Fig. 6.4.

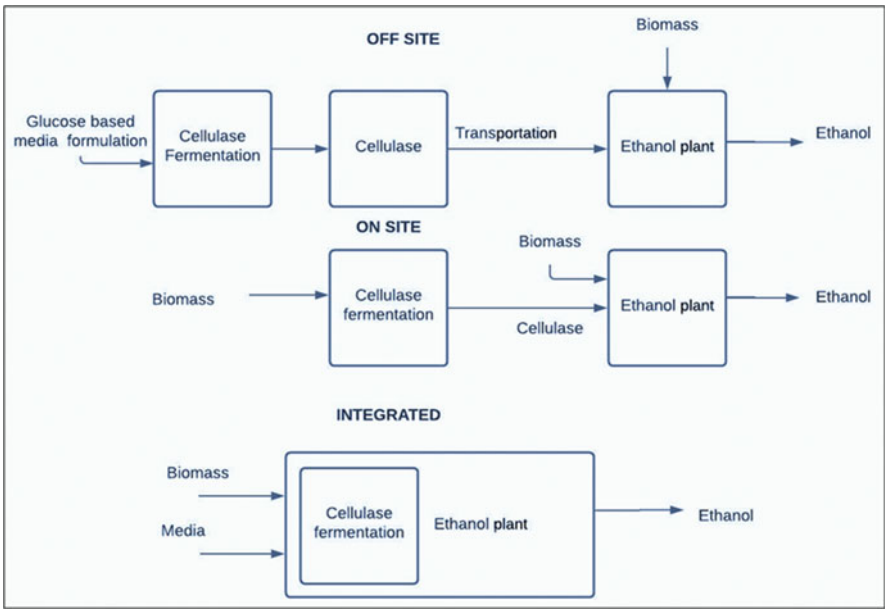

Fig. 6.4 Comparison of offsite, onsite, and integrated biorefinery

Novozyme introduced Cellic CTec3 based on the economic bioconversion of pretreated lignocellulosic substrate (Sun et al. 2015). The techno-economic model for the corn-based ethanol industry revealed the significance of onsite cellulase production for biorefinery. The use of pure glucose, priced $580/t as a carbon source, covers over 50% of cellulases' production cost, which is commercially not feasible (Humbird et al. 2011). Working on a low-cost substrate, non-sophisticated downstream processes, less storage, and transport makes onsite enzyme production eco-friendly and contributes to a circular economy.

6.6.4 Challenges and Perspectives

The circular economic sustainability is challenged by two major threats. These are the lignocellulose biorefinery supply chain and the resistant structure of lignin, which restrict the access of cellulose by cellulases (Garlapati et al. 2020). The last issue significantly affects the cellulose crystallinity and the efficacy of cellulases. Low-cost, energy-efficient pretreatment strategies can resolve the lignin problem. The energy recovery process needs to be accompanied by soil replenishment (Battaglia et al. 2021). In this context, it is essential to re-conceptualise the enzymatic reduction of agricultural lignocellulosic waste for obtaining value-added products through sustainable utilisations. The integrated lignocellulosic biorefinery leads to the circular economic framework through the endorsement of niche markets,

consumer-oriented value generation, and reduction of transportation through supply chain modifications of lignocellulosic feedstocks (Sarsaiya et al. 2019). The ultimate benefits would be a cleaner environment and economic profitability.

6.7 Conclusion

Cellulase plays an important role as it is responsible for the hydrolysis of cellulose into simple sugars, which can be further used as a carbon source for microbial fermentation. Nowadays, valorisation of lignocellulosic waste has gained the attention of researchers, and efforts are made to make this process industrially and commercially feasible as it is eco-friendly and has great potential and a promising future. Cellulases are produced at a large scale using cellulolytic microorganisms either by SSF or SmF. However, industries prefer SmF over SSF as it is easy to monitor and operate. The commonly reported microorganisms for cellulase production are *T. reesei, S. argenteolus, B. licheniformis, Candida cylindracea, Aspergillus oryzae,* etc. Cellulases are a powerful tool for the bioconversion of agro-residues, food, paper, and textile industry waste into beneficial products like surfactants, furfurals, aromatics, phenols, ethanol, butanol, lactic acid, succinic acid, gluconic acid, citric acid, etc. Recently, the use of cellulase is extensively explored for the production of microbial polymers such as nanocellulose, polyhydroxyalkanoates, polyhydroxy butyrate, xanthan, and pullan.

The major challenge behind the use of lignocellulosic biomass in biorefineries is the cost of production. Many strategies have been developed to reduce the cost such as the utilisation of immobilisation techniques for the reusability of cellulase, onsite cellulase production, use of enzyme cocktails instead of a single enzyme, combination of two or more techniques for the pretreatment step, and use of low-cost substrates. Despite many challenges, great progress has been made in the direction of using lignocellulosic biomass as it is a potential renewable alternative for fossil-based resources. Also, advanced tools such as techno-economical analysis (TEA) and life cycle assessment analysis (LCA) provide insight into actual benefits associated with enzyme-based conversion processes over chemical processes. These assessments promise an optimised circular economy approach for maximum fulfilment of societal needs and minimum impact on the environment and ecosystem.

Competing Interests There is no conflict of interest.

References

Abdel-Rahman MA, Hassan SE-D, Roushdy MM, Azab MS, Gaber MA (2019) Free-nutrient supply and thermo-alkaline conditions for direct lactic acid production from mixed lignocellulosic and food waste materials. Bioresour Technol Rep 7:100256

Abraham RE, Wong CS, Puri M (2016) Enrichment of cellulosic waste hemp (*Cannabis sativa*) hurd into non-toxic microfibres. Materials 9(7):562

Adsul M, Sandhu SK, Singhania RR, Gupta R, Puri SK, Mathur A (2020) Designing a cellulolytic enzyme cocktail for the efficient and economical conversion of lignocellulosic biomass to biofuels. Enzyme Microbial Technol 133:109442

Ahmad R, Khare SK (2018) Immobilization of *Aspergillus niger* cellulase on multiwall carbon nanotubes for cellulose hydrolysis. Bioresour Technol 252:72–75

Al-Battashi HS, Annamalai N, Sivakumar N, Al-Bahry S, Tripathi BN, Nguyen QD, Gupta VK (2019) Lignocellulosic biomass (LCB): a potential alternative biorefinery feedstock for polyhydroxyalkanoates production. Rev Environ Sci Bio/Technol 18(1):183–205

Alexandre EM, Silva S, Santos SA, Silvestre AJ, Duarte MF, Saraiva JA, Pintado M (2019) Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction. Food Res Int 115:167–176

Alexandri M, Schneider R, Papapostolou H, Ladakis D, Koutinas A, Venus J (2019) Restructuring the conventional sugar beet industry into a novel biorefinery: fractionation and bioconversion of sugar beet pulp into succinic acid and value-added coproducts. ACS Sustain Chem Eng 7(7): 6569–6579

Amalia AV, Fibriana F, Widiatningrum T, Hardianti RD (2021) Bioconversion and valorization of cassava-based industrial wastes to bioethanol gel and its potential application as a clean cooking fuel. Biocatal Agric Biotechnol 102093

Ariffin H, Abdullah N, Umi Kalsom M, Shirai Y, Hassan M (2006) Production and characterization of cellulase by *Bacillus pumilus* EB3. Int J Eng Technol 3(1):47–53

Astolfi V, Astolfi AL, Mazutti MA, Rigo E, Di Luccio M, Camargo AF, Dalastra C, Kubeneck S, Fongaro G, Treichel H (2019) Cellulolytic enzyme production from agricultural residues for biofuel purpose on circular economy approach. Bioprocess Biosyst Eng 42(5):677–685

Bajaj BK, Sharma M, Rao RS (2014) Agricultural residues for production of cellulase from *Sporotrichum thermophile* LAR5 and its application for saccharification of rice straw. J Mater Environ Sci 5(5):1454–1460

Bansal N, Tewari R, Soni R, Soni SK (2012) Production of cellulases from Aspergillus niger NS-2 in solid state fermentation on agricultural and kitchen waste residues. Waste Manage 32(7): 1341–1346

Battaglia M, Thomason W, Fike JH, Evanylo GK, von Cossel M, Babur E, Iqbal Y, Diatta AA (2021) The broad impacts of corn stover and wheat straw removal for biofuel production on crop productivity, soil health and greenhouse gas emissions: a review. Gcb Bioenergy 13(1):45–57

Bhardwaj N, Verma P (2021) Microbial xylanases: a helping module for the enzyme biorefinery platform. In: Srivastava N, Srivastava M (eds) Bioenergy research: evaluating strategies for commercialization and sustainability, vol 27. Wiley Online, pp 2129–2152

Bhardwaj N, Kumar B, Agrawal K, Verma P (2021a) Current perspective on production and applications of microbial cellulases: a review. Bioresour Bioprocess 8(1):1–34

Bhardwaj N, Agrawal K, Kumar B, Verma P (2021b) Role of enzymes in deconstruction of waste biomass for sustainable generation of value-added products. In: Thatoi H, Mohapatra S, Das SK (eds) Bioprospecting of enzymes in industry, healthcare and sustainable environment. Springer, Singapore, pp 219–250

Bharti AK, Kumar A, Kumar A, Dutt D (2018) Exploitation of *Parthenium hysterophorous* biomass as low-cost substrate for cellulase and xylanase production under solid-state fermentation using *Talaromyces stipitatus* MTCC 12687. J Radiat Res Appl Sci 11(4):271–280

Borrion AL, McManus MC, Hammond GP (2012) Environmental life cycle assessment of lignocellulosic conversion to ethanol: a review. Renew Sustain Energy Rev 16(7):4638–4650

Brindhadevi K, Samuel MS, Verma TN, Vasantharaj S, Sathiyavimal S, Saravanan M, Pugazhendhi A, Duc PA (2020) Zinc oxide nanoparticles (ZnONPs)-induced antioxidants and photocatalytic degradation activity from hybrid grape pulp extract (HGPE). Biocatal Agric Biotechnol 28:101730

Camassola M, Dillon A (2007) Production of cellulases and hemicellulases by *Penicillium echinulatum* grown on pretreated sugar cane bagasse and wheat bran in solid-state fermentation. J Appl Microbiol 103(6):2196–2204

Carrillo-Nieves D, Saldarriaga-Hernandez S, Gutiérrez-Soto G, Rostro-Alanis M, Hernández-Luna C, Alvarez AJ, Iqbal HM, Parra-Saldívar R (2020) Biotransformation of agro-industrial waste to produce lignocellulolytic enzymes and bioethanol with a zero waste. Biomass Convers Biorefin:1–12

Catalán E, Komilis D, Sánchez A (2019) Environmental impact of cellulase production from coffee husks by solid-state fermentation: a life-cycle assessment. J Clean Prod 233:954–962

Chand P, Aruna A, Maqsood A, Rao L (2005) Novel mutation method for increased cellulase production. J Appl Microbiol 98(2):318–323

Chandel AK, Chandrasekhar G, Silva MB, Silvério da Silva S (2012) The realm of cellulases in biorefinery development. Crit Rev Biotechnol 32(3):187–202

Chen H, Su Z, Wang Y, Wang B, Si Z, Lu J, Su C, Ren W, Chen H, Cai D (2020) Lactic acid production from pretreated corn stover with recycled streams. Process Biochem 91:132–140

Chi X, Li J, Wang X, Zhang Y, Antwi P (2018) Hyper-production of butyric acid from delignified rice straw by a novel consolidated bioprocess. Bioresour Technol 254:115–120

Cunha FM, Badino AC, Farinas CS (2017) Effect of a novel method for in-house cellulase production on 2G ethanol yields. Biocatal Agric Biotechnol 9:224–229

Cunha JT, Romaní A, Inokuma K, Johansson B, Hasunuma T, Kondo A, Domingues L (2020) Consolidated bioprocessing of corn cob-derived hemicellulose: engineered industrial *Saccharomyces cerevisiae* as efficient whole cell biocatalysts. Biotechnol Biofuels 13(1):1–15

Daniel GO, John EM, Lee RL (2012) Recent progress in consolidated bioprocessing. Curr Opin Biotechnol 23(3)

Dar MA, Pawar KD, Rajput BP, Rahi P, Pandit RS (2019) Purification of a cellulase from cellulolytic gut bacterium, *Bacillus tequilensis* G9 and its evaluation for valorization of agro-wastes into added value byproducts. Biocatal Agric Biotechnol 20:101219

Das P, Solanki R, Khanna M (2014) Isolation and screening of cellulolytic actinomycetes from diverse habitats. Int J Adv Biotechnol Res 5(3):438–451

Datta S, Christena LR, Rajaram YRS (2013) Enzyme immobilization: an overview on techniques and support materials. 3 Biotech 3(1):1–9

de Aguiar J, Bondancia TJ, Claro PIC, Mattoso LHC, Farinas CS, Marconcini JM (2020) Enzymatic deconstruction of sugarcane bagasse and straw to obtain cellulose nanomaterials. ACS Sustain Chem Eng 8(5):2287–2299

Demirbas A, Taylan O, Kaya D (2016) Biogas production from municipal sewage sludge (MSS). Energy Sources, Part A: Recov Util Environ Effects 38(20):3027–3033

Deswal D, Khasa YP, Kuhad RC (2011) Optimization of cellulase production by a brown rot fungus *Fomitopsis* sp. RCK2010 under solid state fermentation. Bioresour Technol 102(10):6065–6072

Dragone G, Kerssemakers AA, Driessen JL, Yamakawa CK, Brumano LP, Mussatto SI (2020) Innovation and strategic orientations for the development of advanced biorefineries. Bioresour Technol 302:122847

Dutta T, Sahoo R, Sengupta R, Ray SS, Bhattacharjee A, Ghosh S (2008) Novel cellulases from an extremophilic filamentous fungi *Penicillium citrinum*: production and characterization. J Ind Microbiol Biotechnol 35(4):275–282

Ekperigin M (2007) Preliminary studies of cellulase production by *Acinetobacter anitratus* and *Branhamella* sp. Afr J Biotechnol 6(1)

Fang X, Yano S, Inoue H, Sawayama S (2009) Strain improvement of *Acremonium cellulolyticus* for cellulase production by mutation. J Biosci Bioeng 107(3):256–261

Garlapati VK, Chandel AK, Kumar SJ, Sharma S, Sevda S, Ingle AP, Pant D (2020) Circular economy aspects of lignin: towards a lignocellulose biorefinery. Renew Sustain Energy Rev 130:109977

George SP, Ahmad A, Rao MB (2001) Studies on carboxymethyl cellulase produced by an alkalothermophilic actinomycete. Bioresour Technol 77(2):171–175

Gomes D, Domingues L, Gama M (2016) Valorizing recycled paper sludge by a bioethanol production process with cellulase recycling. Bioresour Technol 216:637–644

Grewal J, Khare S (2018) One-pot bioprocess for lactic acid production from lignocellulosic agro-wastes by using ionic liquid stable Lactobacillus brevis. Bioresour Technol 251:268–273

Grigorevski-Lima AL, de Oliveira MMQ, do Nascimento RP, da Silva Bon EP, Coelho RRR (2013) Production and partial characterization of cellulases and xylanases from *Trichoderma atroviride* 676 using lignocellulosic residual biomass. Appl Biochem Biotechnol 169(4):1373–1385

Guo Z-P, Robin J, Duquesne S, O'Donohue MJ, Marty A, Bordes F (2018) Developing cellulolytic *Yarrowia lipolytica* as a platform for the production of valuable products in consolidated bioprocessing of cellulose. Biotechnol Biofuels 11(1):1–15

Han J, Wang L, Wang Y, Dong J, Tang X, Ni L, Wang L (2018) Preparation and characterization of Fe_3O_4-NH_2@4-arm-PEGNH_2, a novel magnetic four-arm polymer-nanoparticle composite for cellulase immobilization. Biochem Eng J 130:90–98

Hamdi C, Arous F, Jaouani A (2020) Actinobacteria: a promising source of enzymes involved in lignocellulosic biomass conversion. Adv Biotechnol Microbiol 13

Heng KS, Hatti-Kaul R, Adam F, Fukui T, Sudesh K (2017) Conversion of rice husks to polyhydroxyalkanoates (PHA) via a three-step process: optimized alkaline pretreatment, enzymatic hydrolysis, and biosynthesis by *Burkholderia cepacia* USM (JCM 15050). J Chem Technol Biotechnol 92(1):100–108

Hong Y, Nizami AS, Pour Bafrani M, Saville BA, MacLean HL (2013) Impact of cellulase production on environmental and financial metrics for lignocellulosic ethanol. Biofuels Bioprod Biorefin 7(3):303–313

Hou W, Bao J (2018) Simultaneous saccharification and aerobic fermentation of high titer cellulosic citric acid by filamentous fungus *Aspergillus niger*. Bioresour Technol 253:72–78

Hu Y, Du C, Leu S-Y, Jing H, Li X, Lin CSK (2018) Valorisation of textile waste by fungal solid state fermentation: an example of circular waste-based biorefinery. Resour Conserv Recycl 129:27–35

Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A, Schoen P, Lukas J, Olthof B, Worley M (2011) Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: dilute-acid pretreatment and enzymatic hydrolysis of corn stover. National Renewable Energy Lab. (NREL), Golden, CO

Husain Q (2016) Magnetic nanoparticles as a tool for the immobilization/stabilization of hydrolases and their applications: an overview. Biointerface Res Appl Chem 6(6)

Ingle AP, Rathod J, Pandit R, da Silva SS, Rai M (2017) Comparative evaluation of free and immobilized cellulase for enzymatic hydrolysis of lignocellulosic biomass for sustainable bioethanol production. Cellulose 24(12):5529–5540

Irfan M, Safdar A, Syed Q, Nadeem M (2012) Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity. Turkish J Biochem/Turk Biyokimya Dergisi 37(3)

Israni N, Shivakumar S (2020) Polyhydroxyalkanoate (PHA) biosynthesis from directly valorized ragi husk and sesame oil cake by *Bacillus megaterium* strain Ti3: satistical optimization and characterization. Int J Biol Macromol 148:20–30

Jaiswal AK, Kumar V, Khakalo A, Lahtinen P, Solin K, Pere J, Toivakka M (2021) Rheological behavior of high consistency enzymatically fibrillated cellulose suspensions. Cellulose 28(4):2087–2104

Jatinder K, Chadha BS, Saini HS (2006) Optimization of medium components for production of cellulases by *Melanocarpus* sp. MTCC 3922 under solid-state fermentation. World J Microbiol Biotechnol 22(1):15–22

Jayasekara S, Ratnayake R (2019) Microbial cellulases: an overview and applications. Cellulose 22

Jiang M, Xu R, Xi YL, Zhang JH, Dai WY, Wan YJ, Chen KQ, Wei P (2013) Succinic acid production from cellobiose by *Actinobacillus succinogenes*. Bioresour Technol 135:469–474

Kanmani R, Vijayabaskar P, Jayalakshmi S (2011) Saccharification of banana-agro waste and clarification of apple juice by cellulase enzyme produced from *Bacillus pumilis*. World Appl Sci J 12(11):2120–2128

Karim Z, Afrin S, Husain Q, Danish R (2017) Necessity of enzymatic hydrolysis for production and functionalization of nanocelluloses. Crit Rev Biotechnol 37(3):355–370

Karnaouri A, Matsakas L, Krikigianni E, Rova U, Christakopoulos P (2019) Valorization of waste forest biomass toward the production of cello-oligosaccharides with potential prebiotic activity by utilizing customized enzyme cocktails. Biotechnol Biofuels 12(1):1–19

Karthik T, Rathinamoorthy R (2017) Sustainable synthetic fibre production. In: Sustainable fibres and textiles. Elsevier, pp 191–240

Kazi FK, Fortman J, Anex R, Kothandaraman G, Hsu D, Aden A, Dutta A (2010) Techno-economic analysis of biochemical scenarios for production of cellulosic ethanol. National Renewable Energy Lab. (NREL), Golden, CO

Khan MN, Luna IZ, Islam MM, Sharmeen S, Salem KS, Rashid TU, Zaman A, Haque P, Rahman MM (2016) Cellulase in waste management applications. In: New and future developments in microbial biotechnology and bioengineering. Elsevier, pp 237–256

Kovács K, Megyeri L, Szakacs G, Kubicek CP, Galbe M, Zacchi G (2008) *Trichoderma atroviride* mutants with enhanced production of cellulase and β-glucosidase on pretreated willow. Enzym Microb Technol 43(1):48–55

Kuhad RC, Deswal D, Sharma S, Bhattacharya A, Jain KK, Kaur A, Pletschke BI, Singh A, Karp M (2016) Revisiting cellulase production and redefining current strategies based on major challenges. Renew Sustain Energy Rev 55:249–272

Kumar B, Verma P (2021a) Biomass-based biorefineries: an important architype towards a circular economy. Fuel 288:119622

Kumar B, Verma P (2021b) Techno-economic assessment of biomass-based integrated biorefinery for energy and value-added product. In: Verma P (ed) Biorefineries: a step towards renewable and clean energy. Clean energy production technologies. Springer, Singapore, pp 581–561

Kumar B, Verma P (2021c) Life cycle assessment: blazing a trail for bioresources management. Energy Convers Manage: X 10:100063

Kumar B, Bhardwaj N, Alam A, Agrawal K, Prasad H, Verma P (2018) Production, purification and characterization of an acid/alkali and thermo tolerant cellulase from *Schizophyllum commune* NAIMCC-F-03379 and its application in hydrolysis of lignocellulosic wastes. AMB Express 8(1):1–16

Kumar RP, Gnansounou E, Raman JK, Baskar G (2019) Refining biomass residues for sustainable energy and bioproducts: technology, advances, life cycle assessment, and economics. Academic Press

Kumar B, Bhardwaj N, Agrawal K, Chaturvedi V, Verma P (2020a) Current perspective on pretreatment technologies using lignocellulosic biomass: an emerging biorefinery concept. Fuel Process Technol 199:106244

Kumar B, Bhardwaj N, Agrawal K, Verma P (2020b) Bioethanol production: generation-based comparative status measurements. In: Srivastava N, Srivastava M, Mishra P, Gupta V (eds) Biofuel production technologies: critical analysis for sustainability. Clean energy production technologies. Springer, Singapore, pp 155–201

Lai TT, Pham TTH, Adjallé K, Montplaisir D, Brouillette F, Barnabé S (2017) Production of *Trichoderma reesei* RUT C-30 lignocellulolytic enzymes using paper sludge as fermentation substrate: an approach for on-site manufacturing of enzymes for biorefineries. Waste Biomass Valorization 8(4):1081–1088

Latifian M, Hamidi-Esfahani Z, Barzegar M (2007) Evaluation of culture conditions for cellulase production by two *Trichoderma reesei* mutants under solid-state fermentation conditions. Bioresour Technol 98(18):3634–3637

Li C, Yang X, Gao S, Chuh AH, Lin CSK (2018) Hydrolysis of fruit and vegetable waste for efficient succinic acid production with engineered *Yarrowia lipolytica*. J Clean Prod 179:151–159

Li C, Lin X, Ling X, Li S, Fang H (2021) Consolidated bioprocessing of lignocellulose for production of glucaric acid by an artificial microbial consortium. Biotechnol Biofuels 14(1): 1–16

Libardi N, Soccol CR, de Carvalho JC, de Souza Vandenberghe LP (2019) Simultaneous cellulase production using domestic wastewater and bioprocess effluent treatment—a biorefinery approach. Bioresour Technol 276:42–50

Liu S, Duncan S, Qureshi N, Rich J (2018) Fermentative production of butyric acid from paper mill sludge hydrolysates using *Clostridium tyrobutyricum* NRRL B-67062/RPT 4213. Biocatal Agric Biotechnol 14:48–51

Liu L, You Y, Deng H, Guo Y, Meng Y (2019) Promoting hydrolysis of apple pomace by pectinase and cellulase to produce microbial oils using engineered *Yarrowia lipolytica*. Biomass Bioenergy 126:62–69

Llimós J, Martínez-Avila O, Marti E, Corchado-Lopo C, Llenas L, Gea T, Ponsá S, Biorefinery. (2020) Brewer's spent grain biotransformation to produce lignocellulolytic enzymes and polyhydroxyalkanoates in a two-stage valorization scheme. Biomass Convers:1–12

López-Mondéjar R, Algora C, Baldrian P (2019) Lignocellulolytic systems of soil bacteria: a vast and diverse toolbox for biotechnological conversion processes. Biotechnoly Adv 37(6):107374

Lu Y, Wang Y, Xu G, Chu J, Zhuang Y, Zhang S (2010) Influence of high solid concentration on enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass. Appl Biochem Biotechnol 160(2):360–369

Ma X, Gao M, Wang N, Liu S, Wang Q, Sun X (2021) Lactic acid production from co-fermentation of food waste and spent mushroom substance with *Aspergillus niger* cellulase. Bioresour Technol 337:125365

Malgas S, Rose SH, van Zyl WH, Pletschke BI (2020) Enzymatic hydrolysis of softwood derived paper sludge by an in vitro recombinant cellulase cocktail for the production of fermentable sugars. Catalysts 10(7):775

Michelin M, Gomes DG, Romaní A, Polizeli MDL, Teixeira JA (2020) Nanocellulose production: exploring the enzymatic route and residues of pulp and paper industry. Molecules 25(15):3411

Mo H, Qiu J, Yang C, Zang L, Sakai E, Chen J (2020) Porous biochar/chitosan composites for high performance cellulase immobilization by glutaraldehyde. Enzyme Microbial Technol 138: 109561

Motaung TE, Linganiso LZ (2018) Critical review on agrowaste cellulose applications for biopolymers. Int J Plast Technol 22(2):185–216

Nargotra P, Sharma V, Bajaj BK (2019) Consolidated bioprocessing of surfactant-assisted ionic liquid-pretreated *Parthenium hysterophorus* L. biomass for bioethanol production. Bioresour Technol 289:121611

Nawaz MA, Karim A, Bibi Z, Rehman HU, Aman A, Hussain D, Ullah M, Qader SAU (2016) Maltase entrapment approach as an efficient alternative to increase the stability and recycling efficiency of free enzyme within agarose matrix. J Taiwan Inst Chem Eng 64:31–38

Ning P, Yang G, Hu L, Sun J, Shi L, Zhou Y, Wang Z, Yang J (2021) Recent advances in the valorization of plant biomass. Biotechnol Biofuels 14(1):1–22

Obruca S, Benesova P, Marsalek L, Marova I, quarterly, b.e. (2015) Use of lignocellulosic materials for PHA production. Chemical 29(2):135–144

Oliveira FM, Pinheiro IO, Souto-Maior AM, Martin C, Gonçalves AR, Rocha GJ (2013) Industrial-scale steam explosion pretreatment of sugarcane straw for enzymatic hydrolysis of cellulose for production of second generation ethanol and value-added products. Bioresour Technol 130: 168–173

Olofsson J, Barta Z, Börjesson P, Wallberg O (2017) Integrating enzyme fermentation in lignocellulosic ethanol production: life-cycle assessment and techno-economic analysis. Biotechnol Biofuels 10(1):1–14

Patsalou M, Samanides CG, Protopapa E, Stavrinou S, Vyrides I, Koutinas M (2019) A citrus peel waste biorefinery for ethanol and methane production. Molecules 24(13):2451

Rabelo SC, Maciel Filho R, Costa AC (2008) A comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production. In: Biotechnology for fuels and chemicals. Springer, pp 563–576

Rabinovich M, Melnick M, Bolobova AJB (2002) The structure and mechanism of action of cellulolytic enzymes. Biochemistry (Moscow) 67(8):850–871

Rajnish KN, Samuel MS, John A, Datta S, Narendhar C, Balaji R, Jose S, Selvarajan E (2021) Immobilization of cellulase enzymes on nano and micro-materials for breakdown of cellulose for biofuel production-a narrative review. Int J Biol Macromol 182:1793–1802

Ribeiro RS, Pohlmann BC, Calado V, Bojorge N, Pereira N Jr (2019) Production of nanocellulose by enzymatic hydrolysis: trends and challenges. Eng Life Sci 19(4):279–291

Ruales-Salcedo AV, Higuita JC, Fontalvo J (2020) Integration of a multi-enzyme system with a liquid membrane in Taylor flow regime for the production and in situ recovery of gluconic acid from cellulose. Chem Eng Proces-Process Intensification 157:108140

Russell A, Ekvall T, Baumann H (2005) Life cycle assessment introduction and overview. J Clean Prod 13(13–14):1207–1210

Safarik I, Pospiskova K, Baldikova E, Safarikova M (2016) Development of advanced biorefinery concepts using magnetically responsive materials. Biochem Eng J 116:17–26

Saha BC, Cotta MA (2008) Lime pretreatment, enzymatic saccharification and fermentation of rice hulls to ethanol. Biomass Bioenergy 32(10):971–977

Saldarriaga-Hernández S, Velasco-Ayala C, Flores PL-I, de Jesús Rostro-Alanis M, Parra-Saldivar-R, Iqbal HM, Carrillo-Nieves D (2020) Biotransformation of lignocellulosic biomass into industrially relevant products with the aid of fungi-derived lignocellulolytic enzymes. Int J Biol Macromol 161:1099–1116

Saliu BK, Sani A (2012) Bioethanol potentials of corn cob hydrolysed using cellulases of *Aspergillus niger* and *Penicillium decumbens*. Excli J 11:468

Salvi DA, Aita GM, Robert D, Bazan V (2010) Dilute ammonia pretreatment of sorghum and its effectiveness on enzyme hydrolysis and ethanol fermentation. Appl Biochem Biotechnol 161(1):67–74

Sarsaiya S, Jain A, Awasthi SK, Duan Y, Awasthi MK, Shi J (2019) Microbial dynamics for lignocellulosic waste bioconversion and its importance with modern circular economy, challenges and future perspectives. Bioresour Technol 291:121905

Scarlata CJ, Davis RE, Tao L, Tan EC, Biddy MJ (2015) Perspectives on process analysis for advanced biofuel production. In: Direct microbial conversion of biomass to advanced biofuels. Elsevier, pp 33–60

Sheldon RA (2020) Biocatalysis and biomass conversion: enabling a circular economy. Philos Trans Royal Soc A 378(2176):20190274

Sillu D, Agnihotri S (2019) Cellulase immobilization onto magnetic halloysite nanotubes: enhanced enzyme activity and stability with high cellulose saccharification. ACS Sustain Chem Eng 8 (2):900–913

Singh A, Bajar S, Devi A, Bishnoi NR (2021) Adding value to agro-industrial waste for cellulase and xylanase production via solid-state bioconversion. Biomass Convers Biorefin:1–10

Siqueira JGW, Rodrigues C, de Souza Vandenberghe LP, Woiciechowski AL, Soccol CR (2020) Current advances in on-site cellulase production and application on lignocellulosic biomass conversion to biofuels: a review. Biomass Bioenergy 132:105419

Soares LA, Braga JK, Motteran F, Sakamoto IK, Monteiro PAS, Seleghim P, Varesche MBA (2019) Bioconversion of sugarcane bagasse into value-added products by bioaugmentation of endogenous cellulolytic and fermentative communities. Waste Biomass Valorization 10(7): 1899–1912

Squinca P, Bilatto S, Badino AC, Farinas CS (2020) Nanocellulose production in future biorefineries: an integrated approach using tailor-made enzymes. ACS Sustain Chem Eng 8(5):2277–2286

Srivastava N, Srivastava M, Alhazmi A, Kausar T, Haque S, Singh R, Ramteke PW, Mishra PK, Tuohy M, Leitgeb M (2021) Technological advances for improving fungal cellulase production from fruit wastes for bioenergy application: a review. Environ Pollut 287, 117370

Subramanian K, Chopra SS, Cakin E, Li X, Lin CSK (2020) Environmental life cycle assessment of textile bio-recycling–valorizing cotton-polyester textile waste to pet fiber and glucose syrup. Resour Conserv Recycl 161:104989

Sukumaran RK, Singhania RR, Mathew GM, Pandey A (2009) Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. Renew Energy 34(2):421–424

Sun FF, Hong J, Hu J, Saddler JN, Fang X, Zhang Z, Shen S (2015) Accessory enzymes influence cellulase hydrolysis of the model substrate and the realistic lignocellulosic biomass. Enzyme Microbial Technol 79:42–48

Teo HL, Wahab RA (2020) Towards an eco-friendly deconstruction of agro-industrial biomass and preparation of renewable cellulose nanomaterials: a review. Int J Biol Macromol 161:1414–1430

Van Zyl WH (1985) A study of the cellulases produced by three mesophilic actinomycetes grown on bagasse as substrate. Biotechnol Bioeng 27(9):1367–1373

Vermelho AB, Supuran CT, Guisan JM (2012) Microbial enzyme: applications in industry and in bioremediation. Enzyme Res 2012

Victoria J, Odaneth A, Lali A (2017) Importance of cellulase cocktails favoring hydrolysis of cellulose. Prep Biochem Biotechnol 47(6):547–553

Vu VH, Pham TA, Kim K (2011) Improvement of fungal cellulase production by mutation and optimization of solid state fermentation. Mycobiology 39(1):20–25

Wang Y, Chen GQ (2017) Polyhydroxyalkanoates: sustainability, production, and industrialization. In: Sustainable polymers from biomass. Wiley VCH, Weinheim, Germany, p 14

Xia L, Cen P (1999) Cellulase production by solid state fermentation on lignocellulosic waste from the xylose industry. Process Biochem 34(9):909–912

Xin F, Geng A (2010) Horticultural waste as the substrate for cellulase and hemicellulase production by Trichoderma reesei under solid-state fermentation. Appl Biochem Biotechnol 162(1):295–306

Xin F, Geng A, Chen ML, Gum MJM (2010) Enzymatic hydrolysis of sodium dodecyl sulphate (SDS)—pretreated newspaper for cellulosic ethanol production by *Saccharomyces cerevisiae* and *Pichia stipitis*. Appl Biochem Biotechnol 162(4):1052–1064

Yadav N, Nain L, Khare SK (2021) One-pot production of lactic acid from rice straw pretreated with ionic liquid. Bioresour Technol 323:124563

Yarbrough JM, Zhang R, Mittal A, Vander Wall T, Bomble YJ, Decker SR, Himmel ME, Ciesielski PN (2017) Multifunctional cellulolytic enzymes outperform processive fungal cellulases for coproduction of nanocellulose and biofuels. Acs Nano 11(3):3101–3109

Yu X, Zhang Z, Li J, Su Y, Gao M, Jin T, Chen G (2021) Co-immobilization of multi-enzyme on reversibly soluble polymers in cascade catalysis for the one-pot conversion of gluconic acid from corn straw. Bioresour Technol 321:124509

Zaki M, Khalil HA, Sabaruddin F, Bairwan R, Oyekanmi AA, Alfatah T, Danish M, Mistar E, Abdullah C (2021) Microbial treatment for nanocellulose extraction from marine algae and its applications as sustainable functional material. Bioresour Technol Rep 100811

Zeng J, Liu L, Li J, Dong J, Cheng Z (2020) Properties of cellulose nanofibril produced from wet ball milling after enzymatic treatment vs. mechanical grinding of bleached softwood kraft fibers. BioResources 15(2):3809–3820

Zhang X-Z, Zhang Y-HP (2013) Cellulases: characteristics, sources, production, and applications. Bioprocess Technol Biorefin Sustain Prod Fuels, Chem, Polym 1:131–146

Zhang Y, Chen J, Zhang L, Zhan P, Liu N, Wu Z (2020) Preparation of nanocellulose from steam exploded poplar wood by enzymolysis assisted sonication. Mater Res Express 7(3):035010

Zhao C, Zou Z, Li J, Jia H, Liesche J, Chen S, Fang H (2018) Efficient bioethanol production from sodium hydroxide pretreated corn stover and rice straw in the context of on-site cellulase production. Renew Energy 118:14–24

Zhou X, Xu Y (2019) Integrative process for sugarcane bagasse biorefinery to co-produce xylooligosaccharides and gluconic acid. Bioresour Technol 282:81–87