Chapter 15 Recent Advancements and Mechanism of Microbial Enzymes in Sustainable **Agriculture**

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Abstract To make the environment sustainable, using microbial technology is an important aspect. Indigenous microbial strains have been investigated for their application in bioremediation and sustainable development. The microbial enzymes are found effective for the bioremediation of the xenobiotic compounds from the environment. The enzymes can be produced extracellularly and intracellularly by microbial cells and catalyze the degradation of the toxic chemicals. Catalysis of the enzymes is performed via the respective amino acids located onto the binding site. The enzymes belonging to the esterase, laccase, dehydrogenase, oxygenase etc. are

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found effective for the bioremediation and sustainable development in agricultural fields. Here in this book chapter, we have discussed the microbial enzymes and their application for the sustainable development.

Keywords Microbial technology · Agriculture · Sustainable development · Enzymes

15.1 Introduction

To fulfill the global food and feed demands, the agriculture is the main component to nourish the global society (Bhatt et al. [2021a,](#page-9-0) [b\)](#page-9-1). The various crops, vegetables, and fruits have been used as a sole source of nutrition by humans globally (Bhatt et al. [2020a](#page-8-0); Goel et al. [2020;](#page-9-2) Kumar et al. [2019](#page-10-0)). The microorganism is the key component of the agricultural system (Bhatt et al. [2020b;](#page-8-1) Suyal et al. [2019a\)](#page-11-0). Microbes are found effective for the transfer of the macro and micro element from the soil to the plant rhizospheric region (Bhatt and Barh [2018](#page-8-2); Bhatt and Bhatt [2021;](#page-8-3) Verma et al. [2021\)](#page-11-1). Plant-microbe interaction makes the beneficial effects into the ecosystem (Ye et al. [2019](#page-12-0); Rawat et al. [2019;](#page-11-2) Kukreti et al. [2020](#page-10-1)). The earlier research focuses on the beneficial effects and depth of molecular mechanism involved into the plant-microbe interaction for the sustainable agricultural development (Kumar et al. [2019;](#page-10-0) Jin et al. [2019;](#page-10-2) Suyal et al. [2019b\)](#page-11-3). Microbial cells are able to produce the various extracellular and intracellular enzymes participated into contrasting biochemical mechanism (Bhatt et al. [2020c,](#page-8-4) [d,](#page-8-5) [e](#page-8-6), [f](#page-8-7)). This biochemical mechanism includes the bioremediation of toxic chemicals, complex reactions into the plant cells, and biodeterioration (Bhatt et al. [2020b,](#page-8-1) [g](#page-8-8), [h,](#page-8-9) [i](#page-8-10), [2021a,](#page-9-0) [b](#page-9-1); Lin et al. [2020;](#page-10-3) Bhandari et al. [2021\)](#page-8-11).

Microbes act as the cellular factory for the production of the enzymes (Bhatt et al. [2020a](#page-8-0), [2021b](#page-9-1)). Microbial enzymes catalyze the reactions on the basis of the preferred substrates. The complex polymer lignin has been degraded by the microbial cells using the enzymes such as lignin peroxidase and laccase. Bacterial and fungal strains have been found effective for the degradation of the wood in ecological niches. Previous researchers investigated the fungi as the potential lignin degrader. Fungal strains belonging to the Basidiomycota, Aphyllophorales, and Ascomycota are considered as the potential wood degrading fungi (Bhatt et al. [2020c](#page-8-4); Anasonye et al. [2015](#page-7-0); Zhang et al. [2020a](#page-12-1)).

Bioremediation using microbial enzymes is the most promising approach for the sustainable agricultural development (Bhatt et al. [2019a](#page-8-12), [b;](#page-8-13) Pankaj et al. [2016a](#page-11-4)). The organophosphate, carbamate, organochlorine, pyrethroids, and neonicotinoids group of the pesticides are degraded by using the microbial enzymes such as hydrolases, dehydrogenases, laccases, monooxygenases, and dioxygenases (Mishra et al. [2020;](#page-10-4) Pankaj et al. [2016b](#page-11-5)). Immobilized enzymes have been documented for their more potential approach as compared to the individual strains (Bhatt et al. [2019c,](#page-8-14) [2020i;](#page-8-10) Pankaj et al. [2015\)](#page-11-6).

15.2 Microbial Enzymes Used in Bioremediation Process

15.2.1 Microbial Oxidoreductases

Microorganisms (bacteria and fungi) use various oxidoreductase enzymes and reduce the toxicity of the various organic contaminants by oxidation coupling reactions. During the reaction, electrons are transferred from the reduced substrate (donor) to another substrate (acceptor) by cleaving the bonds and generating energy. So, in that way the toxic compounds are converted into the less toxic compounds (Mishra et al. [2021\)](#page-10-5). Oxidoreductase enzymes have a wide range of activity for degradation of different organic substrate such as azo dyes, pesticides, lignin, and polyhydrocarbons (Karigar and Rao [2011](#page-10-6); Park et al. [2006;](#page-11-7) Bhatt et al. [2015\)](#page-8-15).

15.2.2 Microbial Oxygenases

Oxygenase enzymes belong to oxidoreductase family (Huang et al. [2021\)](#page-10-7). It oxidizes the reduced substrate by adding oxygen from the molecular oxygen in the presence of co-substrate (FAD/NADH/NADPH). Generally, the oxygenases are subdivided into two groups on the basis of number of molecules of oxygen used for the oxidation of substrate. If one molecule of oxygen is added in the substrate, it is called monooxygenases, while when the two molecules of the oxygen are added in the substrate, it is called dioxygenases. These are the two enzymes which play key role in the metabolism of the various organic substances by increasing the substrate reactivity and solubility in water and help to break down the cyclic ring structure of the chemical compounds by adding oxygen molecule. Most of the enzymes used in the bioremediation process are mono-oxygenases or di-oxygenases (Arora et al. [2009;](#page-7-1) Singh et al. [2021](#page-11-8)).

15.2.3 Microbial Laccases

Laccase (*p*-diphenol:dioxygen oxidoreductase) is a multicopper protein generally found in plants, fungi, and bacteria. Laccase has a wide range of activity that catalyzes the oxidation of reduced substrate with concomitant oxidation reaction coupled to four electron reduction of molecular oxygen to water (Gianfreda et al. [1999;](#page-9-3) Mai et al. [2000;](#page-10-8) Zhang et al. [2020b\)](#page-12-2). Laccase has multiple form of isoenzymes encoded by different genes (Mishra et al. [2020](#page-10-4); Giardina et al. [1995](#page-9-4)). Many microbial cells are reported to produce extracellular or intracellular laccase enzyme. Bacillus subtilis produces laccase which help to degrade the pesticides present in agricultural fields (Gangola et al. [2018](#page-9-5)). Laccase helps to metabolize the organic contaminant and provides the nutrients for the microorganisms (Kim et al. [2002\)](#page-10-9).

Among all the oxidoreductase enzymes, laccase has the biotechnological and bioremediation applications (Karigar and Rao [2011;](#page-10-6) Gianfreda et al. [1999\)](#page-9-3).

15.2.4 Microbial Peroxidase

Microbial peroxidases are highly oxidizing agents that oxidize lignin, lignocellulose, and other phenolic substrate into substances devoid of hydrogen atom. Multiple isoenzymes of the fungal associated peroxidases are found such as lignin peroxidase and manganese peroxidase (Bansal and Kanwar [2013;](#page-8-16) Cocco et al. [2017](#page-9-6)). Degradation of lignocellulose is a very important step for the carbon recycle in land ecosystem. Fungi generally basidiomycetes are known to degrade lignin and help in the carbon recycling process. White and brown rot basidiomycetes digest the lignocellulose as a sole source of carbon. Manganese peroxidase is an extracellular enzyme, generally found in the basidiomycetes, which also helps in digestion of lignin and other phenolic compounds. For initiating the activity of manganese peroxidase, Mn^{2+} acts as substrate and helps to oxidize phenolic compounds (Lin et al. [2020](#page-10-3); Zhang et al. [2020a](#page-12-1); Chowdhary et al. [2019](#page-9-7); Feng et al. [2020](#page-9-8)).

15.2.5 Hydrolases

Hydrolytic enzymes reduce the toxicity of xenobiotic compounds by breaking the linkage in between. Hydrolase enzymes are also known to degrade oil spill and agricultural pesticides degradation (Karigar and Rao [2011\)](#page-10-6). The key feature of the enzyme is its broad range of activity, availability, and high tolerability. The enzymes such as lipase, DNases, proteases, xylanases, and pullulanase are the hydrolytic enzyme generally used in food industry and biomedical sciences. The hemicellulase, cellulase, and glycosidase are the hydrolytic enzymes and actively participate in biomass degradation (Peixoto et al. [2011](#page-11-9); Thakur et al. [2019](#page-11-10)).

The microbial systems are found effective for the development of the sustainable agriculture by using the enzymes. Application of enzymes in agricultural fields accelerates the agricultural wastes and helps in resource recovery from the environment. Here we are enlightening the impact of the microbial enzymes for their resource recovery potential. Accumulation of different kinds of wastes has become a severe environmental and public concern. It is urgent to develop innovative approaches for the removal/degradation of disposal. Microbial enzymes are extremely effective to catalyze the biochemical reaction. Microbial enzymes have achieved importance for their extensive utilization in food, agriculture, and pharmaceuticals. These enzymes are capable of degrading complex compounds into simpler ones through the process of bioremediation. The researchers focus on the resource recovery potential of the microbial enzymes. These enzymes could be used directly for the xenobiotics compound degradation and cleaning of soil and water environments Zhan et al. [2020;](#page-12-3) Ye et al. [2019;](#page-12-0) Huang et al. [2019;](#page-9-9) Danso et al. [2018;](#page-9-10) Fan et al. [2020\)](#page-9-11).

15.3 Importance and Mechanism of Microbial Enzymes in Maintenance of Soil Health

Soil enzymes secreted by microbes are natural mediators which catalyze many processes like organic matter decomposition, soil humus formation, and release of different minerals involved in different cycles and help in the maintenance of soil health and plant growth (Wallenstein et al. [2012](#page-11-11); Khati et al. [2019\)](#page-10-10). Thus, it is crucial to find out the enzymatic activities of soil enzymes and their mechanism to illustrate metabolic prospective of soil fertility (Astner et al. [2020](#page-7-2)). Activity of enzymes is an insightful marker; any changes in soil environment can affect their activity in agricultural farming (Chaudhary et al. [2021\)](#page-9-12). There are different bacterial and fungal enzymes which involve in the breakdown of complex form of mineral nutrients into simpler ones which are easily taken up by plants and enhance their growth (Kukreti et al. [2020;](#page-10-1) Khati et al. [2017\)](#page-10-11). Kwiatkowski et al. [\(2020](#page-10-12)) reported that dehydrogenase, urease, and protease activity improves the fertility of soil and biological properties of soil and involves in nutrient cycling (Kwiatkowski et al. [2020](#page-10-12)).

Cellulose is a largely abundant polysaccharide which is hydrolyzed by cellulase enzyme into D-glucose. It consists of three enzymes such as endo-1,4-β-glucanase which attacks at random on cellulose chain, exo-1,4-β-glucanase which removes glucose/cellobiose from cellulose chain, and β-D-glucosidase which hydrolyses cellobiose into glucose. These enzymes are found in various bacteria and fungi such as Bacillus subtilis, Aspergillus niger, Phanerochaete chrysosporium, Clos-tridium thermocellum, and Poria placenta (Deng and Tabatabai [1994\)](#page-9-13). Amylase enzyme is involved in the breakdown of starch into glucose and maltose which also play an important role in carbon nutrient cycling. This enzyme is secreted by various bacterial and fungal species in soil such as Pseudomonas, Bacillus spp., and Aspergillus niger. Arylesterase enzyme helps in degradation of complex sulfate esters into sulfur which takes part in sulfur cycle. Klebsiella spp., Raoultella spp., and Trichoderma sp. release this enzyme in soil and helps for maintenance of soil fertility and plant development.

Phosphorus is usually entrapped in the complex soil system and becomes unavailable for plant uptake. To enhance the phosphorus bioavailability, phosphatase enzyme is involved. The conversion of organic P to inorganic P by microbial associated *phosphatase* activity leads to P cycling. *Phosphatase* activity can be measured as acid and alkaline phosphatase at pH range 4–6 and 9–11, respectively. After the action of such enzymes, the available P (inorganic P) had good correlation with nutrient transport and plant vigor. Phosphatase enzyme is involved in the conversion of insoluble form of phosphate into soluble form and helps in phosphorus cycling (Khati et al. [2019\)](#page-10-10). Phosphomonoesters and phosphodiesters are involved in

Fig. 15.1 Mechanism of different soil enzymes in nutrient cycling

the breakdown of sugar phosphates and phospholipids and release free phosphate from phosphate diester (Turner and Haygarth [2005](#page-11-12)). Alkaline and acid phosphatase are good indicators of soil microbial activity and correlated to organic content of soil. These enzymes are secreted by Anabaena oryzae, Bacillus, Pseudomonas, and Penicillium (Pankaj et al. [2016a;](#page-11-4) Khati et al. [2019,](#page-10-10) 2017). Various factors are involved for phosphatase activity: (1) if pH increases the activity of alkaline, P increases, while acid phosphatase activity reduces significantly and vice versa; (2) water content of the soil is better linked with alkaline phosphatase activity as compared to the acid phosphatase activity; and (3) salinity stress is better correlated with alkaline phosphatase activity in all seasons.

Urease enzyme takes part in the conversion of urea into ammonia and $CO₂$ with rise in pH and helps in nitrogen cycle which is produced by fungi, bacteria, and yeast such as *Pseudomonas* and *Trichosporon cutaneum*. These enzymes are used as biological indicators because they are influenced by soil factors like organic matter content, heavy metals, and nanocompounds and due to the cropping history (Fig. [15.1](#page-5-0)). Β-glucosidase enzyme is the most common enzyme in soil which catalyzes the hydrolysis of glucosides present in soil. This enzyme activity was observed in various plant species and microbes such as Flavobacterium, Trichoderma spp., and Lactobacillus plantarum (Kwiatkowski et al. [2020](#page-10-12)).

For the health and sustainable soil system, the soil enzymatic activity plays a vital role in nutrient cycling. To establish system for organic matter decomposition to channelizing available nutrient to the plant system, the soil enzyme system plays a key role. Different enzymes are available with the soil system that showed overall health of the soil system. Dehydrogenase (DHA) enzyme is one of them that plays a significant role to indicate the oxidative power of the soil system associated with the existed microbiome (Zhang et al. $2020a$). Dehydrogenase is a subclass of *oxidore*ductase enzymes that catalyze the oxidation and reduction process. DHA activity is directly linked with the soil organic matter (SOM) that is available for microbial activity.

The enzymatic activity of the microbes in soil can be measured by fluorescein diacetate (FDA) hydrolysis assay. In FDA analysis, membrane-bound nonspecific kind of enzymes like esterases, proteases, and lipases hydrolyzes the 3,6-diacetyle fluorescein (FDA). After hydrolysis, a yellow green color is produced that can be quantified by using spectrophotometer at 490 nm (Gilan et al. [2004](#page-9-14)). The total enzymatic activity (TEA) including oxygen utilization, microbial biomass, amount of ATP, etc. can be directly correlated with the FDA hydrolysis (Dzionek et al. [2020\)](#page-9-15). Soil protease enzyme is one of the major enzymatic actions involved in N-cycle due to availability of huge protein content in the soil system. The protein components of organic nitrogen in the soil system are usually hydrolyzed by protease enzyme action. In nitrogen cycle, degradation of protein is an essential component. Protease enzyme is mainly identified in microorganisms that are responsible for conversion of protein to polypeptide to amino acids (de Morais et al. [2018\)](#page-10-13).

15.4 Circular Economy for SPs and Sustainable Development

Synthetic pollutants (SPs) have a detrimental effect on the environment and longterm economic aspects. The existing unsustainable "linear" material and energy flow model are also prominent contributing factors to these issues (Blomsma and Brennan [2017\)](#page-9-16). However, in recent years, the sustainable development strategies have been designed for the reduction of the SPs from the environment that favors economic security without simultaneously degrading the environment. The concept of the circular economy has become a promising alternative model for sustainable development by reducing the SPs and promoting ecological design. The circular economy aims at minimizing waste production, enhancing product life, optimizing reuse, and utilizing energy sources such as biomass (Saldarriaga-Hernandez et al. [2020\)](#page-11-13).

The methods used for bioremediation of the SPs can be based on the cyclical flow of the resources. The bacteria, fungi, and algae are used in a circular perspective for the bioremediation of the SPs from soil and water systems. Microbial strains can use the SPs as a source of nutrition for their growth and development and convert the toxic metabolites into the environmentally accepted form. The metabolic end products can be exploited for the production of useful metabolites and as a resource for recovery for value-added products. Aerobic and anaerobic digestion of SPs generates humus and digestate which could be applied to soil as a fertilizer. During the anaerobic digestion process, biogas that is rich in methane and carbon dioxide is produced, which could be used as a fuel for combustion in transport or energy production (Soobhany [2019](#page-11-14)). Various volatile fatty acids are also formed as intermediates during the anaerobic degradation process and can find various industrial applications (Singh et al. [2012\)](#page-11-15). Bio-electrochemical systems (BESs), integrating microbial–electro-chemical removal mechanisms, have been intensively investigated for the organic compound removal in wastewater and simultaneous generation

of electricity and biofuel. Different microbial strains can also synthesize biopolymers by utilizing various synthetic pollutants (Pagliano et al. [2017](#page-10-14)). New emerging strategies are also allowed production of value-added products such as enzymes (Steiner and Schwab [2012](#page-11-16)), single-cell oil (de Oliveria Finco et al. [2017\)](#page-10-15), and building block chemicals (FitzPatrick et al. [2010](#page-9-17)) from biodegradation end products. The methods used for the conversion of the SPs from the environment when aligned in a sustainable model can generate more fruitful results in the long-term future. However, still, it is a big challenge to convert the SPs into the environmentally accepted form and use them for the production of the value-added products.

15.5 Conclusion

Many xenobiotic compounds are present in our surrounding environment which are released from the different source such as textile industry releases many azo dye and excess application of pesticides in agricultural field. The physical and chemical techniques are available to remove these xenobiotic compounds from the environment. However, these techniques are very costly and expensive. Using the microbial system for the degradation of these xenobiotic compounds is ecofriendly and costeffective technique. Application of indigenous microorganism transforms the toxic xenobiotic compounds into less toxic or nontoxic and environmental accepted form. These microorganisms produce different class of extracellular and intracellular enzymes which help to metabolize the environmental pollutant and use them as a source of energy. Generally, the enzymes such as lipase, esterase, laccase, dehydrogenase, and oxygenase play a key role in bioremediation processes. In a similar way, microbes are also playing key role in the agricultural fields. They produce different plant growth-promoting hormones, solubilize the insoluble compounds, and make them available for the plants and enhance the plant productivity and quality. Therefore, the use of microbial enzymes is important for the development of sustainable environment. In addition to that, this microbial technique is efficient, cost-effective, and ecofriendly.

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