Review

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Posted Date: 28 November 2023

doi: 10.20944/preprints202311.1728.v1

Keywords: Enzymes; Biomass; Waste Management; Waste Treatment; Microorganisms; Applications of Enzymes doi:10.20944/preprints202311.1728.v1

Review

Application of Enzymes in Biomass Waste Management

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Abstract: Enzymes are biological molecules produced by living entities for carrying out biological processes. The application of enzymes for waste treatment has been gaining pace commercially to solve concerns related to agricultural residues, wastewater, replacement of synthetic processes with natural ones, etc. The application of enzymes for waste management has been an environmentally reliable and sustainable process. Treatment of waste with enzymes such as xylanase, proteases, hydrolases, cellulose, peroxidases, chitinases, laccases, etc. has been studied to be effective. These enzymes act upon the waste products and transform them into biodegradable forms that can be recycled, reused and converted to value-added products. They have wide applications and utility as it has been an effective approach, economically cheaper and sustainable techniques. Application of such enzymes for waste management would be beneficial for reducing the quantity of waste, diminishing the negative effects of waste and pollution on the environment, and would be beneficial in bio-converting the waste products into alternate sources of energy. The current chapter focuses on different types of enzymes, their applications for waste management, and their limitations. This chapter also emphasizes the usage of some prominent microorganisms, their secreted enzymes and their proposed mechanisms of action involved with the degradation of the waste products.

Keywords: enzymes; biomass; waste management; waste treatment; microorganisms; applications of enzymes

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1. Introduction

Several anthropogenic activities like agriculture practices, industrialization, etc. are increasing day by day. These activities contribute to increasing the level of pollutants in the environment. These pollutants may be azo dyes, phenols, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, dioxins, as well as heavy metals (Bhardwaj et al. 2023a). There are several processes such as physical and chemical treatment and activated carbon adsorption that have been developed in recent years for removing pollutants from waste. Industrial wastes contain toxic organic as well as inorganic materials. The presence of organic materials in the waste may be treated biologically for the minimization of the overall treatment cost.

Enzymes can play an important role in the treatment of waste. The use of enzymes in waste treatment was first proposed in 1930. They are target-specific and can particularly attack target pollutants. They are used as an efficient catalyst and are used at a particular pH and temperature. Researchers were focusing on the development of new technology for waste treatment which will be faster, cheaper, simpler, and more reliable. Now, they have developed enzymatic processes with the help of biotechnological tools. This technique replaces costly chemicals and does not require specialized machinery. It is a highly acceptable technique in its clean/green and biodegradable nature

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due to several advantages. It falls between the physicochemical and biological processes because it contains chemical processes that are based on the biological catalysis action.

Enzymatic conversion of biomass offers several advantages over chemical and physical techniques. These advantages are:

It can be operated at high as well as low concentrations of contaminant.

It could be operated over a broader range of parameters of pH, temperature, as well as salinity.

There are no shock loading effects.

There is a reduction in the volume of sludge.

The process of controlling is simple.

It is an environmentally friendly technique.

With these potential advantages, researchers have focused on the use of this technique for the treatment of wastewater, solid wastes, and hazardous wastes.

Biomass waste treatment uses enzymes from microorganisms such as bacteria or fungi. They are involved in several metabolic activities and produce different enzymes. These enzymes are specific to their work and involve a series of chemical reactions. Enzymes that are produced by aerobic bacteria (*Alcaligenes*, *Mycobacterium*, *Pseudomonas*, *Rhodococcus*, and *Sphingomonas*) are used in the degradation of pesticides and hydrocarbons while those enzymes produced by anaerobic bacteria that are used in the degradation of compounds like polychlorinated biphenyls (PCBs), dichlorination of trichloroethylene (TCE), as well as chloroform (Sharma 2012).

The pollutants including compounds containing azo dyes, phenols, PAHs, pesticides, polychlorinated compounds, heavy metals, etc. have been studied to create adverse effects of teratogenic nature, carcinogenic potential, mutagenic, as well as toxic effects on the health of humans (Liu et al. 2019; Alam et al. 2023; Bhardwaj et al. 2023b). However, the enzymatic technique is slow, and only some bacterial species are capable enough to produce specific enzymes. So, the researchers prefer genetically engineered microbes for this process. This process converts the pollutants from toxic forms to nontoxic forms (Phale et al. 2019). Thermophilic bacteria *Caldicelluloseruptor bescii* has been studied to directly convert plant biomass into bioethanol. This could be used as a potential agent in the commercial sector for bioethanol production (Chung et al., 2014). Similarly, hemicellulases, cellulases, xylanases, endoxylanases, and β-xylosidases extracted from a variety of ascomycetes *Trichoderma*, *Aspergillus*, etc. have been applied extensively at commercial levels to act as biocatalysts in lignocellulosic biorefineries (Ferreira et al., 2016). A recent study has shown that the co-cultivation and harvesting of microalgae and filamentous fungi were efficiently used for wastewater treatment as well as biofuel production (Chu et al., 2021).

The aim of this chapter is to focus on different types of enzymes and their applications for waste treatment.

2. Enzymes Used for Biomass Conversion, Degradation, and Hydrolysis

Enzymes can help in converting biomass into various useful by-products. Biomass conversion involves the catalysis of complex organic materials, such as plant fibers, feedstocks, agricultural waste, etc. into simple components. These components could further be processed into biofuels, bio-chemicals, and other valuable products (Demirbas 2009; Mahapatra et al. 2021). Several types of enzymes are commonly used in biomass conversion processes (Table 1). Some of the example of enzymes are given below:-

Amylases: They are extracted from fungal and bacterial strains that hydrolyze starch into glucose and maltose. Starch is often used as a feedstock in biomass conversion processes, and amylases are employed at a commercial scale to convert it into fermentable sugars for biofuel/bioethanol production (Castro et al., 2011).

Cellulases: They helps in the breakdown of cellulose into glucose moieties and cellulose is a major component of plant cell walls formed by β-1,4-glycosidic bonds. They consist of three main types: endoglucanases, exoglucanases (or cellobiohydrolases), and β-glucosidases (Dashtban et al.,

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2010). Cellulases have been observed to be important in the biofuel production like cellulosic ethanol and in the conversion of biomass into various biochemicals (Siqueira et al., 2020).

Hemicellulases: They target hemicellulose which is another complex carbohydrate present in plant cell walls. These enzymes include xylanases, mannanases, and arabinases (Zanuso et al., 2021). Bacteria and fungi produce large amounts of hemicellulases. They break down hemicellulose into smaller sugar units, such as xylose, mannose, and arabinose, which can be fermented into biofuels or used as building blocks for biochemical production (Méndez-Líter et al., 2021).

Ligninases: They include lignin peroxidases and manganese peroxidases and are involved in the degradation of lignin which is a complex and highly resistant polymer found in plant biomass. Lignin allows access to the cellulose and hemicellulose components for further enzymatic degradation or chemical processing (Siqueira et al. 2020; Bilal and Iqbal 2021).

Proteases: They are also known as peptidases and are responsible for breaking down proteins into smaller peptides and further to amino acids.

Laccases: They are extracellular enzymes containing multi-copper that consists of glycoproteins with dimeric, tetrameric and monomeric units characterized by bacteria, fungi, and plants (Shekher et al. 2011). Lignin as well as phenolic compounds which are found in banana peels, sawdusts, and rice bran have enhanced production of laccase (Muthukumarasamy et al. 2015).

Table 1. List of the Different Enzymes with Their Sources and Applications.

S. No.	Name of Enzyme	Sources	Application of Enzymes	References
1	Alkylsulfatase	Pseudomonas C12B	Surfactant degradation	Toesch et al. 2014
2	Azoreductase	Intestinal microflora	Removal of azo dyes	Sandhya 2010
3	α-Amylase	Bacillus subtilis	Glucose production and hydrolysis of starch	Kolusheva and Marinova 2007; Presečki et al. 2013
4	Glucoamylase			
5	Cellulase	Trichoderma harzianum, Trichoderma viride	Hydrolysis of cellulose in sludges & municipal solid waste (MSW) to produce alcohol, sugars, and energy	Champagne and Li 2009; Khan et al. 2016; Pandey et al. 2017
6	Cellobio-hydrolase			
7	Cellobiose			
8	Exo-1,4-b-D-glucosidase			
9	Chitinase	Streptomyces anulatus CS242	Production of N-acetyl glucosamine from shellfish waste through bioconversion	Mander et al. 2016
10	Chloro-peroxidase	Caldariomyces fumago	Oxidation of phenolic compounds	Sjoblad and Bollag 2021
11	Cyanidase	Pseudomonas sp.	Cyanide decay	Akcil et al. 2003
12	Cyanide hydratase	Fusarium lateritium	Cyanide hydrolysis	Ebbs 2004
13	Depolymerase	Bacteriophase	Bacterial exopolysaccharide	Knecht et al. 2020
14	Haemoglobin	Blood	Removal of aromatic amines and phenols	Pérez-Prior et al. 2014
15	L-Galactono-lactone oxidase	Candida norvegensis	Conversion of galactose from L-ascorbic acid	Nicell 2003
16	Laccase	Pleurotus (P. ostreatus, P. pulmonarius) and Trametes (T. versicolor, T. hirsuta)	Decolorization of kraft, removal of phenols, bleaching of paper pulp, binding of phenols and aromatic amines with humas, detoxification of wastewater	Khatami et al. 2022
17	Lactases	Bacterial	Dairy waste processing and production of value-added products	Coughlin and Charles 2022
18	Lignin peroxidase (LiP)	Phanerochaete chrysosporium	Removal of phenols and aromatic compounds decolorization of Kraft bleaching effluents	Falade et al. 2017
19	Lipase	Various sources	Improved sludge dewatering	Nimkande and Bafana (2022); Di et al. 2023
20	Lyzozyme	Bacterial		
21	Manganese peroxidase (MnP)	Phanerochaete chrysosporium	Decolorization of synthetic dyes, removal of phenolic contaminants, removal of endocrine disruptive	Bansal and Kanwar 2013

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			chemicals (EDC), degradation of chlorinated alkanes and alkenes, degradation of chlorinated dioxins	
22	Nitrile hydratase	Mesorhizobium sp.	Removal of acrylonitrile	Feng et al. 2008
23	Parathion hydrolase	Azohydromonas australica	Hydrolyzation of organophosphate pesticides	Zhao et al. 2021
24	Pectin Lyase	Clostridium beijerinckii	Pectin degradation	Yadav et al. 2009
25	Pectinmethylesterase	Aspergillus niger		Kohli et al. 2015
26	Phosphatase	Escherichia coli C90	Removal of heavy metals	Chaudhuri et al. 2013
27	Phosphoesterases	Aspergillus sydowii CBMAI 935	Removal of chlorpyrifos, diazinon, parathions	Soares et al. 2021
28	Polyphenol oxidase	Mushroom (Agaricus bisporus)	Removal of phenolic compounds	Li et al. 2021
29	Proteases	Bacillus licheniformis, Aspergillus niger, Chlorella vulgaris	Hydrolyze or breakdown the protein molecules in meat, biodegradation of the industrial sludge	Karn and Kumar 2015; Arslan et al. 2021
30	Tyrosinase	Mushroom (Agaricus bisporus)	Removal of phenolic compounds	Bayramoglu et al. 2013

3. Mechanism of Treatment of Biomass:

The enzymes break down complex organic compounds into simpler components, as depicted in Figure 1. The mechanism involves the following steps:

3.1. Pretreatment of Biomass Wastes

Biomass waste like agricultural residues, wooden chips, residual crops, etc. are pretreated to enhance the enzyme's accessibility to the complex carbohydrates present in biomass. Such techniques include combinations of physical, chemical, or biological means for example, acid or alkali treatment, fungal/bacterial/yeast treatment, milling, steam explosion, etc. These methods are capable of disrupting the biomass structure thereby making it more susceptible to enzymatic degradations (Zhang et al., 2021).

3.2. Enzyme Production

Microorganisms that are capable of producing enzymes suitable for biomass degradation are cultivated in a controlled environment. These microbial cultures are grown in bioreactors under specific conditions that promote enzyme production either naturally or by genetic engineering (Guo et al., 2023).

3.3. Enzymatic Hydrolysis

The pretreated biomass and enzyme solutions are mixed to initiate the hydrolysis process. Enzymes, such as cellulases, hemicellulases, lignin, act on complex carbohydrates of biomass and hydrolyze them into simpler molecules. These enzymes work synergistically to degrade different components of the biomass, releasing glucose, xylose, and other sugars (Huang et al. 2022a).

3.4. Fermentation and Further Processing

The resultant sugar-rich hydrolysate is subjected to fermentation using microorganisms, such as yeast or bacteria, to convert the sugars into desired end products. For example, ethanol-producing microorganisms can ferment the sugars to produce bioethanol, while other microorganisms can convert the sugars into various biochemicals, such as organic acids, enzymes, or bio-based materials (Huang et al. 2022a; Huang et al. 2022b).

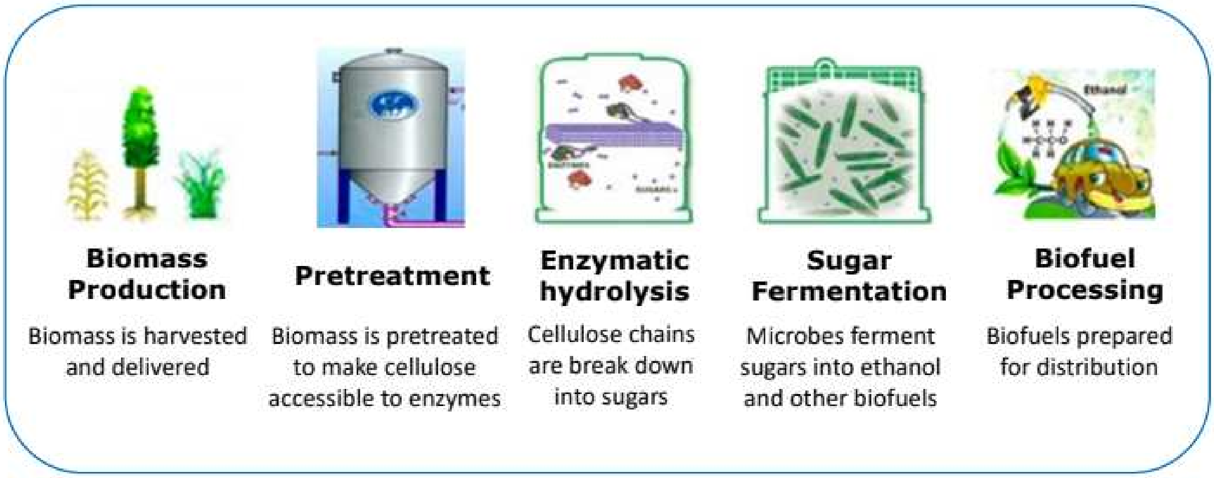

Figure 1. Diagrammatic Representation of the Mechanism of Biomass Wastes Treatment.

4. Application of Biomass Waste Management for Treating Contaminated Wastewater

Water is necessary for all living things (Bhardwaj and Sharma 2021a; Bhardwaj and Sharma 2021b; Bhardwaj 2022; Bhardwaj 2023). Now, the water bodies have been contaminated with the effluent of various industries, institutes, and residential areas. This effluent comprises decomposed organic waste that can generate gases with an awful odor in great amounts. This polluted water contains toxic persistent pollutants which might be hazardous to the environment (Bhardwaj and Jindal 2019; Bhardwaj and Jindal 2020; Bhardwaj et al. 2021; Bhardwaj and Jindal 2022). The usage of enzymes for the treatment of wastewater reduces the toxicity of water and makes the water fit to reusage.

Most of the pollutants act as substrates for certain enzymes. By the action of enzymes, the toxic pollutants are fragmented into smaller fragments that no longer pose a danger to the surroundings. Enzymes can be introduced directly or even mixed with microbes. The entire body of plants or their tissue culture that contains the enzymes in their normal form can be deployed in water bodies. BIO-CAT's enzymes are effectively used to treat organic waste (Sahal et al. 2023).

Certain enzymes are required to catalyze specific reactions in specified concentrations. Oxidoreductases, peroxidases, and oxygenases are used for the removal of inorganic contaminants like biphenols, chlorophenols, benzidines, methylated phenols, phenols, heterocyclic aromatic compounds, and anilines while lipase, urease, amylase, xylanases, cellulase, and protease are used for the removal of organic pollutants. These enzymes are called septic tank enzymes and are utilized for the treatment of wastewater from sewage. Oxygenases have been used for treatment of water bodies that are poisoned by the presence of fossil fuels. Peroxidases are utilized for the treatment of water that is contaminated with dyes, phenols, and hydrogen peroxide. Polyphenol oxidases are used in wastewater treatment plants for the removal of phenol from wastewater and can further be divided into Tyrosinase and Laccase.

Joutey et al. (2013) studied the degradation of persistent organic pollutants (POPs) by using enzymes from microbes and stated that this technique is environment-friendly, cost-effective, and innovative. Enzymes, for example, cytochrome P_{450s} laccases, hydrolases, dehalogenases, dehydrogenases, proteases, and lipases, are involved in the degradation of harmful chemicals (Bhandari et al. 2021).

Cytochrome P_{450} is responsible for synthesizing natural products in living organisms, and it is used in biotransformation of poisonous chemicals present in our ecosystem (Li et al. 2020). This enzyme has an intrinsic capability to degrade xenobiotics (Anzenbacher and Anzenbacherova 2001; Chakraborty and Das 2016). P_{450} BM_3 is used in the degradation of several organic pollutants using Pt/TiO_2-Cu under solar radiation and is formed from *E. coli BL$_{21}$* (Awad and Mohamed 2019).

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Laccase has been observed to possess catalytic capability to degrade the aromatic amine and phenolic compounds (Chandra and Chowdhary 2015). It degrades the PAHs to carbon dioxide (CO_2) (Khlifi et al. 2010) and converts acenaphthylene to 1,8-naphthalic acid and 1,2-acenapthalenedione (Madhavi and Lele 2009). It can detoxify dyes produced by the textile industry (Sondhi et al. 2015).

Dehalogenases belong to the oxidoreductase family and are used for degradation of halogenated compounds. Reductive, oxygenolytic, and hydrolytic mechanisms are used in the breakdown of halogenated compounds (Wang et al. 2018a; Wang et al. 2018b). Halohydrin dehydrogenase (HHDH) and haloalkane dehydrogenase (HADH) are used in the degradation of haloalkane and are expressed in *E. coli* (Xue et al. 2018). 2,4,6-trichlorophenol reductive dehalogenase dechlorinates pentachlorophenol (PCP) into 3-chlorophenol and is isolated from Desulfitobacterium frappieri PCP-1 (Boyer et al. 2003). Alcohol dehydrogenase catalyzes the conversion process of alcohol into ketone or aldehyde. It also catalyzed NAD(P)+-dependent oxidation of aldehydes into carboxylic acids.

Polyethylene glycol dehydrogenase (PEGDH) degrades the pollutants which are released from industries. The enzyme NAD+-dependent polypropylene glycol dehydrogenase (PPGDH) is isolated from the species *Stenotrophomonas maltophilia* and plays a role in oxidization of secondary alcohols (Tachibana et al. 2008). Polyvinyl alcohol dehydrogenase (PVADD) degrades polyvinyl alcohol which is water-soluble contaminants (Hirota-Mamoto et al. 2006). Aldehyde dehydrogenase (ADH) degrades aromatic compounds and catalyzes the conversion of 1-hydroxy-2-naphthaldehyde to 1-hydroxy-2-naphthoic (Ji et al. 2020).

Hydrolases reduce pollutants toxicity by degrading the larger molecules to small molecules. Hydrolytic enzymes like amylases, proteases, lipases, esterases, nitrilases, cellulases, cutinase, as well as peroxidases can be used in the degradation of insecticides, and oil-contaminated soils. They are used in biomedical sciences, and chemical industries (Kumar and Sharma 2019). Hydrolases such as parathion hydrolase or carbamate which are isolated from *Pseudomonas, Achromobacter*, *Flavobacterium*, *Bacillus cereus,* and *Nocardia* have been used for converting diazinon, carbofuran, coumaphos, and carbaryl / parathion through the method of hydrolysis.

Organophosphate pesticides (OPs) degrade through hydrolysis of P-O-alkyl and P-O-aryl bonds (Singh 2014). Malathion is degraded by the action of *Brevibacillus* sp., *Alicyclobacillus tengchogenesis*, *Bacillus cereus,* and *Bacillus licheniformis* (Littlechild 2015). OP acid anhydrolases enzymes, methyl parathion hydrolases (MPH), and OP hydrolases are used for the degradation of Organophosphates (OPs) (Schenk et al. 2016). Cutinase is used for the degradation of polycaprolactone, and it is isolated from the bacterial species *Fusarium solani f. pisi* (Singh et al. 2016). Protease belongs to the hydrolase family and is isolated from *Amycolatopsis* sp., *Aspergillus* sp., as well as *Bacillus* sp. They are low cost and catalyzing enzymes and are used in industries of food, leather as well as wastewater treatment (Kumar and Sharma 2019). They are capable of the degradation of poly(hydroxybutyrate) (PHB) depolymerase *ß*-ester bonds, lipase γ-ω bonds, as well as α-ester bonds (Haider et al. 2019). They convert marine crustacean wastes and keratinous wastes into useful products. Keratinase is isolated from *Stenotrophomonas maltophilia* KB_{13} and used in the degradation of chicken feathers (Bhange et al. 2016). Keratinase is used in the leather industry, and it reduces the chemical (CaO and Na_2S) load in wastewater (Akhter et al. 2020). The enzyme chitinase is isolated from *Bacillus subtilis* and degrades crystalline chitin into N-acetyl-D-glucosamine (Wang et al. 2018a). *Pseudomonas fluorescens* degrades polyurethane (PU) within 4-5 days using the enzyme protease.

Lipases are a well-known biocatalyst and degrade lipids (Casas-Godoy et al. 2012). They are used in the degradation of contaminants such as petroleum, residues of oil, greasy effluents, as well as oil spills (Basheer et al. 2011; Casas-Godoy et al. 2012; Hassan et al. 2018). Lipases which are isolated from species of *Pseudomonas* used for degradation of industrial waste oil (Amara and Salem 2009). Crude lipase which is formed from the species *Bacillus subtilis* is used in soap industries to reduce phosphate-based chemicals (Saraswat et al. (2017). Lipase PL which is isolated from *Alcaligenes* sp., catalyzes the conversion of poly (L-lactide) (PLA) polymers to the monomers. Lipases which are formed from *Lactobacillus plantarum* and *Lactobacillus brevis* are used in the degradation of polycaprolactone (PCL), and polyester (Wang et al. 2022).

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5. Challenges and Future Prospects

Biomass waste treatment using enzymes from microorganisms is an area of ongoing research and development and has gained attention. Researchers are continuously researching to improve enzyme efficiency, process condition optimization, development of more cost-effective and sustainable methods, etc. (Madhavan et al., 2021). Utilizing biomass resources, enzyme diversity and specificity, have been areas of active research. One of the main challenges is scaling-up the biomass waste treatment from the laboratory to industrial-scale processes (Kalak 2023). Advancement in enzyme engineering techniques, protein engineering, meta-genomics studies are required for developing enzymes with enhanced catalytic properties, stability, and specificity (Kate et al., 2022). Biomass waste management using enzymes could also be integrated with bio-refineries, an together aim to produce multiple valuable products that are more sustainable and economically viable such as biofuels, bio-based chemicals, materials, etc. (Leong et al., 2021). Analyzing the properties of biomass waste materials would be helpful for understanding the appropriate methods to utilize them for energy and thereby reducing the consumption of fossil fuels in the future (Savla et al. 2021).

6. Conclusion

There is a need for continuous and vigorous research on usage of enzymes for the treatment of waste. Many industries such as textiles, agro, food & beverages, solid-waste treatment, sugar-mill, etc. are using enzymes for the treatment of wastes. The use of enzymes is a cost-effective, and sustainable technique for the treatment of waste. The usage of enzymes offers many advantages in biomass waste management strategies. Enzymatic processes are generally environmentally friendly, highly specific in their actions, operated under mild conditions, produce minimal waste as compared to traditional chemical methods. Furthermore, enzymes are produced through sustainable and renewable sources, such as microbial cultures. The application of enzymes for biomass waste management on a wide scale/industrial level has challenges. Overall, the application of enzymes for biomass waste management represents a promising approach towards achieving a more sustainable and circular economy.

Author Contribution: All authors have equal contribution.
Acknowledgments: The authors thank Amity University for providing the platform to do this study.
Data Availability: Not applicable.
Conflict of Interest: The authors declare that they have no competing financial interest or personal relationship that could have appeared to influence the work reported in this chapter.

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