Chapter 4
The Pioneering Role of Enzymes in the Valorization of Waste: An Insight into the Mechanism of Action

Anupama Binoy, Revathy Sahadevan, Suchi Chaturvedi, and Sushabhan Sadhukhan

Abstract Finite petro-based reserves and a surge in environmental pollution demands the valorization of waste into revenue streams like biofuels and other industrial commodities. Enzymatic technology provides an eco-friendly platform for the same with higher product yields. Enzymes act as a catalyst in the reaction, and the matter of value addition in this technology is its requirement in low quantity and reusability. They have been included in the valorization of lignocellulosic (woody, agro, and food) waste, treatment of wastewater, and degradation of non-biodegradable hazardous waste. Microbial flora has enormously experimented as well as explored in the conversion of this waste into valuable products. In addition to that, protein engineering and metabolic engineering have been seen as new biotechnological trends in the same field. In this chapter, we will focus on different classes of hydrolytic enzymes based on the structural composition of different types of biomass with special attention to their catalytic activity. The mechanistic action of these enzymes will also be discussed in lieu of their use at various stages in the

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transformation of waste to value-added substances. We will also shed light on the future advancement through the biotechnological revolution in the field of enzyme technology.

Keywords Hydrolytic enzymes · Valorization of waste · Lignocellulosic waste · Wastewater · Biorefinery

Abbreviations

AAO	Aryl alcohol oxidase
BOD	Biological oxygen demand
CAZy	Carbohydrate-active enzyme
CLEAs	Cross-linked enzyme aggregates
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
DyP	Dye-degrading peroxidases
EG	Ethylene glycol
FAD	Flavin Adenine Dinucleotide
GH	Glycoside hydrolases
GHG	Greenhouse gas emission
GLOX	Glyoxal oxidase
GOS	Galacto-oligosaccharides
LiP	Lignin peroxidase
MnP	Manganese peroxidase
MSW	Municipality solid waste
P2O	Pyranose 2-oxidase
PAH	Polycyclic aromatic hydrocarbons
PCR	Polymerase chain reaction
PE	Pectin esterase
PE	Polyethylene
PET	Poly ethylene terephthalate
PG	Polygalacturonase
PGL	Polygalacturonate
PL	Pectin lyase
PMG	Polymethygalacturonase
PMSF	Phenyl methane sulfonyl fluoride
POPs	Persistent environment pollutants
PP	Polypropylene
PS	Polystyrene
PUs	Polyurethane
PVC	Polyvinyl chloride
RNA	Ribonucleic acid
TPA	Terephthalate
VP	Versatile peroxidases;
XYNII	Endo-1,4-ß-xylanase II

4.1 Introduction

The global surge in population and simultaneous automation have resulted not only in depletion of petro fuels reserve but have also piled up different forms of waste globally. In the global waste management outlook, GWMO 2015 stated that the global waste piling accounts for nearly 7–10 billion tonnes which include households, commercial, industrial, and construction-based waste (Wilson and Velis 2015; Agrawal and Verma 2022). Moreover, the major issues in front of humankind have been climate change, greenhouse gas emissions (GHG), depleting resources, and increasing pollution for a long. Waste management has existed in our society for a long in the form of landfills, incineration, composting, etc. but they are least suitable for organic waste due to problems associated with them like the generation of toxic gases such as methane and toxic leachates to the underground water bodies, etc. This calls upon the urgency to frame an economy based on renewable resources fulfilling the shooting energy demands (Kuo 2019; Goswami et al. 2022; Agrawal and Verma 2021) and also builds up sustainable methods to convert our waste into valuable revenue streams. Adapting to a sustainable way can be envisioned by valorizing waste into biofuels (different forms of bioenergy to replace fossil fuels), biomaterials, and other value-added bio-ingredients. This process is commonly associated with the concept of biorefineries where the waste biomass is upgraded and transformed into a spectrum of invaluable and marketable commodities. Waste can be of different forms. The most common and important problem faced during the valorization of waste is the complexity of the composition of waste. Organic or biomass waste is solid or liquid waste that can be found in many forms like agro and food waste, forestry residues, waste generated from food processing industries, etc. The basic units of the biomass waste are rich in protein, sugar, and fat, which indeed make them an ideal feedstock for enzymatic valorization. Enzymes are introduced at various steps in the process of valorization. For instance, the lignocellulosic mass from the agro-waste is subjected to pretreatment by exposing them to delignifying enzymes which removes the protective lignin component. Thus, enabling the hydrolyzing enzymes to easily solubilize the polymers like cellulose, and hemicellulose into their monosaccharides and oligosaccharides. These monomer units are either subjected to fermentation using microbes for the production of biofuels or enzymatic modification like oxidation/phosphorylation for valorizing into valued products (Andler and Goddard 2018; Bhardwaj and Verma 2021). Enzymes are molecular catalysts triggering biochemical reactions. They catalyze the reaction with high substrate and product selectivity at optimum temperature and pressure. Reusing enzymes for several reactions further makes the procedure cost-effective. All this opens up an opportunity for introducing greener processing strategies that are more sustainable for the ecosystem (Kennedy et al. 2006). Enzymes are ubiquitous in all forms of life performing different purposes and therefore can be extracted from them through purification and characterization (Yada 2015; Bhardwaj et al. 2021a; Kumar and Verma 2020a). Systems biology has encompassed a role in understanding the molecular basis of different enzymes. Through various bioinformatics tools and

algorithms, system biology enables us to form a link between different biological components in an organism thereby allowing us to modify and decipher newer enzyme candidates involved in waste valorization as well (Bhatt et al. 2019). Here in this chapter, we will be focusing on different enzymes which participate in hydrolysis of biomass component. As enzymes are very specific to the composition of its substrate, we will also shed light on the composition of different types of biomasses. To better understand the action of hydrolyzing enzymes and their application, it is necessary to take a glimpse at the different characteristics of enzymes and the different factors which affect the activity of enzymes. This chapter will also highlight the advanced enzyme technology and trending opinion on increasing the efficacy of enzymes for valorization of waste.

4.2 Enzymes and Their Characteristics

Enzymes are biological catalysts known to increase the rate of biochemical processes happening inside a living organism (Blanco and Blanco 2017). They were first introduced by Frederick W. Kuhne as molecules of higher molecular weight that help in the fermentation of sugar to alcohol. Enzymes are mostly composed of proteins except for some RNA which exhibit enzymatic characteristics in certain biological processes. Enzymes are associated with cofactors (inorganic metal ions like Cu^{2+}, Zn^{2+}, Mn^{2+}) and/or coenzymes (organic or organometallic complexes) for their activation and function (Renneberg et al. 2017). An enzyme without the cofactor or coenzyme is known as an apoenzyme and the complete active form is referred to as the holoenzyme (Litwack 2018). The accurate structural conformation of enzymes plays a crucial role in determining their activity and any parameters, physical or chemical, that alter the native confirmation will affect the efficiency of their catalytic activity. Based on the type of catalytic reactions, enzymes are categorized into six groups. *Oxidoreductases* catalyze oxidations or reduction reactions. *Transferases* help in the transferring of functional groups between two molecules. *Hydrolases* catalyze hydrolysis (lysis in the presence of water). *Lyases* are involved in the removal or addition of groups to form or reduce double bonds. *Isomerases* catalyze the internal arrangement of atoms in a molecule to form isomers and finally, *lipases* catalyze condensation reactions to form bonds between carbon–sulfur, carbon–carbon, or carbon–nitrogen (Blanco and Blanco 2017; Kumar and Verma 2020b).

4.2.1 Enzyme–Substrate Interaction

An enzyme facilitates a suitable environment for the substrates to form products at an enhanced rate. The substrate undergoes non-covalent interaction with the enzyme in a specific site called the active or substrate-binding site of the enzyme to form a

substrate–enzyme complex. This enzyme–substrate complex later transforms into a product and releases the enzyme back. The catalytic action of enzymes is favored at optimal pH, temperature and ionic strength, etc. and any alterations will affect the catalytic activity of the enzymes. For example, a temperature rise will cause an alteration in the enzyme's native conformation (denaturation) which will reduce their catalytic activity. Small molecules can also affect the activity of enzymes and are known as inhibitors. The important difference between competitive and noncompetitive inhibitors is their preference for the binding site on enzymes. Competitive inhibitors compete for the active site on the enzyme whereas noncompetitive inhibitors engage with sites other than that of the active site. Both the inhibitors eventually bring conformational changes in the structure of the enzyme and facilitate inhibition of its activity. The active site of an enzyme has a unique and specific sequence of amino acids which in turns increases substrate specificity and selectivity. Enzyme specificity is first explained by the "Lock and Key model" introduced by Emil Fischer in 1894. The assumption of the rigid structure of enzymes was falsified later in 1958 through the "Induced Fit Model" proposed by Daniel Koshland where the concept of the transition state of the enzyme–substrate complex was introduced. According to this model, the conformation of the enzyme's active site as well as in some cases conformation of the substrate itself will undergo small changes during the formation of the enzyme–substrate complex (Blanco and Blanco 2017).

4.2.2 *Enzyme Thermodynamics and Kinetics*

An enzyme, just like any catalyst increases the reaction rate by lowering the activation energy of the reaction (Fig. 4.1).

The kinetics of the enzyme–substrate is explained by Leonor Michaelis and Maud Menten in 1913. Michaelis-Menten theory is based on the following reaction between enzyme (E) and substrate (S) to yield the product (P) through the formation of the enzyme–substrate complex (ES).

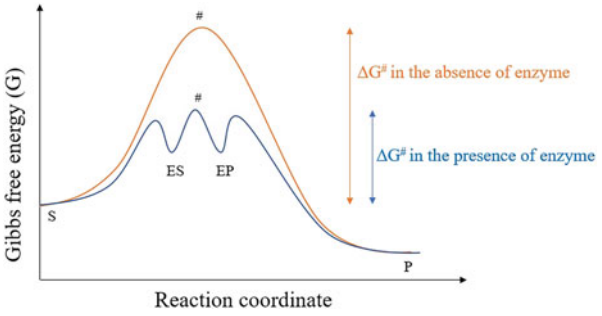

Fig. 4.1 Energy profile diagram of an enzyme-catalyzed and uncatalyzed reaction. Where S represents a substrate, ES is the enzyme–substrate complex, EP is the complex of enzyme and product just before their degradation to product, P, and # represents the transition state

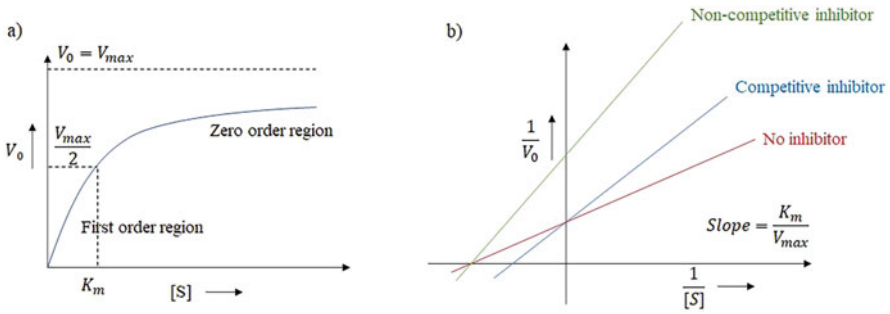

Fig. 4.2 The graphical representation of (**a**) Michaelis-Menten equation; (**b**) Lineweaver-Burk plot in the absence of inhibitor and the presence of competitive and non-competitive inhibitors

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\mathrm{ES}] \xrightarrow{k_2} \mathrm{P} + \mathrm{E} \tag{4.1}$$

Michaelis and Menten derived an equation for the rate of the catalytic action of enzymes based on the assumption that the formation of ES and the reverse reaction to E and S is faster than its conversion to P and E and then applied the steady-state approximation for ES complex. The Michaelis-Menten equation is given as,

$$V_0 = \frac{V_m[S]}{[S] + K_m} \tag{4.2}$$

where V_0 is the rate at which ES converts into a product, V_m is the maximum reaction rate at the saturated concentration of the substrate, [S] is the concentration of substrate, and K_m is Michaelis constant, it is the concentration of the substrate needed to acquire $V_m/2$. The graphical representation of this equation, V_o *vs* [S] plot is given in Fig 4.2a. This equation is applicable to single enzyme–substrate interaction at constant enzyme concentration. At a low concentration of substrate, the enzymatic reaction follows first-order kinetics where the rate of reaction is proportional to substrate concentration. At a high concentration of substrate, the reaction follows zero-order kinetics, which means the rate of reaction is independent of substrate concentration.

Enzyme activity in the presence of inhibitors is well understood from the Lineweaver-Burk plot which is a graphical representation of the reciprocal of the Michaelis-Menten equation, as shown in Fig 4.2b.

$$\frac{1}{V_0} = \frac{1}{V_{\max}} + \left(\frac{K_m}{V_{\max}}\right)\frac{1}{[S]} \tag{4.3}$$

4.3 Chemical and Structural Factors Guiding Enzyme Hydrolysis

Biomass waste is biodegradable organic matter produced directly or indirectly by the energy utilized from the sun through the process of photosynthesis. The source of waste is very diverse and varied in terms of its composition. They are mainly composed of constituents like lignocellulosic biomass, starch, chitin, triglycerides, proteins, etc. (Tuck et al. 2012; Kumar and Verma 2020b). Due to the high fixed carbon content in them, these feedstocks have been in past and are even now used to generate energy and heat by burning them which could pose threat to the environment by the release of toxic air pollutants such as PAH (polycyclic aromatic hydrocarbons), particulate matters, POPs (persistent organic pollutant) (Sivertsen 2006). Policymakers have directed the industries to focus on the sustainable valorization of worldwide generated biomass so that such a resourceful matter is not just dumped as waste or burned into ashes. Currently, they are utilized as major feedstock in biorefineries for biofuels production, biogas, and biofertilizers.

4.3.1 Composition of Lignocellulosic Biomass

Lignocellulosic biomass is categorized based on the source of its generation. It could be organic matter from woody terrestrial forest residues, herbaceous residues from agriculture (corn cobs, sugarcane bagasse, rice and wheat husk, fruits and vegetable residues generated from fields and market), green waste from Municipality Solid Waste (MSW), animal and human sources, aquatic organic mass, and organic mass generated by anthropogenic ways as well. The sustainable utilization of biomass offers a huge advantage as they are widely available worldwide, reduces the overall cost of fuels by introducing them as an alternative source, and finally, contributes to the reduction of greenhouse gas emissions. Moreover, the overall production and process cycle of sustainable alternatives exhibit a zero-carbon dioxide balance. Lignocellulosic biomass is structurally composed of cellulose, hemicellulose, protein, lipids, etc. They also contain some active ingredients like antioxidants, polyphenols, lignin, pigments/carotenoids, etc. These constituents are arranged in layers to form the complete lignocellulosic biomass structure with lignin being the outermost layer, hemicellulose occupying the middle space, and cellulose placed at the core of the mass (Fig. 4.3).

Interestingly, cellulose is the major substrate in the biorefineries for biofuels and chemical commodities production. The percentage of these components varies based on the type and source of lignocellulosic biomass. Almost 15% of the total lignocellulosic mass comprises protein also. The lignin component renders a high resistance to cellulose access by the hydrolytic enzymes. Various pretreatment methods have been adopted to remove the lignin part to give more access to hydrolytic enzymes to degrade the high polysaccharide component of cellulose and

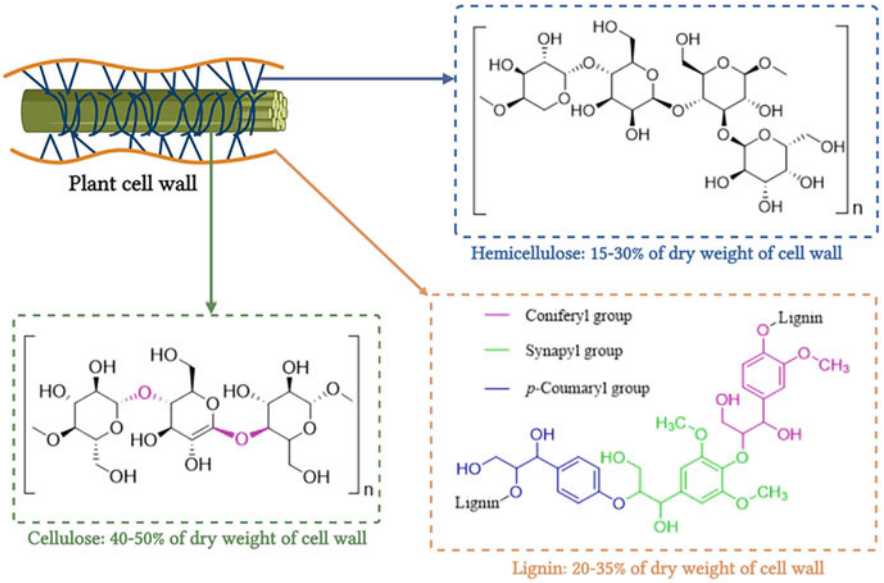

Fig. 4.3 Structural representation of lignocellulosic biomass: The plant cell wall is composed of a lignocellulosic structure where lignin forms the outermost covering, followed by hemicellulose and the most internal part is made up of cellulose components

hemicellulose. Depending upon the chemistry of the components present in the lignocellulosic biomass, enzymes are selected to degrade it.

4.3.1.1 Cellulose

Cellulose is an important cell wall component of the plant. It is a high molecular weight polysaccharide composed of D-glucose units linked with each other through *β*-1,4-glycosidic bonds forming the basic repeating unit called cellobiose, 4-*O*-*β*-D-glucopyranosyl-D-glucopyranose (structural basis of cellulose) (Fig. 4.4a). A cellulose primary chain is composed of almost 500–1400 monomer units. These primary chains are further arranged in a parallel array to form the higher structural unit called microfibril. Several microfibril units form cellulose fibril, the higher structural unit (Robak and Balcerek 2018; Bhardwaj et al. 2021b). The extensive intramolecular (O_6–O_2 and O_3–O_5) and intermolecular (O_3–O_6) hydrogen bonds and Van der Waals forces in cellulose structures give its crystalline nature, high tensile strength, and recalcitrance to hydrolysis. The amorphous cellulose corresponds to regions where the above-mentioned molecular bonds are disrupted giving twists and torsions to the structure resulting in interspersed disordered regions in cellulose. The structural or crystalline region of cellulose is highly packed not allowing even a single water

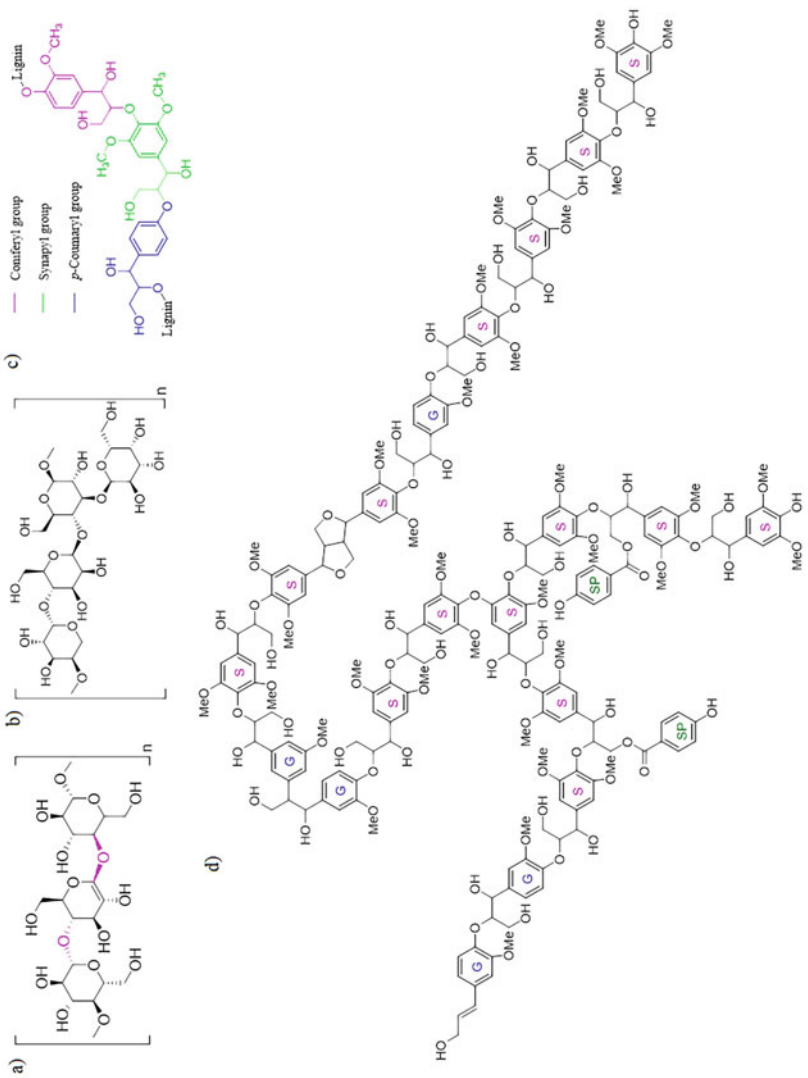

Fig. 4.4 Chemical structures of components present in lignocellulosic plant's cell wall: (**a**) basic skeletal structure of cellulose: Glucose units combined by *β*-(1,4)-glycosidic linkages; (**b**) basic skeletal structure of hemicellulose having *β*-(1,4)-xylose-*β*-(1,4)-agarose-*β*-(1,4)-mannose-*β*-(1,4)-glucose-*α*-(1,3)-galactose motifs; (**c**) basic skeletal structure of lignin component; and (**d**) Structure of lignin in poplar plant. Where S is the syringyl group, G is the guaiacyl, and SP is the synapyl basic groups

molecule to enter, but the distorted amorphous region is very easily accessible to enzyme hydrolysis (Betts 1991).

4.3.1.2 Hemicellulose

Hemicellulose stands second to cellulose in terms of its abundance on the planet. In comparison to cellulose, hemicellulose is highly branched with almost 500–3000 sugar units as monomers. Hemicellulose is a heteropolymer comprising of side chains with xylans as pentoses, mannans, and glucomannans as hexoses or arabinogalactans and galactans as galactose units. Apart from monosaccharides, they also accompany typical uronic acids like D-glucuronic, 4-*O*-methylglucuronic, and D-galacturonic acids (Fig. 4.4b).

Xylan: Xylans are water-soluble polysaccharides made up of repeating units of *β*-D-xylopyranose linked by *β*-(1,4)-glycosidic bonds. This primary chain also comprises other carbohydrates such as xylose, mannose, arabinose, rhamnose, or 4-*O*-methylglucuronic acid.

Mannan: Mannans are the most important constituent of hemicellulose which helps the hemicellulose to bind to the cellulose counterpart. They are widely found as a component in the angiosperm cell wall. *β*-D-mannopyranosyl units are formed by *β*-1,4 linkage along with a small ratio of galactans in the linear chains. There are four different types of mannans present: galactomannans, galacto-glucomannans, glucomannans, and linear-mannans.

Galactans: Galactans are made of galactose as repeating units linked through *α*-1, 3, and *β*-1,6 linkages to form 4-*α*-D-galactopyranosyl and 3-*β*-D-galactopyranosyl attached in alternate fashion. They are long polymeric chains not commonly found in all forms of plants. They are majorly found in some algae, seeds, buds, or flowers (Li et al. 2013).

4.3.1.3 Lignin

Unlike cellulose, lignin has an irregular three-dimensional structure with no specific repeating units. Lignin acts as the protective and cementing cover in plants which helps arrange the fibers together to enhance the compactness of the wood thereby making it more resistive. It helps in gluing hemicellulose with cellulose, and it resists the access of enzymes to cellulose by acting as a physical barrier. It is an amorphous organic compound comprising phenylpropanoid units with three different types of *p*-hydroxycinnamyl alcohol: coniferyl alcohol, sinapyl alcohol, *p*-coumaryl alcohol (Fig 4.4c, d). Overall lignification is species specific and is obtained by several cross-linking reactions between the radicals formed by oxidation and resonance delocalization in phenylpropanoid monomeric units. Lignin is synthesized in the plants via shikimic acid pathway. The structural integration of different cellulosic components makes it very recalcitrant to hydrolysis by enzymes. The cellulose and hemicellulose are attached through hydrogen bonds, meanwhile, lignin forms five different types of

lignin carbohydrate bonds to bind to hemicellulose: γ-esters esters, benzyl ethers, phenyl glycosides, ferulate/coumarate esters, and hemiacetal/acetal linkages (Giummarella et al. 2019; Agrawal and Verma 2020a, b). The biorefineries arena focus on the effective valorization of lignocellulosic materials into valuable products by introducing controlled cleavage of carbon–carbon and carbon–oxygen bonds present in the recalcitrant lignin on the outermost coat. The focus is also maintained on getting rid of various impurities (organic and inorganic).

4.3.2 Starch

Food is stored in plants in the form of starch in seeds, roots, and a little amount in the residual biomass. It is composed of two types of polysaccharides, namely amylose and amylopectin. Amylose is formed by polymerizing D-glucose via α-1,4 linkages linearly, on the other hand in amylopectin, which has branched formation is formed by α-1,4 glycosidic bonds linearly, and α-1,6 glycosidic linkages for branched chains (Whistler and Daniel 1984).

4.4 Enzymes and their Application in Waste

Despite the sustainable availability of biomass for conversion into bioenergy, the process is expensive and time-consuming as it requires lengthy downstream processing for the collection of final products. Before subjecting the different components of waste to hydrolysis, pretreatment of the complex material is required which is the most expensive process in the transformation. It is done to remove the recalcitrant lignin component which remains the major hindrance in exposing the buried cellulose to the saccharification process. The pretreatment involves subjecting the lignocellulosic mass to either high pressure, temperature, or chemical treatment or enzymatic hydrolysis. The chemical pretreatment includes the use of chemicals like organic solvents, concentrated acids/bases, or neoteric solvents which are very harsh and corrosive. Additionally, the process becomes more tedious as many steps are required to separate the final products from the chemicals used in pretreatment. The highly concentrated and corrosive acids and alkali are damaging to the equipment too. There are a lot of unwanted products formed during the process of pretreatment with chemicals that can act as inhibitors to the microbial enzymes used for fermentation. Therefore, the use of hydrolytic enzymes proves to be useful as it poses less cost and also reduces the difficulties faced in the downstream process (Manisha and Yadav 2017). As discussed earlier, the most resourceful feedstock for the production of green fuel is biomass waste, and it has been in regular consideration and experimentation under the biorefinery concept (Azapagic 2014). To switch to industrial symbiosis, i.e., waste from one sector is used as feedstock in another

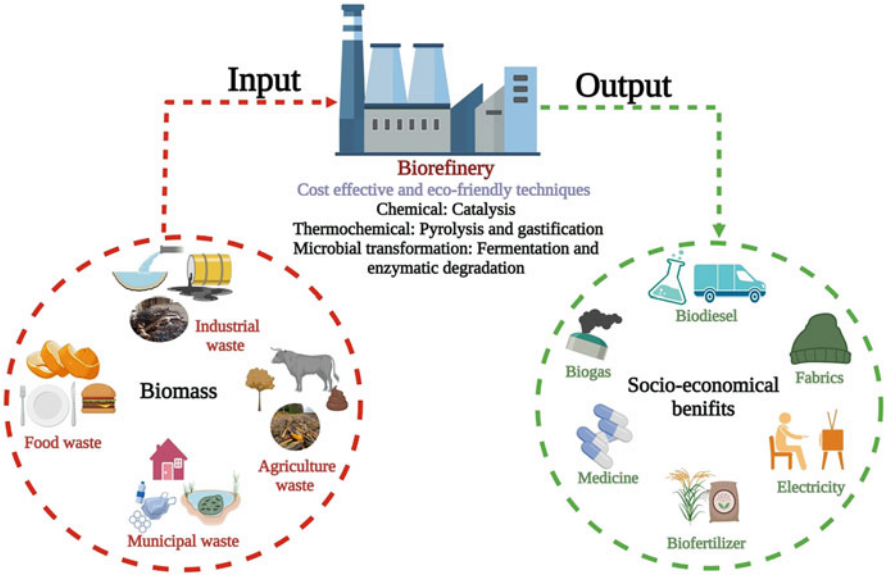

Fig. 4.5 A model of biorefinery concept: Biomass feedstocks from various sources are taken as raw material in biorefinery and converted into various forms of bioenergy and other value-added products

industry; therefore, it is very necessary to identify, characterize, and quantify the residues present in the waste stream (Fig. 4.5).

Microorganisms and their enzymes have long been core tools in the biofuel refineries, being present in all stages, starting from pretreatment, hydrolysis, and fermentation. They can be introduced as biocatalysts with the substrates where they are produced in situ by microorganisms or they are added ex situ in purified form or as enzyme cocktails. In this section, we will be considering the important enzymes, especially the hydrolytic type in saccharification of polymers into monomers and will also discuss the mechanism of their actions.

4.4.1 Hydrolytic Enzymes Involved in the Valorization of Lignocellulosic Waste

The main source of lignocellulosic biomass is the organic residues obtained from human activities such as agricultural waste and food processing industries. Moreover, solid municipal waste which mostly comprises paper and organics can be included as an important lignocellulosic waste stream for valorization into valuable products. The biopolymers included in lignocellulosic biomass constitute cellulose, hemicellulose, and lignin. The major enzymes required to saccharify these polymers

are cellulases, hemicellulases, and lignin-degrading enzymes. These enzymes are classified under the single-family of glycoside hydrolases (glycosidases or carbohydrases), E.C 3.2.1, and are involved in the catalysis of *O*-glycosidic bond hydrolysis (van Wyk et al. 2017; Bhardwaj et al. 2020). The gene corresponding to this class of enzyme is present in all living organisms except in some Archaeans and some unicellular parasites. Glycoside hydrolases cleave the glycosidic bonds via two different mechanisms based on the status of the anomeric configuration during the reaction. The net inversion of an anomeric configuration is achieved as a result of a one-step double displacement reaction between the acidic and basic amino acid groups whereas the retention of the anomeric configuration happens via a two-step double displacement reaction involving acid/base and nucleophilic assistance provided by amino acid residues (Naumoff 2011). All the information concerning genomic, structural, and functional aspects of glycoside hydrolases and their family members is available in a highly curated, knowledge-based database known as the carbohydrate-active enzyme (CAZy) database. This database states that glycoside hydrolases (GHs) are categorized into 135 different families and 14 clans. This classification was based on their overall structural confirmation, amino acid sequence, and function (Lombard et al. 2014).

4.4.1.1 Cellulases

Cellulase is a class of enzymes that hydrolyzes the β-1,4-glycosidic bonds in polysaccharides like cellulose to glucose units and is grouped among glycoside hydrolases (GH). Cellulose from fungus has two domains, namely a catalytic domain, which performs the catalytic activity, and a cellulose-binding domain, which anchors the enzyme to the cellulose substrate. Both the domains are linked together through a linker domain. The hydrolytic action depends upon the synergistic action of three major enzymes, cellobiohydrolase/exoglucanase (E.C 3.2.1.176)/(E.C 3.2.1.91), endocellulase/endoglucanase (E.C 3.2.1.4), and β-glucosidase (E.C 3.2.1.21) (Horn et al. 2012; Kostylev and Wilson 2014). Endocellulases hydrolyze the amorphous area of cellulose to release long-chain oligomers with non-reducing ends which are then acted upon by exocellulase or cellobiohydrolases on the β-1,4-glycosidic bonds liberating β-cellobiose. Cellobiohydrolases act on the oligomers from the reducing ends whereas exocellulase act on the non-reducing ends. β-glucosidase hydrolyzes the smaller glucans or disaccharide cellobiose into the monomeric glucose (Juturu and Wu 2014) (Fig. 4.6a).

Several microorganisms have been found to produce cellulose enzymes. Among bacteria the most important are *Clostridium* species, *Pseudomonas* species, and *Trichoderma reesei* whereas the major cellulose-producing fungi belong to *Aspergillus species*, *Fusarium species*, *Penicillium species*, *Schizophyllum commune*, and *Melanocarpus species.* In anaerobic bacteria, cellulose occurs as cellulosomes, an extracellular aggregated enzyme structure. Endocellulase or endoglucanase belonging to the family glycoside hydrolases (GH) 5 comprises a single catalytic subunit made up of 335 amino acids folding into the active enzyme. The structural

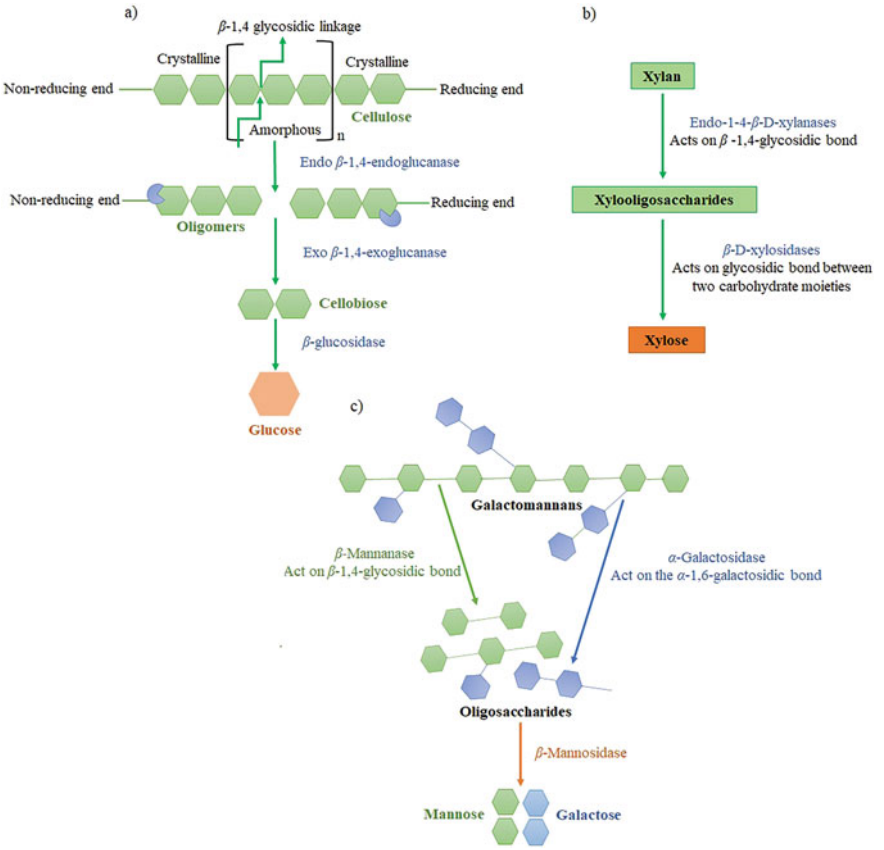

Fig. 4.6 Mechanism of action of cellulases and hemicellulases: (**a**) Endocellulase, exoglucanase, and β-glucosidase act in synergy for the degradation of cellulose. (**b**) The mechanism of action of endoxylanase and β-glucosidase on the degradation of xylan to xylose; and (**c**) synergistic action of β-mannanases, β-mannosidase, and α-galactosidases on the degradation of various units in mannan component of hemicellulose

architecture of endoglucanase has eight $(\beta/\alpha)_8$ barrel-shaped loops along with a short double-stranded anti-parallel β sheet and three single turns helices. The catalytic substrate-binding site has two glutamate amino acid residues at positions 133 (acid-base) and 240 (nucleophile) which are highly conserved and decisive in the first step of the reaction (Lo Leggio and Larsen 2002) (Fig. 4.7a). Cellobiohydrolases the exoglucanases belong to the family GH 7. The three-dimensional structure of cellobiohydrolases is made up of 431 amino acid residues and exhibits a β-jelly roll structure with two anti-parallel β-sheets to each β-jelly roll. Each β-sheet curves to form concave and convex shapes which are connected through four α-helices. Amino acid glutamine at positions 207 and 212 at the active site of the enzyme participates in the acid-base reaction mechanism (Fig. 4.7b) (Muñoz et al. 2001). β-

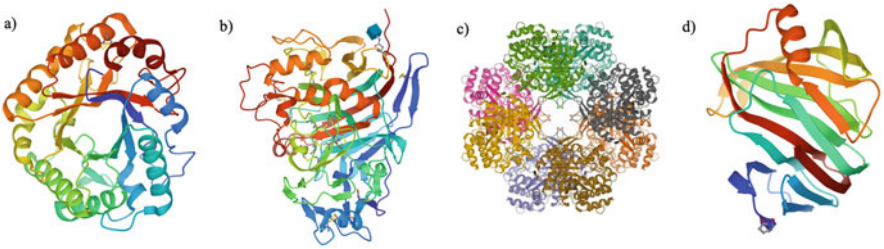

Fig. 4.7 Crystalographic structure of (**a**) endoglucanase enzyme obtained from *Thermoascus aurantiacus* (PDB I.D: 1GZJ) (Lo Leggio and Larsen 2002); (**b**) exoglucanase enzyme obtained from *Phanerochaete Chrysosporium* (PDB ID: 1GPI) (Muñoz et al. 2001); (**c**) *β*-glucosidase enzyme obtained from *Bacillus polymyxa* (PDB ID: 1BGA) (Sanz-Aparicio et al. 1998); (**d**) endo-1-4-β-D-xylanases enzyme obtained from *Trichoderma reesei* (PDB I.D: 1ENX (Törrönen et al. 1994)

glucosidase from the strain *Bacillus polymyxa* (BglA) exists in a tetramer of dimers arranged in an octameric confirmation. The enzymes form aggregates due to their intracellular localization. The substrate-binding site of the enzyme accommodates Glutamine at 166 and 352 which acts as acid/base and nucleophile in hydrolysis reaction, respectively. The substrate binding is also influenced by Histidine residue at 121 as well as tyrosine residue at 296 positions (Sanz-Aparicio et al. 1998) (Fig. 4.7c). Cellulases have been utilized widely in various industries like brewery distilleries, textile processing, paper pulp industries, detergent production, cattle feed processing, and recently been introduced in the production of biofuels.

4.4.1.2 Hemicellulases

This class of enzyme is also known as hemicellulose degrading enzymes. They are involved in the depolymerization of components present in the hemicellulose portion of lignocellulosic biomass such as galactans, xylans, mannans, and arabans. Mannanases, *α*-glucuronidases, and *α*-arabinofuranosidases are widely discussed as well as utilized enzymes.

Xylanases: Xylanase enzymes constitute two major enzymes which include endo-1-4-*β*-D-xylanases (EC 3.2.1.8) and *β*-D-xylosidases (E.C. 3.2.1.37). The endoxylanases hydrolyze xylan into xylooligosaccharides which are further acted upon by xylosidases to yield monomeric xylose (Fig. 4.6b). The structural analysis of endo-*β*-1,4-xylanase II (XYNII) from *Trichoderma reesei* reveals that the enzyme exists as a single domain with 190 amino acid residues folded into two anti-parallel *β*-sheets arranged parallel to each other (Fig. 4.7d). The active site cleft is formed by twisting the β-sheets, and it accommodates two glutamic acid residues at positions 86 and 177 (Törrönen et al. 1994). Some of the accessory enzymes like acetyl xylan esterase (E.C 3.1.1.72), *p*-coumaric esterase (3.1.1.B10), *α*-glucuronidases (E.C 3.2.1.139), *α*-l-arabinofuranosidases (E.C. 3.2.1.55), and ferulic acid esterase (E.C

3.1.1.73) are necessary to hydrolyze the remaining component or side chains of the hemicellulose structure such as glucuronic acid, galacturonic acid, arabinose, galactose, ferulic and coumaric acids (Bhardwaj et al. 2019; Gírio et al. 2010; Beg et al. 2001). The action of α-glucuronidase, α-l-arabinofuranosidases, and acetyl xylan esterase is to remove acetyl and phenolic side branches whereas *p*-coumaric esterase and ferulic acid esterase cleave the ester bonds in xylose. The synergistic enzyme activity of all the xylanases proves significant in opening up the xylan component of the lignocellulosic biomass (Moreira and Filho 2016). The presence of xylanases has been found in many organisms ranging from microorganisms like fungi, bacteria, and yeast to crustaceans, insects, and seeds (Beg et al. 2001). Microbial xylanases are preferred over animal sources. The most notified, experimented, and applied xylanases are from the organisms from the genus *Bacillus, Chaetomium, Nonomuraea, Arthrobacter, Clostridium, Thermomonospora, Dictyoglomus, Fusarium, Streptomyces, Aspergillus,* etc. (Bhardwaj et al. 2019; Sunna and Antranikian 1997).

Mannanases: These enzymes are involved in the depolymerization of mannans which are an integral part of the hemicellulose portion of the cell wall. 1,4-β-D-mannohydrolases or β-mannanases (E.C 3.2.1.78) are endo-acting mannanases that cleave the internal glycosidic bonds on the linear chains liberating short oligosaccharides like β-1,4-manno-oligosaccharides. On the other hand, β-1,4-D mannopyranoside hydrolases or β-mannosidases (E.C 3.2.1.25) are exo hydrolases that choose to act on the non-reducing ends of mannobiose to degrade it into individual mannose units. Lastly, β-1,4-D-glucoside glucohydrolases or β-glucosidases (E.C 3.2.1.21) act on the products liberated from the cleavage of glucomannan and galactoglucomannan specifically cleaving the β-1,4-glucopyranose units from the non-reducing terminal (Dhawan and Kaur 2007; Moreira and Filho 2008). α-galactosidases (E.C 3.2.1.22) and acetyl mannan esterases (E.C 3.1.1.6) are some of the accessory proteins which are required to excise the additional side chains or groups present occasionally on the mannans (Malgas et al. 2015) (Fig. 4.6c).

Mannanase is classified under different GH families (like GH 1–3, GH 5, 26, 27, 113, etc.). Their primary structure is different while they share common spatial arrangements. They all have a canonical $(\beta/\alpha)_8$-barrel protein fold in their active site and based on that they have been included in clan GH-A. The central active site cleft contains two glutamate residues at the C-terminal side (Dawood and Ma 2020). Mannanase is the second most important industrial enzyme after xylanases and has been explored in various industries like textile and paper industries, pharmaceuticals, food, feedstock industries, etc. The bacterial degraders for mannanases among Gram-positive bacteria are from *Bacillus* species, and *Clostridia* species, whereas from Gram-negative bacteria are from *Vibrio*, *Pseudomonas, Klebsiella,* and *Bacteroides.* Among fungal counterparts *Aspergillus, Agaricus, Trichoderma,* and *Sclerotium, Penicillium* species are mostly reported. Actinomycetes from *Streptomyces* species have also been shown to be mannan degraders (Dhawan and Kaur 2007; Chauhan et al. 2012).

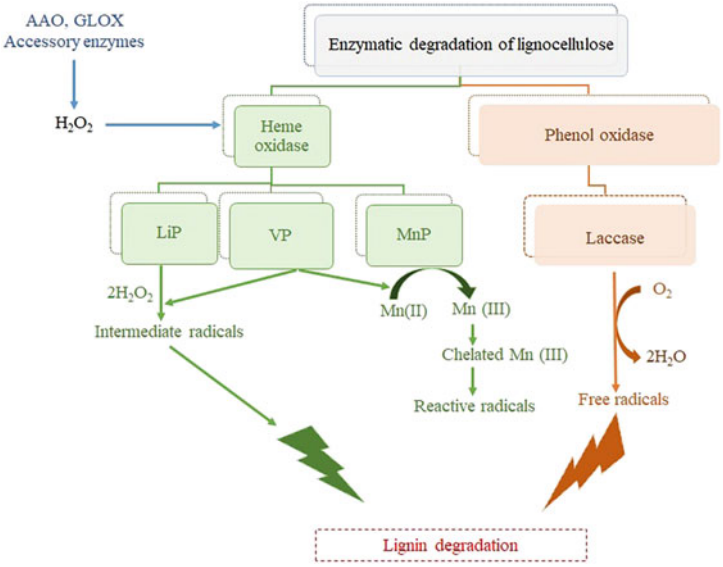

Fig. 4.8 Lignin hydrolysis by lignin-degrading enzymes: Diagram depicting the mechanism of action of different lignin-degrading enzymes for hydrolyzing lignin component

4.4.1.3 Lignin-Degrading Enzymes

They catalyze the conversion of lignin present in lignocellulosic biomass into small aromatic molecules. Lignin is a phenolic polymer containing phenylpropanoid aryl-C3 units links. These can be degraded by the synergistic action of two groups of enzymes, namely lignin-modifying and lignin-degrading auxiliary enzymes. The former includes laccase or phenol oxidases (E.C 1.10.3.2), lignin-modifying peroxidases such as lignin peroxidase (LiP) (E.C 1.11.1.14), versatile peroxidases (E.C 1.11.1.16), dye-degrading peroxidases (E.C 1.11.1.19), and manganese peroxidase (MnP) (E.C 1.11.1.13). All the peroxidases have a unique prosthetic group in the form of protoporphyrin IX (Pollegioni et al. 2015). Auxiliary enzymes in lignin degradation on the other hand include glucose oxidase (E.C 1.1.3.4), cellobiose dehydrogenase (E.C 1.1.99.18), glucose oxidase (E.C 1.1.3.4), aryl alcohol oxidase (E.C 1.1.3.7), pyranose 2-oxidase (E.C 1.1.3.10), and glyoxal oxidase (E.C 1.2.3.5) along with some other enzymes like alkyl aryl etherase, and aryl alcohol dehydrogenase (Bilal et al. 2019; Zhang et al. 2020a, b; Levasseur et al. 2008; Agrawal and Verma 2020a, b) (Fig. 4.8).

Basidiomycetes white-rot fungus is extensively investigated for the production of these auxiliary enzymes (Garcia-Ruiz et al. 2014). Hofrichter and Ullrich stated an action of a new enzyme heme-thiolate haloperoxidases, catalytically identical to other heme-containing oxidoreductases (cytochrome P450 monooxygenases and

catalases), as lignin degrading in cultures of *Ceriporiopsis subvermispora* (Hofrichter and Ullrich 2006).

Although the crystal structure and catalytic cycle of all the ligninolytic enzymes are well-reviewed (Chen et al. 2012; Pollegioni et al. 2015; Wong 2009; Janusz et al. 2017), we have attempted to briefly touch on some of the important aspects in understanding the mechanism of these enzymes.

Laccases: Laccases fall in the group of oxidoreductases that uses oxygen as an oxidizing agent. The four copper ions at the active center of laccases help the enzyme in oxidizing most of the phenolic and aromatic compounds present in the lignin (Mai et al. 2000; Pollegioni et al. 2015). Some metal ions and organometallic compounds are also been reported as the substrate of laccases (Garcia-Ruiz et al. 2014; Zimmerman et al. 2008). All these four copper ions at the T2/T3 site (the T1 Cu and the tri-nuclear Cu cluster (T2 Cu, T3α Cu, and T3β Cu)) have a different electro-paramagnetic resonance which is key to their unique reaction with the random polymeric nature of lignin. The fungal laccases are known to comprise ~520–550 amino acids residues with glycosylation as primary modifications. The three-dimensional structure of fungal laccases demonstrates three tightly arranged cupredoxin-like domains having β-barrel symmetry. The third domain holds the T1 Cu near the surface of the protein, and the T2 Cu, T3 (α and β) Cu are located at the junction of the first and the third domain (Mehra et al. 2018; Sitarz et al. 2016). Laccases are extracellularly, intracellularly as well as periplasmically produced depending on the type of microorganisms producing them. It is found mostly in fungal and bacterial cells.

Lignin Peroxides (LiP): LiPs are generally known to oxidize phenolic and non-phenolic organic compounds instead the enzyme specificity is relatively poor. The structural analysis of LiP isolated from *Phanerochaete chrysosporium* revealed its globular nature. The active site pocket is formed of two domains organized of eight α-helices (major and minor) with restricted β components enclosing a heme-chelating ferric ion (Choinowski et al. 1999). The three-dimensional structure of LiP is further stabilized by four disulfide linkages, two calcium ions, and two glycosylation-specific post-translational sites. Although their enzymatic mechanism is similar to other peroxidases in the same class, they stand effective catalytically due to their very high redox potential when it comes to oxidizing the recalcitrant lignin component (Sigoillot et al. 2012).

Manganese Peroxidases (MnP): MnP was isolated and studied initially from the fungi *Phanerochaete chrysosporium*. Supplementation of Mn ions and other organic compounds like 2-hydroxybutyrate, malonate, glycolate, or glucuronate in the growth medium stimulated the production of MnP in white-rot fungus (Mester and Field 2006). These molecules in particular stabilized the structure of the enzyme. A molecule of heme (iron protoporphyrin IX) is sandwiched between the two domains formed by α-helices very similar to that of the structure of LiP. Very close to the heme porphyrin lies the binding site of Mn^{2+} ion which constitutes one aspartate and two glutamate γ-carboxylic groups. Slightly different from the structure of LiP, MnP consists of five disulfide bridges and two Ca^{2+} ions. The active cycle of MnP varies

from that of LiP, in the oxidation of lignin compounds involving the conversion of $Mn^{2+}$to Mn^{3+}ions (Niladevi 2009).

Versatile Peroxidases (VPs): This enzyme represents a cocktail of LiPs and MnPs. It also constitutes a heme porphyrin group close to the catalytic site. The active center is made of 11–12 α-helixes with four disulfide bridges, two Mn (II) binding sites and two Ca(II) binding sites. This enzyme is capable of oxidizing methoxybenzenes and various non-phenolic lignin compounds with high affinity. The multi-step reaction mechanism of VPs is similar to other peroxidases.

Dye-decolorizing peroxidase (DyP): These enzymes are different from the classical lipid-modifying peroxidases like LiP, MnP, and VPs. They can decolorize a range of molecules including dyes, β-carotene, and aromatic sulfides (Zámocký et al. 2015). They were first investigated and isolated from the cultures of the fungus *B. adusta* (Fernandez-Fueyo et al. 2015). DyPs can oxidize non-specifically all peroxidase substrates and also possess hydrolase and oxidase activity. The structural analysis of DyPs demonstrates the presence of two domains. The catalytic site lies in the cavity of the two domains accommodating the heme cofactor (Colpa et al. 2014).

Glyoxal Oxidases (GLOX): Lignin components like methylglyoxal and glyoxal can be oxidized by the GLOX. These enzymes proceed with the oxidation of their substrate with the formation of extracellular hydrogen peroxide (Yamada et al. 2014). They are moreover required to regulate the peroxidase activity of the lignin-modifying peroxidases. The active center of GLOX is occupied by a copper ion which helps in the aldehyde oxidation of its substrate (Yin et al. 2015).

Aryl Alcohol Oxidase (AAO): This enzyme is a member glucose-methanol-choline oxidase/dehydrogenase family. Structurally, this enzyme is a monomer with two domains non-covalently bound with the FAD cofactor (Fernandez et al. 2009). AAO substrates include various aryl-alcohols (phenolic and non-phenolic), aliphatic (polyunsaturated) primary alcohols, or aromatic secondary alcohols present in the lignocellulose biomass. It also oxidizes the radical intermediates produced by laccase enzymes like guaiacol, sinapic acid, etc. (Mathieu et al. 2016). The oxidative dehydrogenation reaction of AAO is an NADP-dependent reaction and produces H_2O_2 (Ferreira et al. 2010).

Pyranose 2-Oxidase (P2O): These oxidoreductases are involved in the oxidation of aldopyranose compounds. It is produced periplasmically and transported in membrane-associated vesicles (De Koker et al. 2004; Prongjit et al. 2009). They are homotetrameric and have three major conserved regions, namely the binding site for FAD, the substrate-binding region, and the flavin attachment loop. The threonine hydroxyl of Thr169 present at the active site is very important for the oxidation of sugars and flavin molecules. The P2O catalyzes the oxidation of its substrate at the C2 position via a Ping-Pong type reaction mechanism at pH 7 (two half-reactions). The end products are the 2-keto-sugars and hydrogen peroxide. First, a hydride equivalent from the sugar substrate is donated to the protein-bound flavin with the generation of a reduced FAD (FADH) and the 2-keto-sugar, and secondly, a reduced flavin is oxidized by donating two electrons to O_2 thereby forming H_2O_2 (Pitsawong et al. 2010).

4.4.2 Hydrolytic Enzymes Involved in the Valorization of Food Waste

Food waste origin is marked starting from the agriculture sector, packaging, and retail, finally till household consumption. These waste streams generated are rich in biodegradable organic matter, which due to their peculiar chemical characteristics like high biological and chemical oxygen demand, content-rich in carbon and nitrogen, are deleterious to the environment when discarded in landfills and aquatic streams. They may cause aquatic pollution, harmful toxic leaching into ground and surface water, altered soil composition, etc. The food processing industries and so their waste is categorized based on the food material they process. Baiano et al. (2014) assessed and estimated the approximate percentage of waste generated by different food processing units or industries (Baiano 2014). According to them, the beverages manufacturing industry generates 26% of food waste, dairy industries make up 21%, fruit/vegetable, cereal edible oils manufacturing and processing as well as meat and fish product processing and preservation industries constitute (12.9%), (3.9%), (8.4%) of food waste, respectively. However, valorization of these compounds to revenue streams like biofuels, other food, and non-food commodities is possible by introducing enzymes catalysis at various steps of their conversions (Andler and Goddard 2018). For instance, esterification of different waste components like oil, sugar, starch, and even flavonoids help in enhancing their values through converting or valorizing them into value-added products like biodiesel, surfactants, biodegradable plastics, and this indirectly prevents their direct disposal to wastewater treatment facilities.

Pectinases: The industries processing vegetables and fruits are rich in crude dietary fibers, carbohydrates, polyphenols, flavonoids, triglycerides, or plant-based fatty acids, etc. The by-product of these industries can be widely valorized through enzymatic and also through other physico-chemical extraction into value-added revenue streams like novel pharmaceuticals, animal feeds, etc. (Mourtzinos and Goula 2019; Fierascu et al. 2020). For instance, soluble and insoluble dietary fibers from citrus fruit pulp have been investigated by a group of researchers to replace fat content in ice cream because of their high phenolic and carotenoid content and more importantly because of their high water and fat retention capacity (de Crizel et al. 2014). Fierascu et al. (2020) have also extensively reviewed the current opinions on utilizing the waste generated from fruits processing industries into useful commodities (Fierascu et al. 2020). Pectinases are important fibrinolytic enzymes, and they are widely utilized in beverages industries for clarification as well as enhancement of color purposes. These enzymes help dissolve pectin structures (Micheli 2001) and are categorized based on their mechanism of bond cleavage: (1) pectin esterases and (2) depolymerase enzymes. Depending upon the substrate on which the pectinases act, pectin esterases are of two types, i.e., pectin acetylesterases (E.C 3.1.1.6) and pectin methylesterase (E.C 3.1.1.11). Pectin depolymerase, on the other hand, are hydrolases, for example, polygalacturonase, PG (E.C 3.2.1.15), and lyases or transeliminase comprising pectin lyase, PNL (E.C 4.2.2.10), and pectate lyase, PL

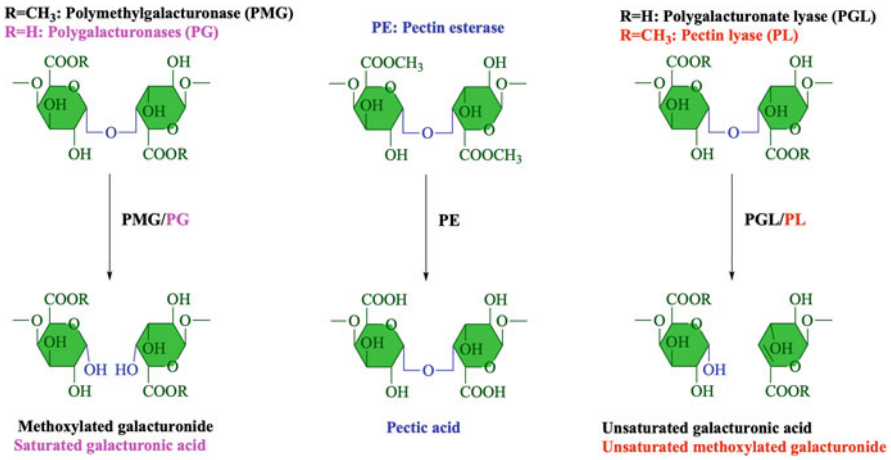

Fig. 4.9 Action of pectinases: Mechanisms of action of polymethylgalacturonase, polygalacturanase, pectin esterases, polygalacturanase, and pectin lyase on their specific pectin substrate

(E.C 4.2.2.2) (Sharma et al. 2013). Pectin methylesterases (E.C 3.1.1.11) convert the methyl groups into pectic acid and the depolymerase enzyme further disintegrates pectic acid into simpler carbohydrates (Fig. 4.9).

Pectinases in common consist of only one domain termed parallel β-helix making a right-handed cylinder. The structure contains three parallel β-sheets. These β-sheets arrange themselves to form a prism-like structure with two β-sheets forming an anti-parallel sandwich while the third one remains perpendicular to the axis. The active site of the enzyme is formed on the exterior side of the parallel β-helix. This structural model is most similar and closest to the structures demonstrated in the referred articles through crystallographic studies in pectinases from various strains (Jenkins et al. 2001; Pickersgill et al. 1994, 1998; Petersen et al. 1997).

Inulinase: Inulinase acts upon the β-2,1-linkage between the fructose units present in the inulin molecule. The polyfructose chain is terminated with a glucose unit attached with α-1, β-2-glycosidic linkage. Inulin is a stored carbohydrate found commonly in roots and tubers of plants like onion, garlic, and also there are reports of bacterial inulin which are comparatively highly branched in nature. Inulinase enzyme is classified into two classes: an endo-inulinase called 2,1-β-D-fructanfructohydrolase (E.C 3.2.1.7) and an exo-inulinase called β-D-fructanfructohydrolase (E.C 3.2.1.80) (Neeraj et al. 2018). The structural analysis of inulinase from *Aspergillus awamori* reveals the presence of two catalytically important residues (Asp41 and Glu241) at the substrate-binding site of the enzymes. These residues play a vital role in the double displacement reaction at the initial hydrolysis step (Nagema et al. 2004). Modern nutrition prospects recommend artificial sweeteners in the place of sucrose in diet to people looking for maintenance of weight/weight loss and lowering blood sugar levels in diabetes. Fructose products

have also made their place as sweeteners in prebiotics popularly known as Greek yogurt. Here's where Inulinase yields its importance in industries generating fructose or fructose oligosaccharides as artificial sweeteners. Inulinase has also been established in the production of a plethora of commercially important products like citric acid, tetrahydrofuran, mannitol, sorbitol and 2,3-butanediol, etc. (Chi et al. 2009).

Lipolytic Enzymes: Wastewater generated from the slaughterhouse, poultry, and fish farms are rich in biodegradable organic matter like oil and grease waste which can result in high BOD and COD. As a consequence, there is an increase in the growth of filamentous microorganisms (bulking), floating and clogging of sludge in the treatment plant, and unpleasant odor. This demands the hydrolysis of the organic matter before its release into the treatment plants for further processing. Even though there are various methods like dissolved air flotation systems, tilted plate separators, grease-trap, and physical-chemical treatment (aerobic and anaerobic pretreatments) employed for the removal of these biodegradable organic matters from the wastewater, their proper implementation counters several setbacks. Therefore, effluents from several origins can be subjected to enzymatic hydrolysis by lipase. Treatment of domestic wastewater rich in oil and grease with lipase obtained from *Candida rugosa* (Jaeger and Reetz 1998) and *Pseudomonas aeruginosa* (Dharmsthiti and Kuhasuntisuk 1998) has been vastly investigated. Lipases are chemically known as triacylglycerol hydrolases (E.C. 3.1.1.3) which catalyze the hydrolysis of triacylglycerol. The active site of lipases is decorated with amino acids like serine, aspartate or glutamate, and histidine (Mateos et al. 2021). They exist as monomeric proteins folded to form β-sheets in the center enclosed by α-helix. The lipase activity is considered maximum at the oil-water interface which is dependent upon its change in conformation from closed to open form upon coming in contact with a hydrophobic surface. Apart from hydrolysis, lipases can catalyze other reactions like esterification, transesterification, acidolysis, and aminolysis.

Lactases: Dairy industries are also major contributors of proteins, fatty acids, and lactose in liquid waste. The major portion of which is considered to be deproteinized cheese whey obtained from cheese producing industries all over the world, which despite no toxic content is a major concern to environmental preservation and safety (Lappa et al. 2019). The lactose component in cheese whey has been elaborately investigated for the production of several commodities like bioethanol, artificial sweeteners, green plastics/polyhydroxyalkanoate polyesters, etc. (Koller et al. 2012), and there are reports of its valorization into disinfectants, electron donors for electricity generation through single chamber microbial fuels cells as well (Banaszewska et al. 2014; Pescuma et al. 2015; Yadav et al. 2015). Lactases are known as β-D-galactohydrolase/β-galactosidases (E.C 3.2.1.23), and they catalyze the cleavage of lactose into glucose and galactose. The most demanding prebiotics, lactulose, and galacto-oligosaccharides (GOS) are produced by lactose through the action of β-galactosidases. Lactose is transgalactosylated by β-galactosidases to produce a mixture of non-digestible forms of mono-, and oligosaccharides that form the GOS. In the reaction, lactose acts both as the donor and acceptor of

transglycosylated galactose. Whereas in lactulose synthesis, lactose only acts as a donor of galactosyl glucose, and the fructose acts as an acceptor.

Protease: Proteases are classified into different groups based on the activity of proteases under various parameters like acidic, alkaline, or neutral conditions as well as the composition of their substrate-binding site (Panda et al. 2013). Alkaline serine protease (E.C.3.4.21–24.99) is most active at pH ranging from neutral to alkaline (Singhal et al. 2012; Varela et al. 1997). Other proteases are the neutral and acidic proteases (Razzaq et al. 2019). Proteases are proven to be a significant tool in bioremediation as well as valorization of waste to value-based products (Page and Cera 2008). The most exploited and studied microorganism for the production of alkaline protease and neutral proteases are *Bacillus species* and acidic proteases are known to be produced by the fungus. These proteases can be efficiently inactivated by PMSF (phenyl methane sulfonyl fluoride). Protein-rich waste is responsible for increasing the biological oxygen demand of aquatic waste. As discussed in earlier sections, the waste aquatic stream is drowned with effluents from food processing industries which constitutes waste from dairy products, waste from poultry, and fish industries as well as textile and leather industries. Leather industries involve alkaline protease with keratinolytic activity to hydrolyze keratin content present in the hair residues for increasing the surface area of the skin. They are also used in bating and clearing undesirable pigments during the preparation of clean skin and hides (Bhaskar et al. 2007; Shankar et al. 2011). Alkaline protease preparations of *Bacillus species* are enormously used in poultry industries to get rid of the feather waste generated in the slaughterhouse. The keratinolytic capability of these proteases has been best utilized in cleaning drainage pipes clogged with hair residues. A cocktail of protease preparation from *Streptomyces species, Bacillus subtilis*, and *Bacillus amyloliquefaciens* along with thioglycolate mixture is available commercially in the market. Alkaline proteases find a crucial place in the degradation of plastics as well as X-ray photographic sheets, particularly for the silver recovery. The active site of the alkaline protease comprises catalytic triad formed by Aspartate and Histidine residues along with Serine residue. Proteases based on their structural and sequence similarity can be obtained and assessed from the database called MEROPS (Rawlings et al. 2006) database. Table 4.1 comprehensively covers the application of different enzymes in the valorization of waste.

4.4.3 Hydrolytic Enzymes in Biodegradation and Valorization of Non-biodegradable Plastics Waste

The monomers utilized for the preparation of most plastics, ethylene, and propylene are derived from petroleum. The most commonly used are polyethylene, polystyrene, polyurethane, polypropylene, polyvinyl chloride, poly (ethylene terephthalate), etc. Out of the total production worldwide, only a small fraction around 20% is getting recycled, thus plastic remains a long-lasting, and major threat to the

Table 4.1 Listed applications of different hydrolytic enzymes in the valorization of waste and their microbial sources

Sl. No.	Name of the enzymes	Microbial source	Industry	Application	Reference
1	Cellulase	Bacteria: *Clostridium species, Pseudomonas* species, *Bacillus species* Fungus: *Aspergillus species*, *Penicillium species, Melanocarpus species*, *Trichoderma species, Schizophyllum commune* Actinomycetes: *Cellulomonas species, Streptomyces species, Thermomonospora species*	Pulp and paper industry, Textile industry Agricultural, and Food Industry Other	Deinking, pulping, bleaching, improve the quality and strength of pulp fibers, reduce the use of energy and chlorine, production of biodegradable sanitary napkins, papers, etc. Bio-stoning of jeans, softening of garments, biopolishing of textile fibers, enhancing quality, stability, and absorbance property of textile fibers, removal of excess dye from fabrics, and restoration of color brightness. Improve seed germination, plant growth and flowering, disease and plant pathogen control, and improve soil quality. Bioethanol production, bio-detergent production	Pere et al. (2001), Kuhad et al. (2011), Singh et al. (2016)
2	Xylanase	Bacteria: *Arthrobacter* species, *Geobacillus species, Pediococcus acidilactici, Bacillus species, Paenibacillus species, Rhodothermus marinus, Staphylococcus aureus, Pseudomonas species* Fungus: *Talaromyces species Thermomyces lanuginosus*, *Thermoascus sp,*	Agricultural, Food Industry, Pulp and paper industry, Textile industry, and Pharmaceutical industry	Improve fermenting process, improve animal feed digestibility, biogas, and bioethanol production, clarification, and maceration of fruit juice, brewing, hydrolysis of agro-residues. Bio-bleaching of pulp, Deinking Textile processing, desizing, and bioscouring. XOS production.	Beg et al. (2001), Bhardwaj et al. (2019)

		Melanocarpus albomyces Chaetomium species, *Fusarium species Humicola* species *Paecilomyces sp., Scytalidium sp.*			
3	Mannanase	Bacteria: *Agaricus bisporus, Aspergillus species, Bacillus species, Bacteroides species, Clostridium species* Fungus: *Aspergillus species, Agaricus species, Trichoderma species*, *Sclerotium species*	Pulp and paper industry, Textile industry, Agricultural and Food Industry	Bleaching of softwood pulps using enzymes, eliminates the use of chlorine and hydrogen peroxide, and enhance the brightness of paper. Desizing and bioscouring, reduce the viscosity of the print paste. Oil extraction from coconut, food additives, oil drilling, improve the nutritional value of animal feed.	Dhawan and Kaur (2007)
4	Aryl alcohol oxidase	Bacteria: *Sphingobacterium* species Fungus: *Thermothelomyces thermophilus, Coprinopsis cinerea, Phanerochaete chrysosporium*	Food and beverages, Polymer industry, and Pharmaceutical industry	Food flavors, e.g., vanillin. Production of bio-based polymer. Production of health beneficial compounds, e.g., to cuminaldehyde (therapeutic effects against cancer and diabetes).	Urlacher and Koschorreck (2021), Serrano et al. (2019), Li and Jiang (2004)
5	Lactase	Bacteria: *Bifidobacterium infantis* *Fungus: Lactobacillus thermophiles, Kluyveromyces fragilis, Aspergillus foetidus*	Food and beverages	Production of lactose-free products reduces the crystallization of ice creams and condensed milk	Saqib et al. (2017), Porta et al. (2010)
6	Alkaline protease	Bacteria: *Bacillus pumilus* Fungus: *Streptomyces fungicidicus*, *Myceliophthora species*	Leather Industry, Cosmetic sector, Pharmaceutical Industry and	Helps in effective bating. Formulation of cosmetic products. Development of ointment compositions, gauze, and new bandage material. Detergents and silver recovery.	Baweja et al. (2016), Sharma et al. (2019), Razzaq et al. (2019)

(continued)

Table 4.1 (continued)

Sl. No.	Name of the enzymes	Microbial source	Industry	Application	Reference
			Other applications		
7	Esterase	Bacteria: *Lactobacillus casei, Pseudomonas species* Fungus: *Aspergillus niger*	Pharmaceutical industry, Paper and pulp industry	Production of chiral drugs, detoxifying toxic residue. Reducing pitch problems during paper manufacture.	Panda and Gowrishankar (2005)
8.	Lignin-degrading enzymes				
	Lignin peroxidase, Manganese peroxidase (MnP), and Glycol oxidase (GLOX)	Fungus: *P. chrysosporium Trametes versicolor, Phlebia radiate, Trichoderma viride, Trametes suaveolens, Phanerochaete sordid* Bacteria: *Bacillus anthracis and Bacillus cereus* Fungus: *Rigidoporus Lignosus* and *Ceriporiopsis subvermispora* Bacteria: *Streptomyces lividans* Fungus: *P. chrysosporium, Pycnoporus cinnabarinus, Bjerkandera adusta, Dichomitus squalene*	Textile industry, Cosmetic industry, Pulp and paper industry, Polymer industry, Food industry, and Other application	LiP and MnP: Decolorize dyes and degradation of a xenobiotic compound. LiP: Decolorize synthetic melanin an alternative to hydroquinone cream. Lip and MnP: Biopulping, biobleaching, delignification in the paper industry. Lip and MnP: Polymerization of acrylamide into a thermoplastic resin (polyacrylamide). LiP and MnP: Delignification of lignocellulose. GLOX: Oxidize aldehydes to a variety of organic acids, carboxylic acids, pyruvic acid, oxalic acid, and formic acid, source of H_2O_2 production during lignin degradation	Marco-Urrea and Reddy (2012), Abadulla et al. (2000), Joon Sung (2020), Agarwal et al. (2018), Iwahara et al. (2000), Daou and Faulds (2017), Falade et al. (2017), Januz et al. (2017)

9	Pectinase	Bacteria: *Bacillus subtilis, Saccharomyces cerevisiae* Fungus: *Aspergillus niger*, *Rhodotorula* species	Food and beverages, Textile industry, and Other applications	Reducing the viscosity and turbidity of freshly collected juice, removing the mucilage from the coffee beans. Bioscouring of cotton fibers, degumming, and retting of fiber crop, oil extraction. Purification of plant viruses.	Nighojkar et al. (2019), Sharma et al. (2019)
10	Insulinase	Bacteria: *Streptomyces species* Fungus: *Kluyveromyces marxianus, Aspergillus niger.*	Food and beverages	Production of metabolites, e.g., lactic acid, sorbitol syrup, and high fructose syrup and oligosaccharides	Singha et al. (2017)

environment, especially to the aquatic streams (Kaushal et al. 2021). Plastics are categorized into two ways: (1) Thermoplastics whose chemical composition remains unchanged at high temperature and have mostly linear carbon chain backbone and (2) Thermosets which are made of other elements along with carbon possess highly cross-linked anatomy and their chemical conversion at high temperatures is irreversible. One of the most common thermoplastics used worldwide is polyethylene (PE). It is composed of ethylene as a monomer unit and is highly crystalline in structure which makes it recalcitrant to biodegradation. Based on the pattern of the linear chain and different densities, PE can be low-density PE (LDPE), high-density PE (HDPE), and also low molecular weight PE (LMWPE) Polypropylene (PP), yet another thermoplastic made by polymerizing propylene gas. PP differs from PE in having a methyl group instead of one hydrogen atom at an alternate carbon atom in the linear carbon backbone. This chemical structure gives it more rigidity in comparison to PE. Both these plastics are included in the category of polyolefin due to their inert and resistant nature to most heating, biological and chemical treatments. It is abundantly utilized as packaging plastics in various industrial sectors (Zheng et al. 2005). Polyvinyl chloride is yet another synthetic plastic that is used in rigid or plasticized forms. They are formed by polymerizing vinyl chloride or chloroethene in linear form. The pollution caused by PVC plastics is noticeable as burning these plastics emits hydrogen chloride fumes which pose serious health hazards. They are more prone to microbial degradation in comparison to other plastics due to the high percentage of plasticizers added to them (Webb et al. 2000). Polystyrene is synthesized by polymerizing styrene as repeating units. They could be thermoplastics or thermosets. They are also widely used in packaging industries due to their foam-like appearance (Tokiwa et al. 2009). Polyurethane and PET (polyethylene terephthalate) both have improved thermostability as they are hetero-atomically branched. PET is the most abundantly produced and used plastic in the modern era. Researchers have claimed that global warming caused by enormous CO_2 emissions and promiscuous usage of PET plastics are two of the most alarming situation in the biosphere (Wang et al. 2020). It is a high molecular weight thermoplastic composed of terephthalate (TPA) and ethylene glycol (EG) via an ester bond. This polymer has great tensile strength, and durability and its production cost are also low. Their structure contains large aromatic rings which make them rigid and resistant to biodegradation (Webb et al. 2013). Polyurethane has heteroatoms with carbamate linkage which could be either ester or ether bonds. They form the major constituents of microplastics making them the most concerning issues in the aquatic system (Shah et al. 2013). Mitigation of these plastics from the environment is carried over by certain physical and chemical methods like incineration, recycling, and dumping them into landfills. All of these methods are not environmentally friendly and they are not even cost-effective. The introduction of plastic hydrolyzing enzymes has opened up hopes for eco-friendly treatments to get rid of this dire environmental pollutant (Verma et al. 2016) (Table 4.2). Varieties of microorganisms like fungi, bacteria, actinomycetes, and algae have been investigated as well as reported to exhibit plastic polymer degrading capacity. Plastic degrading enzymes are mostly obtained from microbial organisms, and therefore they are studied under two categories: intracellular and

Table 4.2 Plastic degrading enzymes and their microbial sources

Sl. No	Enzyme	Microorganism	Plastic substrate	References
1	Laccase	*Rhodococcus ruber* C208 (Mesophilic bacteria) *Bacillus cereus* (Bacteria)	Polyethylene (PE)	Santo et al. (2013), Vimala and Mathew (2016)
2	Manganese peroxidase	*Phanerochaete chrysosporium* ME446 (White-rot fungus) *IZU-154* (Fungi) *Bacillus cereus* (Bacteria) *Penicillium simplicissimum* (Fungi)		Iiyoshi et al. (1998), Sowmya et al. (2015)
3	Lignin peroxidase	*Streptomyces (Bacteria) Phanerochaete chrysospo*rium MTCC-787 (Fungi)		Jeon and Kim (2015), Mukherjee and Kundu (2014)
4	Alkane hydroxylase	Recombinant AH from *Pseudomonas species* E4 expressed in *Escherichia coli* BL21 (Bacteria)		Yoon et al. (2012)
5	PETase	*Chlamydomonas reinhardtii* (Green algae)	Polyethylene terephthalate (PET)	Kim et al. (2020)
6	Cutinase-like enzyme/ IsPETase	*Ideonella sakaiensis* 201-F6 (Bacteria)		Han et al. (2017)
7	Lipase	*Pseudomonas chlororaphis* (Bacteria)	Polyurethanes	Stern and Howard (2000)
8	Polyurethanase	*Serratia marcescens* (Bacteria)		Mankoci et al. (2019)
9	Protease and esterase	*Pseudomonas fluorescens* (Bacteria) *Pseudomonas chlororaphis* (Bacteria)		Hung et al. (2016), Shah et al. (2008), Nakajima-Kambe et al. (1995)
10	Polyurethanase (PUase)	*Curvularia senegalensis* (Fungi)		Crabbe et al. (1994)
11	Polyhydroxyalkanoate depolymerase	*Alcaligenes faecalis* (Gram-negative, rod-shaped bacteria)		Gamerith et al. (2016)
12	Polyester Polyurethane (PUR) esterase	*Comamonas acidovorans TB-35* (Bacteria)	Polyester polyurethane	Akutsu et al. (1998)
13	Phenylacetaldehyde Dehydrogenase	*Pseudomonas fluorescens ST (Bacteria) Pseudomonas putida S12 (Bacteria) Xanthobacter species 124X (Mesophilic bacteria)*	Polystyrene	Oelschlägel et al. (2018)

(continued)

Table 4.2 (continued)

Sl. No	Enzyme	Microorganism	Plastic substrate	References
14	Cytochrome P450 CPY152A1	*Bacillus subtilis* (Gram-positive, catalase-positive bacteria)		Shoji et al. (2007)
15	Cytochrome P450 CPY152B1	*Sphingomonas paucimobilis* (Gram-negative bacteria)		Fujishiro et al. (2012)
16	AlkB (alpha-ketoglutarate-dependent hydroxylase	*Pseudomonas putida* GPo1 (Bacteria)		Hou and Majumder (2021)
17	Alkane monooxygenase	*Geobacillus thermodenitrificans* NG80–2 (Thermophilic bacteria)		Li et al. (2008)
18	Hydroquinone peroxidase	*Azotobacter beijerinckii* HM121 (Lignin decolorizing bacteria)		Nakamiya et al. (1997)

extracellular enzymes. Extracellular enzymes are involved in the depolymerization of long-chain polymers into smaller fractions, viz. oligomers, dimers, etc. Whereas the intracellular enzymes participate in the final conversion of intermediates into the forms which can be assimilated by the microbes as a sole source of carbon. As a result of this process, a valuable emission gas, i.e., methane is released as metabolic products which can be used as fuels and can further be utilized as precursors for the production of organic acids (Amobonye et al. 2021). Furthermore, the wax moth *Galleria mellonella* is known to depolymerize plastics with the help of their gut microbiota containing the fungus *Aspergillus flavus* (Zhang et al. 2020a, b). All these enzymes involved in degrading plastics are hydrolases that catalyze the cleavage reaction in the presence of water (Müller et al. 2005). Esterases, cutinases, laccases, lipases, and PETases are the most extensively studied hydrolytic enzymes concerning the degradation of plastics. Microbial valorization of plastics into value-added chemicals is elaborately reviewed in Ru et al. (2020). The reviewer explained elaborately the microbial metabolic pathway involved in the depolymerization of ester/urethane-containing plastics, aromatic plastics, and linear aliphatic plastics into its monomer constituents and their further assimilation by microbes for the production of value-added chemicals. The chemical structure such as linkages in petro plastics, linearity or branching in carbon chain, type of linkage (ester, ether, or carbamate linkage between the monomers), presence of hydrophobic functional groups, and physical properties like rigidity (crystalline/amorphous) and density plays a significant role in engineering enzymes suitable for biodegradation (Mohanan et al. 2020). Ongoing and present studies for identification of plastic degrading microorganisms and modification of these microbial enzymes through genetic engineering provides a wide opportunity to efficiently recycle or remove

plastic from the environment. Yet advantageous is when these plastics can be converted into more valuable and marketable products.

4.5 Recent Biotechnological Trends in Increasing the Efficacy of Enzymes in the Waste Valorization

Enzymes have been central in various industries like food processing, beverages distilleries, leather, textile, and paper industries for a long time (Sheldon and Woodley 2017). Their involvement in the production of biofuel and value-added products is tremendously surging in recent times (Chapman et al. 2018). Consequently, for obtaining optimal bioprocesses involving enzymes, further enhancement of enzyme stability and functionality is indispensable. Biotechnological breakthrough offers a great deal for enhancing the power of existing enzymes as well as identifying newer enzyme candidates.

4.5.1 Techniques to Decipher Newer Biocatalyst Candidates

Conventionally, enzyme discovery was done by cultivating microorganisms, fractionating cell-free extract, followed by the screening of the enzyme activity, and then recovered enzymes through purification are subjected to mass spectrometric analysis after trypsin digestion. The identified short peptides are then utilized to decipher the corresponding gene from the genomic DNA. Although these methods are dependent on the use of cultivable microorganisms, a significantly important set of enzymes were discovered using this process. State-of-the-art tools are now assessable to scrutiny and tap the vast microbial biodiversity present in nature (Rinke et al. 2013). The introduction of omics such as metagenomics and metatranscriptomics presents a big potential to analyze the diversity of complex microorganisms. These tools help in the development of genomic libraries from environmental DNA for function or sequence-based similarity screening of the enzymes (Gilbert and Dupont 2011; Uchiyama and Miyazaki 2009). For example, a collection of hydrolytic enzymes such as amylase, lipase, oxidoreductase, and epoxide hydrolase have been deciphered using this technique (Knietsch et al. 2003; Rondon et al. 2000). Several bioinformatics strategies such as in silico data mining, the Catalophore™ approach, and de novo enzyme design tools are also helpful in this process (Handelsman 2004). Progressive success in developing methods for genome sequencing like next-generation sequencing has opened up newer approaches to hunt for putative enzymes. Here, genome hunting is based on either searching for the open reading frame or homology alignment of sequences.

4.5.2 Isolation of Enzymes from Extremophiles

Enzymes that can withstand extreme parameters like higher temperature and pH as well as harsh chemicals like a high concentration of salts, metal ions, organic solvent, etc. have great value at industrial levels. There is a large diversity of organisms in the extremophilic regions and as most of these organisms have not been yet cultivated in pure cultures, the characterization of their enzymes from them is comparatively difficult (Pikuta et al. 2007; Cavicchioli et al. 2011). The bacterial isolates obtained from the extremophiles have displayed properties of different hydrolytic enzymes such as amylase, protease, lipase, and xylanase. Extreme thermophiles are widely present in bacterial species like *Thermus, Thermotoga, Clostridium, and Bacillus. Pyrococcus, Thermococcus, or Methanopyrus* belongs to hyperthermophilic Archaea. Hydrolytic enzymes such as Amylase, Xylanase, Lipase, and Protease enzymes are isolated from some halophilic bacterial species like *Halobacterium, Halobacillus, and Halothermothrix* (Moreno et al. 2012).

4.5.3 Genetic Engineering or Recombinant DNA Technology for the Production of Recombinant Protein in a Microbial Host

To obtain desired efficacy in the expression of enzymes, gene-based technology like recombinant DNA technology can be used. In this technology, the desired gene or gene of interest is inserted into the organism *via an* appropriate vector. The gene of interest can be manipulated through the addition of the desired sequence in the endogenous gene or deletion or knockout of undesirable sequence through recombining genes and elements. Rational redesigning and direct evolution are the two different methods that are adapted for modifying enzymes to their desired characteristics. Rational redesigning utilizes site-directed mutagenesis to target amino acid substitution effectively at the active site of the protein for evolving the enzyme into a more efficient one. Whereas the direct evolution method includes repeated oligonucleotide-directed mutagenesis, random mutagenesis through error-prone polymerase chain reaction (PCR), or modification through chemical agents (Manisha and Yadav 2017; Wiltschi et al. 2020) (Table 4.3).

4.5.4 Immobilization of Enzymes

The major confrontation in enzyme technology and its application in industries is bulk production and the question of reusability. These problems can be easily dealt with the immobilization technique. Enzymes can be immobilized by tethering or encapsulating them in an appropriate material that has desired physical, chemical,

Table 4.3 Genetically engineered microbes for increasing the efficacy of expressed hydrolytic enzymes in the valorization of waste

Sl. No.	Enzymes	Improved properties	Organism	References
1	Cellulase	Increased the inherent ability of *Lactiplantibacillus plantarum's* lignocellulose degradation	*Thermobifida fusca*	None and Yadav (2017)
2	β-glucosidase	Improve enzyme activity and thermostability	*Trichoderma reesei*	Lee et al. (2012)
3	Xylanase	T_m improved by 25 °C	*Thermotoga thermarum*	Yang et al. (2017)
4	Laccase	3-fold improved kcat and thermostability	*Bacillus* HR03	Mollania et al. (2011)
5	Lipase	2-fold increase in amidase activity	*Pseudomonas aeruginosa*	Fujii et al. (2005)
6	Lipase B	20-fold increase in half-life at 70 °C	*Candida antarctica*	Siddiqui and Cavicchioli (2005)
7	Pyranose 2-oxidase	Increased thermostability and the catalytic properties	*Trametes multicolor*	Spadiut et al. (2009)
8	Endoglucanase	Increase hydrolytic activity on cellulosic substrate	*Thermoascus aurantiacus*	Srikrishnan et al. (2012)

electrical, or mechanical properties. These materials increase the stability as well as efficacy in terms of better catalytic activity of the immobilized enzymes. Moreover, immobilization can reduce the steps required to separate them from the reaction mixture as well allows substantial reusability without affecting the activity allowing them to be compatible in a continuous process. To examine the enzyme activity of immobilized enzymes two kinetic parameters, namely the Michaelis constant, K_m, and maximal reaction velocity, V_{max} is often examined and compared with the non-immobilized counterpart. The three widely used immobilization techniques are encapsulation or entrapment, carrier-bound attachment, and the formation of cross-linked enzyme aggregates (CLEAs). Encapsulation or entrapment technique, as the name suggests is the immobilization of enzymes using material of varying degrees of porosity and permeability (Bezerra et al. 2015; Jesionowski et al. 2014). Various materials such as a variety of carriers, for example, sol–gels, hydrogels, polymers as well as nanomaterials have been experimented with immobilization of enzymes via encapsulation technique. Carrier-bound immobilization of enzymes is done by physisorption or chemisorption of enzymes on prefabricated organic or inorganic materials. Materials like metal oxides, nanomaterials, ceramic, or silica gels are used for this purpose. Chemisorption technique is preferred over physisorption as covalent attachment reduces the chances of enzyme leaching from the matrix. CLEAs technique is a very recently utilized technique where the soluble enzyme is aggregated using precipitating agents like alcohol, acetone,

ammonium sulfate, etc., and then subsequently cross-linked or co-polymerized with cross-linking agents like glutaraldehyde (Sheldon 2010). Hence, immobilizing enzymes can be well exploited for obtaining desired characteristics to bring increased efficacy in the use of enzyme technology in the industrial arena as well as biorefineries.

4.5.5 Cell Surface Engineering

Genetic technological advances have let us modify microorganisms to express our genes of interest (Table 4.3). Cell surface engineering is another biotechnology tool where the particular microorganism is tailored to express the desired number of enzymes on its surface. This is technique is advantageous in biorefineries where there is a need for multiple enzymes for the conversion of biomass to biofuels. The most utilized and engineered organism includes *Saccharomyces cerevisiae*. Naturally, yeast can ferment sugars to produce alcohols, but it does not possess the enzyme required for saccharification of complex sugars present in the cell wall of the biomass. Cell surface engineering enables the arming of yeast and other organisms with a cocktail of enzymes that help the production of biofuels and other value-added products (Kuroda and Ueda 2013; Ueda 2016; Ueda and Tanaka 2000).

4.6 Conclusions

Developing sustainable approaches toward building a circular economy and safeguarding mother nature has become the need of the hour. Toward achieving this, biomass waste from various sectors like agro-forest, solid municipal waste, food manufacturing, and processing industries can be utilized and profitably and competently converted to bioenergy and value-added products. To reduce the losses incurred by the use of traditional chemical processes in the treatment of waste biomass, enzymes are introduced for the economic and easier hydrolysis of the different waste components. The major portion of waste generated is composed of biodegradable biomass as well as non-biodegradable plastics. Different microbial sources of hydrolytic enzymes are exploited for the valorization of these wastes into value-added products and bioenergy. Cellulases, hemicellulases, lignin peroxidases, pectinases, amylases, proteases, etc. enzymes are widely utilized in this concern. The biorefinery concept is structured to use the by-product from one industry as feedstock for another industry to produce more value-added goods in addition to conversion of waste to bioenergy. Various technological advances like genetic engineering, cell surface engineering, and immobilization of enzymes are exploited to increase the efficacy of the hydrolytic enzymes obtained from microbial sources. Identification of new sources of these enzymes through metagenomic analysis is relevant and very necessary to keep the reservoir filled. In addition to that further

improvement of these enzymes could be done through techniques like metabolic engineering and chemical modification of the enzyme.

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