



Microbial Enzymes Based Technologies for Bioremediation of Pollutions

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Abstract

Environmental pollution comes from a variety of sources. With the development of human civilization, the development of technology, and the increasing population, the world is now facing environmental pollution. Since environmental health has a direct effect on human health, therefore environmental protection is one of the most essential human problems. Removal of pollutants is a significant issue that, if not paid enough attention to, the next generation will face serious problems. Chemical and biological methods can be used to remove contaminants, but since the use of chemical methods will result in wastes that can cause contamination, the use of biological treatment methods such as bioremediation is a better and less dangerous to remove contaminants. In the bioremediation process, fungi or bacteria and their enzymes are used to clean and purify pollution. In this waste management technique living organisms or their enzymes uses to remove or neutralize contaminants. Mechanisms of enzymes that related bioremediation such as hydrolases and oxidoreductases have been widely studied. This chapter investigates information on the microbial enzymes from different microorganisms involved in the biodegradation of a broad range of pollutants.

Keywords: Microbial enzymes, Bioremediation, Pollution, Environment

1 Introduction

The quality of life on earth is directly related to the overall quality of the environment. Unfortunately, with development in technology, science, and industry, a large number of nuclear waste and raw sewage is dumped or let out into the environment and therefore causing an earnest difficulty for human survival on earth. The advancement of science and the more significant presence of industries in life have caused entrance much pollution in the distribution environment. Disposal of waste and pollution in the traditional way causes a lack of space for the subsequent disposal of the rest of the waste. In the past, garbage was traditionally drilled in the pit and then disposed of with waste. This method of waste disposal was faced with a lack of space each time the waste was discharged. New waste disposal technology also uses chemical decomposition and incineration at high temperatures, such as UV oxidation methods and base-catalyzed dechlorination. Although these methods can be useful in reducing pollutants, they still have problems [1-3]. It is essential to control the types of pollutants and prevent the diseases' spread [4]. Any substance that inadvertently enters the environment is known to be a pollutant. The damage that pollutants cause to the environment through natural or human resources is pollution. Human societies, due to their increasing activities, cause water, air, and soil pollution, which somehow enter the pollution to human food chain. Urbanization and industrialization are also spreading a lot, which causes a lot of pollutions [5]. Environmental pollution, including soil and water pollution, is caused by industrial and agricultural processes that pose serious risks to human health and have devastating consequences for the ecosystem [6-8].

Enzymes are the materials that can change the reactions rate and reaction activation energy; however, they do not

present in the reaction. Enzymes can be active in wide ranges of environmental conditions changes e.g., changes in pH and temperature [9]. Enzymes, due to their catalytic role, have an essential position in metabolic and biochemical reactions. Among biological agents, enzymes have a remarkable ability to detoxify and effectively convert contaminants because they can break down contaminants very quickly. In general, enzymatic methods promise to remove contaminants in the form of biodegradation [10-13]. There are many microbes such as bacteria, fungi, and yeast can produce enzymes. Microbial enzymes that produce by microorganisms have high performance, efficiency, and easy way of production. Microorganisms are the primary source of enzymes, because to reinforce enzyme production, genetic manipulation on microorganisms cells such as bacteria cells can occur [14-16]. One of the ways to control pollution is bioremediation. Bioremediation is a process that uses microorganisms or plants or their enzymes for treating the pollutions [17, 18]. Only some pollutants are biodegradable, and bioremediation is a process in which biological destruction occurs with the interaction between microorganisms and pollutions. For the bioremediation process, the environmental conditions should be suitable for the growth of microbes, and enough nutrients accessible for microbes. The aim of the chapter is to express the use of modern methods of enzyme microbes to treat contamination. Enzymatic microbes are biocompatible and biodegradable contaminants

2 Enzymes

Enzymes are the categories of biological catalysts that have at least one polypeptide section [1]. The use of enzymes for eliminating contamination, resolves many of the disadvantages

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posed by eliminating contamination by microorganisms. Also, the use of enzymes has superiority rather than traditional methods and microbial remediation. Microbial metabolism inhibitors that affect the inhibition of microbes do not affect the inhibition of enzymes. Enzymes could continue to function under severe and restrictive conditions imposed on microbial activity. Enzymes can also affect deficient concentrations of pollutants and are active in the presence of antimicrobial agents. Microorganisms prefer to destroy contaminants that decompose quickly, but enzymes are smaller than microorganisms, so they have more movement and can destroy contaminants with a high degree of hardness. Some of the enzymes such as hydrolases, dehalogenases, transferases, and oxidoreductases involved in refining pollution [10, 19, 20]. For example, esterases, haloalkanes, and glutathiones are in the categories of hydrolases, dehalogenases, transferases, and oxidoreductases, respectively. Esterases are in the category of hydrolases enzymes, that they can form ester bonds by catalyzing the cleavage. Esterases have the ability to degrade natural compounds or industrial pollutants, due to stereoselective action [21]. Haloalkanes are in the category of dehalogenases enzymes. These enzymes can catalyze halogenated aliphatic pollutants such as chlorine, bromine, and iodine, and convert to alcohol, halide, and a hydrogen ion. Haloalkanes have industrial and bioremediation applications [22-24]. Glutathiones are in the category of transferases enzymes and found in the cytosol. These enzymes have a wide range of abilities in induction biological stress, and they are involved in antioxidant attacks [25, 26]. Oxygenases belong to the oxidoreductases group of enzymes. These enzymes can transfer oxygen from an oxygen-containing molecular and could oxidize the substrates. Oxygenases can degrade or detoxification compounds [27-31]. According to Figure 1, enzyme have the polyelectrolyte wall; the molecule that must be degraded could be exposed to the polyelectrolyte wall and allowed to penetrate.

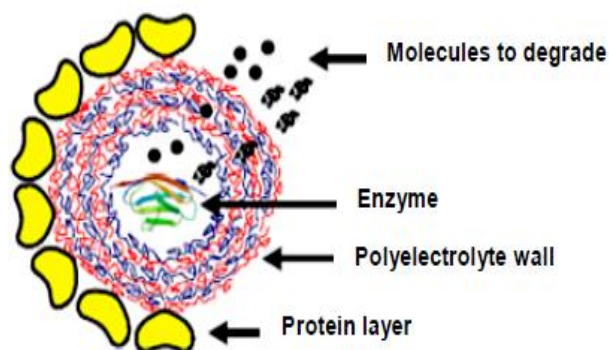


Figure 1: Degradation of molecules by enzymes [32]

Active sites of enzymes involved in catalytic processes; in other words, enzymes by creating covalent or noncovalent bonds, could involve in catalytic processes. Enzymes could involve in the bioremediation of pollutants by the biodegradation process. Enzymes could include in the six section that includes oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Oxidoreductases enzymes could change the place of electrons and protons from a transferor to a receptor. The transferases enzymes could transfer one of the functional groups from a transferor to a receptor. The hydrolases enzymes help for the splitting of bonds by water. The lyases enzymes could catalyze the split of bonds by removing them. Isomerase enzymes help for retuning

of geometric or structural. Ligases enzymes help for joining each other of two molecules [1]. Mohsenzade et al. surveyed enzymatic activity in some fungi for application in bioremediation of pollution of petroleum. In this work, three enzymes include Catalase, Peroxidase, and Phenol Oxidase, were selected, and their activity checked in the fungal. The ability for bioremediation of fungal enzymes was surveyed on the soil of petroleum. Results show that enzymes of fungi had good resistance for the removal of pollutants. In other words, they had the good ability to grow petroleum pollutants. In the bioremediation process, they could decrease the number of pollutants. The highest enzymatic activity belonged to *Aspergillus terreus* fungi [33]. Redox enzymes have the ability to transmission pollutions in the infected sites; for example, chromate reductases are in categories of redox enzymes such as *Pseudomonas putida* and *Escherichia coli* that have the ability to catalyzing Cr (VI) to Cr (III), which has less solubility and toxicity. In the redox cycle, one of the electrons transfer to oxygen and could produce reactive oxygen species (ROS). The existence of ROS and oxidative stress, reduces contaminated chromium, and reduces pollution [34, 35]. Extracellular enzymes are the category of enzymes that are secreted by microbes. These enzymes can convert a composition from a tenacious mood to a more biodegradable state. Extracellular enzymes have the ability to increase the degradation rate [9]. Novotny et al. used Ligninolytic fungi as extracellular enzymes for bioremediation of water and soil pollutants. Ligninolytic fungi's included Mn-dependent peroxidase, lignin peroxidase, and laccase that investigated for degradation of hydrocarbons, polychlorinated biphenyl mixture, and dyes. Results showed a high amount of Mn-dependent peroxidase could remove Reactive Orange 16 azo. Mn-dependent peroxidase, lignin peroxidase, and laccase could degraded soil pollution such as anthracene and pyrene. These enzymes have better efficiency in liquid media compared to soil media [4].

2.1 Microbial enzymes

It has been a long time that industrial product makers utilize microbial enzymes as the main catalysts to transport raw materials into end products [36]. Microbial enzymes act as biocatalysts and catalyze biochemical and metabolic reactions in an environmentally friendly manner. Microbial enzymes are known as special enzymes due to their various industrial and medical applications, having stability, owning catalytic role, and ease of production [37, 38]. Microbial enzymes have many advantages in comparison with chemical catalysts; for this reason, microbial enzymes, including very interesting biocatalysts that have been widely studied [39]. Microbial enzymes have applications in many areas, such as food production, pharmaceuticals, agriculture, fermentation, and chemical processes [40]. Microbial enzyme generation focuses on simple hydrolytic enzymes that can degrade natural polymers such as pectin, starches or proteins, starches. These enzymes include pectinases, proteases, and amylases. The microorganisms for further use of enzymes, the enzymes secreted into the nutrient medium. These extracellular enzymes for feeding the microorganisms, separate the huge molecules of the substrate into smaller molecules [41]. For example, oxidoreductases are the type of microbial enzymes which exist in microbes, plants, and animals. In enzyme Commission (EC), oxidoreductases category as EC 1. These enzymes have the ability to exchanging of electrons between electron donor molecules and electron acceptors substrates. In this process, oxidative and reductive reactions occur and oxidizing and reducing materials could be active or transform [42]. These enzymatic reactions require the presence of electrons or

hydride ions [43]. Oxidoreductases are transferred with cofactors such as heme groups; flavin group includes flavin mononucleotide and flavin adenine dinucleotide and metal ions [43, 44]. Generally, oxidoreductases, that can produce smaller molecules, versus bacteria are reactive [45]. Cheriyan et al. investigated enzymatic bioremediation for cashew nut shell liquid contamination. In this work, oxidoreductases and proteases enzymes were used for bioremediation of cashew nut shell liquid (CNSL). Peroxidase enzymes reduced the color of the cashew nut shell liquid solution, and protease degraded the phenolic of solution [46]. As shown in Figure 2, there are different types of oxidoreductases include oxidases, dehydrogenases, and hydroxylases.

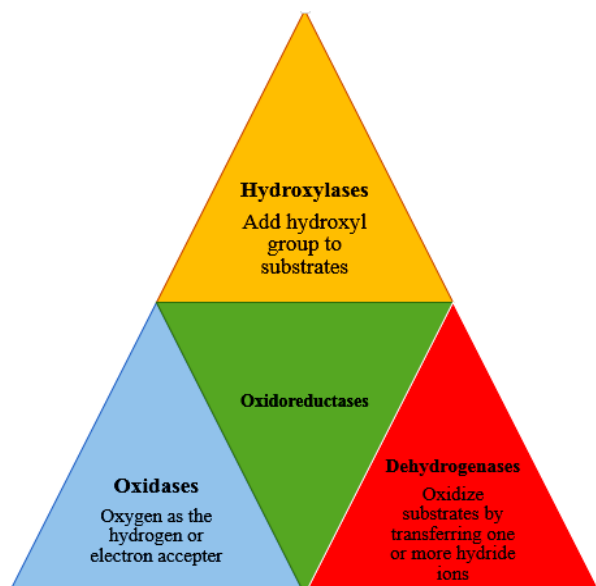


Figure 2: Types of oxidoreductases enzymes [42]

2.1.1 Oxidases

Oxidases can catalyze the reactions by using dioxygen molecules in the role of electron acceptor [47]. In other words, oxidases use molecular oxygen as an electron acceptor [48]. Oxidases use different materials such as metals and cofactors for transferring electrons. These materials include flavin or alcohol-based metals or amine oxidases [49].

2.1.2 Dehydrogenases

Dehydrogenase enzymes catalyze the reaction with coenzymes such as nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP), flavin group, and flavin mononucleotide (FMN) [47]. All living microorganisms contain the dehydrogenase enzyme, which transports hydrogen atoms from organic transporters to electron acceptors substances [50].

2.1.3 Hydroxylases

Hydroxylases enzymes have active sites containing metal that can catalyze the reactions [51, 52]. The metal in the activator role changes the oxidation state of the substrate. Iron metal is commonly used to change the oxidation state, and the metal also transmits its electrons through the electron transfer chain [52]. Iron metal can transfers oxygen to compound or form OH radicals [53]. In Table 1, application of oxidoreductases enzymes are shown.

3 Bioremediation

Bioremediation is a process that occurs by using microorganisms that pollutants could transit or degrade into

low-degree pollution. Bacteria, fungus, algae, and plants use in this method. In other words, enzymes of these microorganisms attack the pollutants and convert them to low-risk pollutants [1, 58]. In bioremediation after degradation of pollutants, the society of microorganisms reduced, and the presence of microorganisms don't create more pollutions [4]. Bioremediation is a new technology which can be used for treatment of a different group of environmental pollutants with other physical and chemical methods of treatment, simultaneously [59].

Table 1: Microbial enzymes applications

Enzyme	Application	Ref
Oxidoreductases	Biotransformation of lignocellulosic biomass, Derivatization of carbohydrates, Food industry, Environmental protection, Synthesis of organic materials, Synthesis of nanomaterials and polymers, Synthesis of medicinal materials, Biocatalysts for bio-based economy, Textile industry, Cleansing applications, Biodegradation, Biodetoxification and bio-decontamination, Biosensors and bioreporters, Medical applications, Coenzymes regeneration, Medical applications, Oxyfunctionalization of organic substrates	[43, 54-57]

Zamanpour et al. investigated bioremediation and photolysis of enrofloxacin. Structured packing rotating biological contactor was designed and created as a biological setup. The removal rate reached a maximum of 70% only for the biological unit. Different concentrations of enrofloxacin were examined on the biological unit. At the starting point of the process removing of the drug were equal to 70%, but by spending the time, it decreased to 40% [60]. The bioremediation process happens with the presence of microorganisms which this method has some advantages and disadvantages are tabulated in Table 2. In Figure 3 shown a bioremediation process that plants, bacteria, and fungi could remove pollutions and create green earth with a low amount of pollutions.

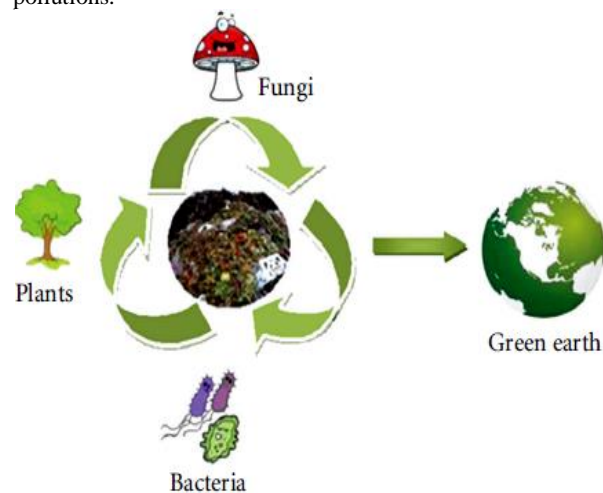


Figure 3: bioremediation process [1]

Ali et al. investigated the bioremediation of soils with crude oil. Desert soil (17.3%, w/w) was saturated with crude oil. Results after one month showed that 53-60 % of the oil was eliminated. After five months, 14-20 % of the oil was eliminated. Microorganisms such bacteria's presented in a polluted site that could be act in oil saturation conditions [68].

Table 2: Advantages and Disadvantages of Bioremediation process [1, 4, 61-67]

Advantages	Low cost
	Environmental friendly
	Easy method
	A safe method for the environment
	Socially acceptable method
	Combination with other treatment methods
	Removing operating and transportation costs
	Minimal disruption in the ecosystem
	Madding directly on the site
	Self-sustaining
Disadvantages	Not producing waste
	Having the capability to degrade many Contaminants
	Needing an average level of fund investiture
	Low speed of the process
	Using special species of bacterias and fungi
	Limited to biodegradable compounds
	Hard to do in piloted field and difficult of implementing the method on an industrial scale
	Needing more efforts for training and education to general success acceptance
	Retransfer of pollutants previous stabilized by changing geochemical and hydrological situations
	Migration of probable pollutant and its transfer via the environment
	Required to process a control formalize for a long-term conservation due to long time of bioremediation process
	Needing more nutrients or oxygen for doing the bioremediation process on clay soils
Destroying products and may creating products with more toxicity	
Limitation of the method to biodegradable products	
Needing for essential site factors for achievement include appropriate levels of nutrients and contaminants, suitable environmental growth conditions, and the presence of metabolically capable microbial populations	
Complicated controlling volatile organic compounds (VOCs) in an ex-situ process	

3.1 Microbial bioremediation

Microbial bioremediation are a process that use spent biomass, enzymes, or microorganisms for removing environmental pollutions. Pollutants in the various environmental sections, always have contact with microorganisms due to the microorganisms' existence everywhere. Microbes allow the pollutant to be channeled into the normal microbial metabolic pathway for biotransformation and degradation. Microbes transform or break down the pollutants via their intrinsic metabolic processes with or without slight pathway modifications. In the bioremediation process, by using naturally occurring microbial catabolic capabilities, most of the synthetic compounds such as metals, radionuclides, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and hydrocarbons (e.g., oil) can be accumulated, transform or degrade [19, 69]. Microbial bioremediation includes proceccos such as aerobic process, anaerobic process, and combination of these two methods. Cellules that need the oxygen molecules presence during the cellular processes named aerobic, and cellulose that do not require for the presence of oxygen molecules named anaerobic [4, 70]. In figure 4, illustration how microbes, remove pollutions into the environment, is shown. According to this figure, microorganisms include microbes adsorb pollutions into their active cites and degrade the pollutions and convert them in other forms and finally remove the pollutions. The bioremediation process is a complicated method and depends on biological and environmental factors shown in Table 3. These factors detect the kinetics of degradation. Amount of humidity effects on the rate of pollutions metabolism.

3.2 Bioremediation of hospital biomedical waste

Hospital wastes like anatomic wastes, solid wastes, disposals, sharps, etc. are produced via different process occur in the hospitals such as research activities and immunization. These generated wastes can be harmful to human and contains infectious diseases if not administered with a suitable method. Bacterias have the ability to remediate liquid waste that maybe become a good option for remediation of medical waste [71, 72]. Hospital biomedical waste, which mainly consists of organic matter, can be amplified by microbial hydrolytic enzymes that have high potential. Bacteria capable of producing hydrolytic enzymes such as amylase, protease, and lipase, are needed to remove hospital contaminants [73]. In Figure 5 shown stages of bioremediation of hydrolytic bacteria isolated from medical waste. According to the figure, at first should choose the hydrolytic bacteria that has more ability to be used as a bioremediation agent [74].

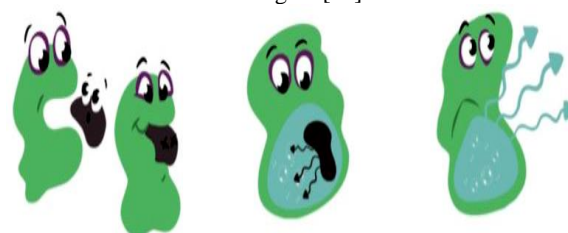


Figure 4: Bioremediation process [11]

Table 3: Effective factors on microbial bioremediation

Environmental factors	Occurring biodegradation process in optimal pH
	The optimum temperature for detection of the speed of bioremediation process
	Amount of humidity that effecting on the rate of pollutions metabolism
	Effect of soil structure on biodegradation factors and adverse effect of moisture in the structure
	Solubility in water
	Availability of essential nutrients for microbial growth and biodegradation rate
	Site Characteristics for detection of the horizontal and vertical amount of pollution
	Redox potential
	Amount of oxygen for reinforcement of hydrocarbon metabolism
	Contaminant concentration
	Type of microorganism
	Type of pollutions
	Geological and chemical conditions at polluted sites
Biological factors	Mutation
	Horizontal gene transfer
	Enzyme activity
	Interaction

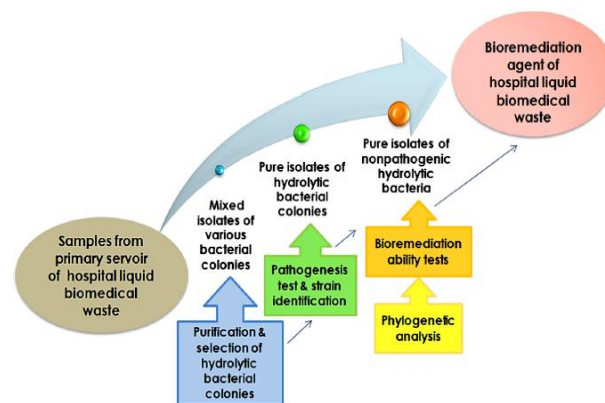


Figure 5: Bioremediation of hydrolytic bacteria isolated from medical waste [74]

4 Conclusion

The pollution of water and soil created by petroleum hydrocarbons and industrial chemicals, is an essential matter in the whole world. Because of the high usage of mentioned materials, they are involved as environmental pollutions in numerous terrestrial and aquatic ecosystems. The use of bioremediation method for removing these pollutions, provides an economical and safe economic method to commonly used physical-chemical treatment. Physical and chemical technologies have high costs when using on a large scale. By utilizing the massive variety of metabolic pathways and biological activities presented by microorganisms, new strategies can be encountered. Using the activity of enzymes is an essential step in the utilization and degradation of pollutions. Many enzymes from plants, fungi, and bacteria have been reported to be involved in the biodegradation of toxic organic pollutants. Bioremediation is a nature-friendly biotechnology and cost-effective that is applied by microbial enzymes. The research activity in this field would help to develop advanced bioprocess technology to achieve new useful substances and reduce the toxicity of the pollutants.

Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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