Microbial Peroxidases and their applications

Divya Ghosh, Saba Khan, Dr. Sharadamma N.

Divya Ghosh is currently pursuing master's program in Life Science in Mount Carmel College, Bangalore, India, Ph: 9774473747; E-mail: ghoshdivya497@gmail.com; Saba Khan is currently pursuing master's program in Life Science in Mount Carmel College, Bangalore, India, Ph: 9727450944; E-mail: sabakhan23.03@gmail.com; Dr. Sharadamma N is an Assistant Professor in Department of Life Science, Mount Carmel College, Autonomous, Bangalore, Karnataka- 560052. E-mail: sharadamma.n@mccblr.edu.in

Abstract: Peroxidases are oxidoreductases that can convert many compounds into their oxidized form by a free radical mechanism. This peroxidase enzyme is produced by microorganisms like bacteria and fungi. Peroxidase family includes many members in it, one such member is lignin peroxidase. Lignin peroxidase has the potential to degrade the lignin by oxidizing phenolic structures in it. The microbes that have shown efficient production of peroxidase are Bacillus sp., Providencia sp., Streptomyces, Pseudomonas sp. These microorganisms were optimized to produce peroxidase efficiently. These microbial strains were identified by 16S rDNA and rpoD gene sequences and Sanger DNA sequencing techniques. There are certain substrates on which Peroxidase acts are guaiacol, hydrogen peroxide, etc. The purification of peroxidase was done by salt precipitation, ion-exchange chromatography, dialysis, anion exchange, and molecular sieve chromatography method. The activity of the enzyme was evaluated with different parameters like enzyme activity, protein concentration, specific activity, total activity, the effect of heavy metals, etc. The quantification of protein was done by Lowry's method and kinetic studies with enzyme immobilization being done. However, despite the enzyme's versatility and its environmental usage, many limitations like, heterologous production, catalytic stability, and redox potential must be controlled to execute peroxidases for transformation at a large scale and also in the bio-elimination of pollutants. This review article emphasizes the novel potentialities of peroxidase to catalyze the transformation of different toxic pollutants which exist in the environment and aims to decrease their impact on nature.

Keywords: Lignin, Peroxidase, Guaiacol, Hydrogen peroxide

1. Introduction

Hazardous aromatic compounds are usually found in wastewater discharged from textile, paper, and pulp industries, coal, plastics, resins, petroleum refining industries iron and steel manufacturing units, and agricultural activities. Lignin is defined as a structurally complex molecule that can be entirely degraded by only some microorganisms. Lignin is an industrial by-product that is found mainly in pulp and paper waste, agricultural residue, and also in hydrolytic industries [1], [2], [3]. Lignin comprises phenolic compounds, which are further composed of three groups, i.e., guaiacyl (G), syringyl (S), and hydroxyphenyl (H). These undergo polymerization via cross-linking carried out by an enzymatic reaction. The reaction gives rise to β-O-4 bonding and C-C and C-O bonding [4]. Microbial cells are considered as factories that operate as a collection of efficient molecular machines. Microorganisms make use of enzymes to perform several different functions. Hydrogen peroxide is a comparatively simple and mild oxidizing reagent. Hydrogen peroxide is a highly advantageous reagent as its breakdown leads to the production of water and oxygen as by-products and no inorganic salts are produced, unlike other oxidizing reagents. Peroxidases are enzymes that are known to catalyze oxidation-reduction reactions, thus belong to the oxidoreductase class of enzymes. Peroxidase includes a group of specific enzymes such as NAD peroxidase, fatty acid peroxidase, glutathione peroxidase, and non-specific enzymes from the different sources is simply known as peroxidases. Peroxidases may be or may not be heme-containing enzymes. It catalyzes the transfer of oxygen from hydrogen peroxide to a substrate thereby oxidizing the substrate and reducing hydrogen peroxide in the process. Superoxide and hydroxide radicals are toxic molecules that are found in aerobic organisms [5]. The molecular weight of peroxidase is 11.45 kDa. Hydrogen peroxide acts as an electron acceptor for catalyzing different oxidative reactions. The specificity and biological functions vary with the source of the enzyme. For example, extracellular peroxidase is a crucial component in extracellular lignin-degrading microorganisms responsible for the initial attack of lignin by a non-specific oxidative mechanism.

It has been found that peroxidase has a great potential for bio remedial processing of wastewater that is contaminated with phenols, bioleaching in the paper industry, textile dye

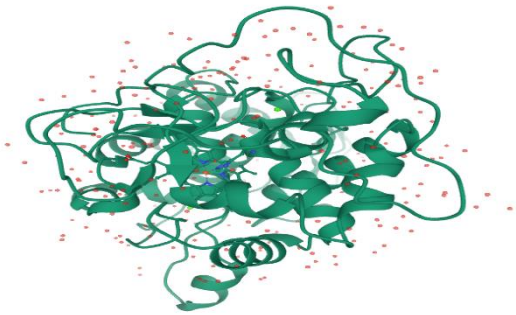

Figure 1: 3D structure of lignin peroxidase

degradation, cresols, chlorinated phenols, etc. Generally, peroxidases, from several different sources are non-specific. It provides the microorganism its unique ability to degrade a broad array of environmental pollutants [6].

1.1 Sources of peroxidase

Peroxidases (EC 1.11.1.7) represent a huge family of isoenzymes that are obtained from various sources such as plants, animals, and microbes, hence are widely distributed in nature. Peroxidases that have been utilized in the decomposition of pollutants, to generate animal feedstock, agricultural, paper, and pulp industries, dye decolorization industries, biosensors, etc. are extracted from microbes such as bacteria, fungi, and yeast [5].

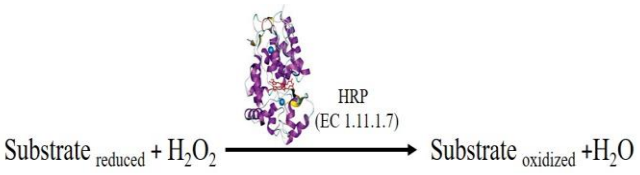

Figure 1: A general reaction catalyzed by HRP

Figure 2: HRP catalyzed reaction

1.2 Characteristics of peroxidase

Peroxidases reduce hydrogen peroxidase for example and oxidize various organic and inorganic compounds. Hence it is classified under the oxidoreductase class of enzymes. Peroxidases are considered as heme-containing enzymes or proteins which possess iron (III) protoporphyrin IX as the prosthetic group. It has a molecular weight ranging from 30-150 kDa. While some are categorized as non-heme peroxidases. Peroxidase represents a group of specific enzymes such as NADH peroxidase, iodine peroxidase, and glutathione peroxidase, and several non-specific enzymes that are called peroxidases [5].

2. Microbial source

Table 1: Microorganisms and their Habitats

Microorganisms	Habitat	Reference
Bacillus sp. FALADE-1-KX640922	The scrapings of rock from the Hogsback forest in Eastern cape, South Africa	Shashikumar *et al.*, (2014)
BL-27	The soil polluted with petroleum in the Shengli oilfield (China)	Dan Wang, Jiahui Lin, Weidong Wang., (2019)
Streptomyces sp. Strain BSII#1	The sediment of the Bwanda Hotsprings, Lochinvar National Park, Zambia	Prof. Don Cowan and Drs Mark Taylor, Moola Mutondo and Heide Goodman (2008)
Providencia spp.	From the laboratories of the biological department in Baghdad University	Rajkumar, R., Yaakob, Z., Takriff, M.S, and Kamarudin, K.F., (2013)
*Pseudomonas sp.*SUK1	A soil sample was collected from contaminated sites of the textile industry from Solapur, India	Kalyani *et al.*, (2008)

3. Enzyme production

The peroxidase was produced by the *Bacillus sp*. FALADE-1-KX640922 by submerged fermentation in which 100 ml of

lignin fermentation: K_2HPO_4 (4.55 g/l), KH_2PO_4 (0.53 g/l), $MgSO_4$ (0.5 g/l), NH_4NO_3 (5 g/l), yeast extract (0.1 g/l) & 0.1 % lignin w/v. Then about 2% of bacterial suspension was inoculated at pH 7 in media with media which is uninoculated is kept as control. This then allowed for the incubation period of about 48 hours in optimum conditions and the crude enzyme was isolated. The soil sample was inoculated in an MSM media containing crude oil as a carbon source, incubated for about 7 days then the bacterial colonies were streaked on the plates of LB agar then allowed to grow at 45 ºC for 12 hours [8]. The *Bacillus subtilis* was inoculated in the production media for about 48 hours at 37 ºC in a shaking incubator and the crude enzyme is isolated thereafter. The *Streptomyces spp*. spore or hyphal suspension was inoculated into modified phenoxazinone media and kept for incubation at 30 ºC for 2 days. Initially, the size of the inoculum was 5% (v/v) at a small scale then the inoculum was increased to 10% for baffled bioreactor and large scale which was further scaled up in airlift fermenter and bubble column bioreactor without any inducer at room temperature. The bacteria like *Pantoea agglomerans*, *P. aerogenosa*, *Bacillus subtilis*, *S. aureus* were preserved at 4 ºC and subcultured at a week interval. Then the inoculum was added into the media for the growth of bacteria kept in a shaking incubator [9]. The inoculation of the bacteria *Pseudomonas sp*. SUK1 was done at 30 ºC for 24 hours into the medium. The cells were collected by the centrifugation process and suspended in sodium phosphate buffer. The cell suspension was mixed properly by the homogenization process and then the sonication is done to disrupt the cell. The supernatant was used as the crude enzyme.

4. Enzyme purification

The enzyme was purified with different methods to accumulate the highest degree of protein with high purity. The purification of *Bacillus subtilis* was performed by the methods which are salt precipitation method, dialysis method, and ion-exchange chromatographic method. The isolated *Providencia spp*. was purified by ammonium sulfate precipitation [10] and ion-exchange chromatography [11]. The *Pseudomonas sp*. SUK1 produced crude enzyme peroxidase which was initially loaded on DEAE cellulose and then it was eluted by using the gradient of NaCl. The ion-exchange chromatography is also done to purify the protein which is followed by size exclusion, which allows more of the activity of the enzyme to be seen with 17-fold and yield of 1.67% which was initially 3.11-fold and yield was 3.60%.

5. Assay of enzyme activity

The activity of the enzyme peroxidase was accessed using the ability of it to degrade the compounds of lignin like guaiacol & veratryl alcohol. The inoculum of 5µl was inoculated into the guaiacol and veratryl alcohol plates which has components like K_2HPO_4 (4.55 g/l), KH_2PO_4 (0.53 g/l), $MgSO_4$ (0.5 g/l), NH_4NO_3 (5 g/l), yeast extract (0.1 g/l), guaiacol /veratryl alcohol (0.1% v/v) and agar (15g/l). then plates of agar were allowed for incubation at 30 ºC for about 168 hours which was then flooded with the gram's iodine to determine the degradation zone around the colonies produced on the plates. It can also be accessed using the spectrophotometric method in which the OD is taken or recorded at 420nm wavelength, pH-6, and 20 ºC [8]. The *Pseudomonas sp*. SUK1 was estimated for protein concentration by Lowry's method [12] through the use of a standard that is bovine serum albumin. The enzyme activity was deduced by using a substrate named n-propanol, which will be acted upon by the enzyme to convert to the product in which the reaction mixture contains 3 components like n-propanol, H_2O_2, citrate buffer. For the *Streptomyces spp*. the assay used was 2,4-dichlorophenol (2,4-DCP) assay [13]. The reaction mixture contained the tris-HCL, H_2O_2, amino antipyrine, 2,4-dichlorophenol, and enzyme extract. The activity was determined by the conversion of the substrate by the enzyme. For the *Providencia spp*. the activity of the enzyme was estimated by using the spectrophotometric method at 420nm and increasing the absorbance by using the citrate phosphate buffer [14].

6. Optimization studies on peroxidase production

Peroxidase production was optimized for the *Bacillus sp*. FALADE-1-KX640922 by maintaining conditions such as the pH range from 3-11, temperature range from 20-45 ºC, and the agitation rate from 0-200 rpm. The manipulation of the fermentation medium composition was done by adding a source of carbon with the 1 mM of "lignin monomers:

guaiacol, veratryl alcohol, vanillin, vanillic acid, and ferulic acid". More modification is done by adding ammonium nitrate, ammonium chloride, and ammonium sulfate which act as inorganic sources of nitrogen [15]. For the BL-27 strain, the temperature ranges from 5 - 65 ºC, pH 1-12, and salinity from 0-10% w/v. for the *Bacillus subtilis* the optimum pH is 6 and the temperature is 37 ºC. the activity of the enzyme is increased by the manganese sulfate while its activity is decreased by the inhibitor like PVP. The optimum conditions for Streptomyces *spp*. strain BSII#1 grow well at temperature 37 ºC with pH- 8·0 and many inducers like veratric acid, anisaldehyde, veratryl alcohol, pyrogallol or guaiacol (each at 0·1, 0·5 and 1 mmol l^{-1} concentration) were also introduced in the medium for the maximum yield of enzyme peroxidase. For *the Pseudomonas sp.* SUK1, maximum peroxidase production was observed at pH-3 and the optimum temperature was noted to be 40 ºC which if exceeded will decrease the activity of the enzyme with the high rate [16], [17].

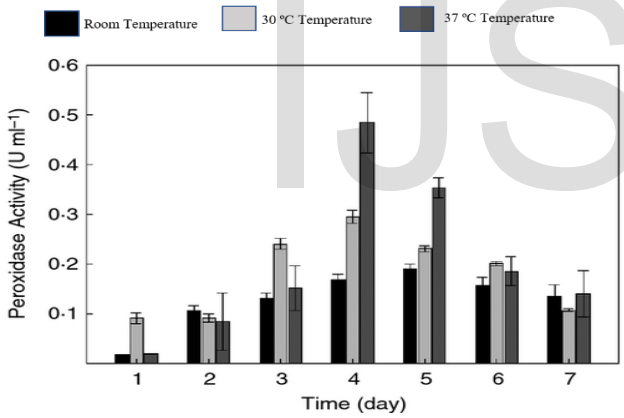

Figure 3: **Effect of temperature on peroxidase activity in *Streptomyces spp*. strain BSII #1** [48]

7. Kinetics for the enzyme production and bacterial growth

The *Bacillus sp*. FALADE-1-KX640922 was allowed to grow in LFM under the optimal conditions like pH-8, temperature-30 ºC, and 150 rpm. The culture of bacteria was then withdrawn at about 24 hours to 120 hours interval and then allowed for the assay of peroxidase activity and determination of protein concentration was done through the Bradford method. The growth of the cell was deduced by absorbance at 600 nm [18]. For the *Bacillus subtilis*, the kinetic experiments were done in shake flask cultures. The culture was kept in 100 ml of M_9 minimal medium for the visible growth because the medium contained the carbon sources. The concentration of glucose starting from 5 till 150 mg/l (5, 10, 15, 20, 50, 100 and 150 mg/l); sucrose (5, 10, 25, 50 and 100 mg/l), xylose (100, 150, 200 and 250 mg/l), starch (100, 150 and 200 mg/l), cellulose (300, 400, 500 and 600 mg/l) and lignin (400, 500, 600, 700, 800 and 900 mg/l). Then the flasks were all allowed to be maintained at 30 ºC and 150 rpm. The calculation of specific activity for all the carbon sources was done. Through the given equation below the substrate utilization, the constant and maximum specific growth rate was calculated.

"**$\mu = -ks(\mu/s) + \mu max$**,

where ks is the substrate utilization constant and μmax is the maximum specific growth"

In *Pseudomonas sp*. SUK1 the different kinetic factors were studied with the use of n-propanol. The Km was estimated to be 0.061 mM and the Vmax value was 2.7 × 102 μM. The Km value if is lower indicates the highest affinity of the enzyme towards the substrate which in this case was 0.061mM [19]. The affinity of guaiacol was found to be more than that of hydrogen peroxide as the k_m value for guaiacol is lower than that of peroxide [20].

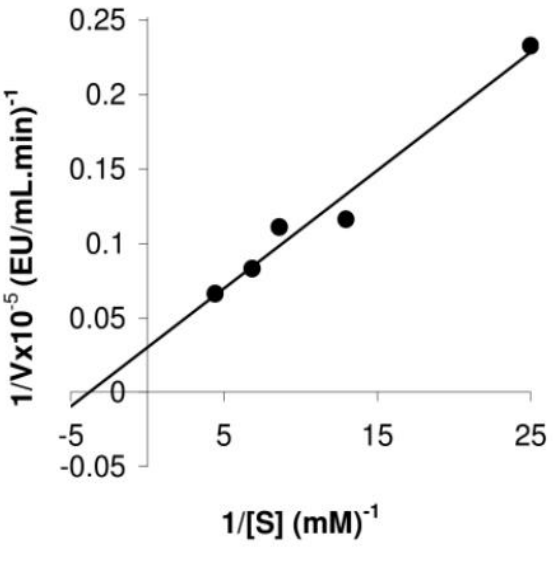

Figure 4: Lineweaver-Burk double reciprocal plot of guaiacol and hydrogen peroxide [21].

8. Effects of metal ions on peroxidase

Table 2: EC_{50} values of different metal ions which ranges from 6.0 to 24.51mM

Heavy metals	EC_{50} values (mM)
Fe^{2+}	12.58
Fe^{3+}	9.48
Co^{2+}	12.59
Sr^{2+}	24.51
Pb^{2+}	6.00
Hg^{2+}	7.32
Ni^{2+}	10.57
Al^{2+}	18.69
Zn^{2+}	13.57

The trace elements are needed by the living organisms but in an amount that could be beneficial but not detrimental. It has been noted that the Pb^{+2} has the highest inhibitory effect while Sr^{+2} has the minimum inhibitory effect on the enzyme. But as the concentration of each metal ion is increased, the inhibitory effect is also seen to have significantly increased causing the activity or efficiency of the peroxidase enzyme to be less. For example, mercury and cadmium have a collaborative toxic effect on enzyme activity [21].

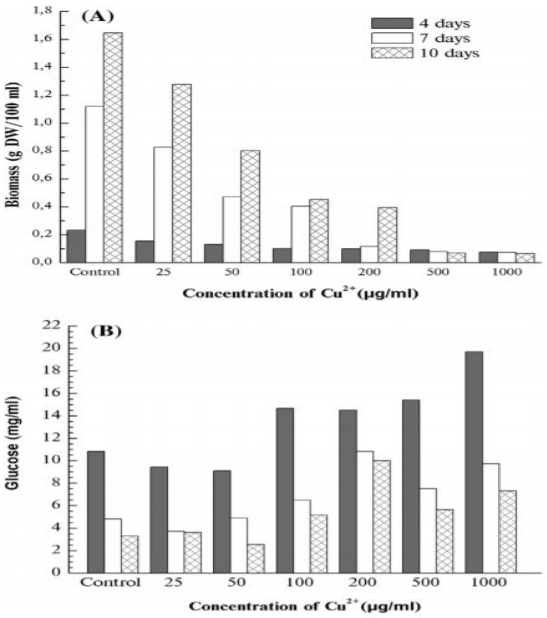

Figure 5: Graph (A) shows biomass yield against the increasing concentration and (B) shows glucose uptake by the cell against the concentration of Cu^{+2} of *T. trogii* [22].

The concentration of Cu^{+2} ion if increased while in the growth of the culture causes a significant decrease in the growth/ biomass yield in fungal strain compared with the control. In the concentration of 25µg/ml there was a very little decrease in the growth but when the concentration of Cu^{+2} was increased from 50 to 200µg/ml it was found that the decrease in the growth was 2 - 4.2 times more than that of control.

The Cu^{+2} and manganese are essential for microbial growth as they act as active centers by being the cofactors for enzymes [24]. But if their concentration is more it will have toxic effects on the growth of microbes.

The glucose uptake by the cell was also seen to decrease when the concentration of Cu^{+2} was increased compared to control where it was up taking the glucose properly for growth and enzyme production.

For the lignin peroxidase, the concentration of Cu^{+2} if increased causes a decline in the activity of it but at the low concentration, it has efficient activity [25].

9. Phylogenetic analysis

The phylogenetic analysis in the *Bacillus* FALADE 1 was done with the help of the neighbor-joining technique [26] in

the software named MEGA 7.0 [27] while the physical and chemical properties of the protein were determined by another software named generous 10.2.2. The creation of a phylogenetic tree for the strain BL-27 was with the help of the maximum likelihood method and general time-reversible model. The phylogenetic tree concludes that the similarity between the strain BL-27 and the *Bacillus subtilis* is found to be 99% so it was kept under *B. subtilis* genus. For the *Bacillus subtilis*, the phylogenetic tree was made from the gyrA sequence of nucleotide with the help of neighbor-joining, Fitch-Margoliash, and maximum parsimony algorithms. It is concluded that the gyrA sequence is the one that provides the accurate and rapid classification and identification of *Bacillus subtilis* from the other related species. In the *Pseudomonas sp*. SUK1 with the help of 4 sequences (16SrRNA, gyrB, rpoB, rpoD) the phylogenetic tree is constructed by making individual dendrograms by the methods like NJ, MP, and ME methods. The phylogenetic tree was constructed with the help of the neighbor-joining method for Streptomyces spp. And analyzed by MEGA 5.2. the phylogenetic tree was made by the use of software MEGA 7 software for the BL-27 strain.

10. Peroxidase application to manage environmental pollutants

10.1 Role of peroxidases in pulp and paper industries

Paper and pulp are generally made out of cellulosic fibers or other plant materials. Synthetic materials are also used to provide unique and special qualities to the finished products. Pulping is done to remove lignin without losing fiber strength thereby removing impurities and freeing the fibers. Pulp mill waste and black liquor which is a pulping by-product cause hazardous ecological problems and high pollution load. A dark, black, and viscous alkaline waste is called black liquor. It is composed of various extracts namely lignin, cellulose, phenolics, resins, fatty acids, and tannins. [28]. This alkaline waste consists of 10% - 15% of the total waste but usually lends to 90% - 95% of the total pollution load leading to high pH, BOD, COD, and color which makes it environmentally toxic [29]. This waste can be efficiently eliminated through bioremediation by the use of microbes such as fungi, bacteria, algae, and enzymes as a biological treatment or in combination with other physical or chemical methods. Over extensive research, it has been observed that peroxidase from a few generations of white-rot fungi can be used for degrading wastes due to their non-specific extracellular enzymatic system involved in lignin biodegradation. [30].

It is a laborious task to analyze the biochemical mechanisms of microbial lignin degradation. This difficulty is faced due to the structural complexity of the lignin molecule. Lignin does not contain readily hydrolyzable bonds recurring at periodic intervals along a linear backbone like other biopolymers. Rather, lignin is a three-dimensional, amorphous polymer that possesses several stable carbon-carbon and ether linkages between phenylpropanoid monomeric units. Hence, it is difficult to design experiments to assure the specific enzymatic transformations which occur during microbial decay of lignin. Theoretically, however, one of the ways to overcome this problem of chemical complexity is to study the microbial degradation of simple lignin model compounds of known chemical structure. Except for cellulolytic thermophiles, most bacterial strains possess the ability to degrade lignin-derived oligomers or aromatic monomers. The strains can convert high-molecular-weight polymers to low molecular weight compounds which are followed by uptake to provide a source of energy. Several degrading pathways exist within bacteria where low molecular weight compounds are digested through stepwise reaction to achieve an important intermediate like protocatechuate (PCA) and catechol.

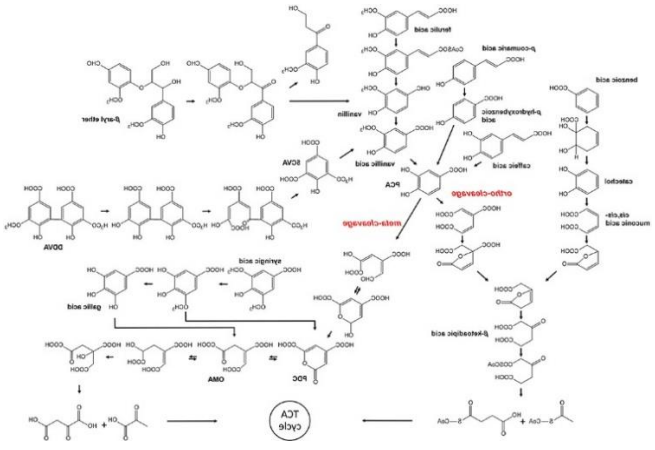

Figure 6: Pathway for degradation of lignin-derived compounds by bacteria [49].

Various soil bacteria can degrade aromatic compounds via the β-ketoadipate pathway and produce renewable chemicals. Within this pathway, many practically useful intermediates

can be found, including vanillin, which is extensively used in the food industry as a flavor additive.

10.2 Peroxidases in dye and dye-based textile effluent treatment

Dye wastes are considered xenobiotics. Xenobiotics are the most problematic group of pollutants as they do not hold a good biodegradability profile. It has been declared as one of the major environmental contaminants released from anthropogenic sources. [31]. Dyes find applications in textile industries such as textile dyeing, paper printing, as an additive in petroleum products, and color photography. These synthetic dyes are emitted into the industrial effluents which leads to the pollution of the environment. Microbes such as fungi and bacteria have emerged as an extremely valuable alternative to achieve biodegradation of hazardous pollutants [32]. Several peroxidases isolated from bacteria have been used for the decolorization of synthetic dyes. For example, *Bacillus sp*. VUS isolated from soil contaminated with textile effluents showed the ability to degrade various dye [33]. The contaminants of dyeing and bleaching industries have been found seeping into the ground thereby rendering the groundwater unsuitable for consumption.

10.3 Polycyclic aromatic hydrocarbons degradation by peroxidase

Peroxidase are effective in degrading polycyclic aromatic hydrocarbons (PAHs) which is a xenobiotic. Overuse of fossil fuel has resulted in the accumulation of PAHs. They survive for a longer period in the environment due to the property of low aqueous solubility and high hydrophobicity. [34]. PAHs pose carcinogenic, mutagenic, and teratogenic effects. However, many extracellular peroxidases, isolated from ligninolytic microbes play a major role in the complete mineralization of xenobiotic compounds. PAHs are degraded and transformed by MnP and LiP in the presence of hydrogen peroxide, hence leading to the formation of less mutagenic and easily degradable quinones and hydroxylated derivative [35].

10.4 Removal of endocrine disruptive chemicals (EDCs) by peroxidases

EDCs are defined as substances found in nature such as in food sources, personal care products, and manufactured products which interfere with the function of the endocrine system of the body. In other words, EDCs act as agonists or antagonists of hormones. EDCs act as hormone mimics and trick the body to recognize them as hormones while some EDCs block the naturally occurring hormones. Several classes of oxidative enzymes have shown the potential to efficiently remove EDCs that resist conventional wastewater treatment. EDC can also be oxidized by manganese peroxidase [36].

10.5 Mineralization of Polychlorinated biphenyls (PCBs) and pesticide by peroxidases

Polychlorinated biphenyls abbreviated as PCBs have been defined as “A group of man-made organic chemicals composed of carbon, hydrogen and chlorine atoms” [37]. PCBs, possess the unique characteristics of non-flammability [38] high electrical resistivity, stability towards heat and pressure, and low degree of reactivity which makes them worthy for a wide range of applications. PCBs are a potent pollutant and are released only through anthropogenic activities. PCBs can be mineralized to less toxic substances by the use of peroxidase [39].

10.6 Action of peroxidase as biosensors

"Biosensors are analytical devices that convert a biological response into an electrical signal." Biosensors merge bio-recognition elements with physical transducers to detect target compounds. Biosensors can be used for the continuous monitoring of a contaminated region [40]. They have high specificity and sensitivity. H_2O_2 is a potent conciliator of biochemistry. It may be involved in the etiology of aging and neurodegenerative diseases for example Parkinson's disease. H_2O_2 plays a vital role in neurochemistry and hence its concentration has been studied with keen interest [41]. A new third-generation biosensor for H_2O_2 has been established by cross-linking HRP onto an electrode that is modified with multiwall carbon nanotubes. It also determines biological effects like cytotoxicity, genotoxicity, endocrine disruption effects, etc along with the determination of specific chemicals

[42]. Enzyme biosensors are developed based on the selective inhibition of specific enzymes. Biosensors find a wide range of applications. For example, whole-cell biosensors are used in the determination of BOD and the detection of pesticides. HRP based biosensors have been developed where polyvinyl pyrrolidone nanofibers are spun along with the integration of horseradish peroxidase [43].

10.7 Differential degradation and detoxification of aromatic pollutants by two different peroxidases

It has been previously accepted that peroxidase, in general, acts upon all the substrates, or this case on aromatic pollutants in a similar manner under the same condition. However, currently, it has been reported that different peroxidases obtained from different sources can degrade pollutants under different conditions. Soybean peroxidase (SBP) and chloroperoxidase (CPO) are two different types of enzymes used in research where two different thiazole compounds were subjected to degradation. SBP completely degrades Thioflavin T (ThT) whereas CPO produces a chlorinated form of ThT without the actual degradation. It has also been seen that SBP-degraded ThT is non-phytotoxic while ThT treated with CPO is toxic. A carcinogenic dye, sulforhodamine B has been used as a model aromatic pollutant to study non-enzymatic oxidative degradation [44]. The optimum concentration for SBP is 50 pM to degrade sulforhodamine B while CPO optimum concentration was found to be 440 pM. Due to the high CPO concentration, the substrate could be less efficiently degraded [45], [46]. CPO mediated degradation of SRB dye significantly increased from 55%-90% with a rise in temperature from 20 ºC to 40 ºC while the efficiency of SRB dye degradation decreased to approximately 25% at 60 ºC. However, there were no observable significant differences in SBP based degradation of the SRB dye when the temperature raised from 20 ºC to 80 ºC [47]. These facts have proven that SBP is a robust and thermostable enzyme. The experiments performed has shown that CPO and SBP exhibit different thermal stabilities.

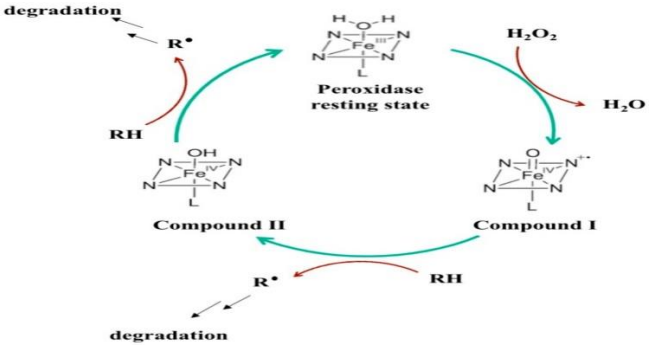

Figure 7: Diagrammatic representation of the catalytic cycle of heme-peroxidases. RH: organic pollutant; R•: a radical form of the organic pollutant [44].

11. Conclusion

The *Bacillus* FALADE 1 was able to produce the peroxidase enzyme at alkaline pH, mesophilic temperature, and high agitation speed. This bacterium showed a high potential of producing peroxidase in a huge amount with a specific activity of 8.32 U/mg than the other producers. It is also good in the ability to attain the optimum production of peroxidase in only 48 hours which is a very short incubation period.

The BL-27 strain was isolated from hydrocarbon-contaminated sites that need an optimum pH of 7, temperature 45 ºC and 10 g/l of NaCl will be able to produce peroxidase which can degrade crude oil.

The purified enzyme activity in *Bacillus subtilis* was found to be maximum at pH 6 and temperature was found to be room temperature. The concentration of the substrate is increased to a certain concentration after which the effect is constant. The activity of the enzyme is also enhanced by the increased amount of manganese sulfate which acts as an activator while the enzyme's activity is decreased by the use of inhibitors like PVP which is used in high concentration. The purity of the enzyme was then confirmed by running it on the SDS-PAGE in which the proteins were separated based on the size. The size was approximately found to be 44 KDa. The purified enzyme was immobilized by sodium alginate. So finally, it is concluded that the enzyme peroxidase was obtained from the bacteria *Bacillus subtilis* which is a good source for it.

In the *Streptomyces sp*. strain BSII#1 the inducer which is veratryl alcohol is used to induce the formation of the

peroxidase enzyme in large amounts and it was also observed that the aeration when increased caused an increase in the amount of peroxidase production.

In *Providencia spp*. the peroxidase enzyme was produced with optimum conditions and then purified and analyzed by the molecular method.

In *Pseudomonas sp*. SUK1 the peroxidase was produced in the optimum condition with its purification through different means and then observed for the activity of the peroxidase by n-propanol, p-cresol, and veratrole.

The demand and production of new chemicals increase with urbanization, industrialization, etc. It often exceeds the safety measurement tools and remediation technologies that exist. The concept of bioremediation has emerged as an effective process in tackling such pollutants by detoxification or elimination. The enzyme peroxidase has extreme potential for bioremediation of wastewater contaminations such as cresols, dyes, EDCs, phenols, and other industrial effluents. Extensive research and rapid progress in determining the effectiveness of biodegradation of environmental pollutants by peroxidases have led to the path of exploration of sustainable bioremediation strategies. The importance of peroxidase in the process of detoxification or elimination of environmental pollutants depends on its ability to catalyze peroxidase reduction reaction, oxidation of various organic and inorganic compounds, and also the polymerization of toxic compounds. However, the fascinating perspective from enzyme bio-catalysis for biodegradation is yet to address an open environmental issue. Based on literature evidence, enormous improvement has been done in the detection and removal of harmful pollutants through the use of sophisticated instruments. Environmental protection is governed by three main factors. These factors are environmental legislation, ethics, and education. All three are crucial as they influence national-level environmental decisions and personal-level environmental values and behaviors.

References

[1] Gottlieb, S., and Pelczar, M. J. Jr: "Microbiological aspects of lignin degradation". *Bacteriol. Rev.* 15:55.

[2] Reddy, N., and Yang, Y. "Biofibers from agricultural byproducts for industrial applications". *Trends Biotechnol*, 23, 22–27, 2005.

[3] Santos, R. B., Hart, P., Jameel, H., and Chang, H.-M. "Wood-based lignin reactions important to the biorefinery and pulp and paper industries". *Bioresources* 8, 1456–1477, 2013.

[4] Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., Schatz, P. F., et al. "Lignins: natural polymers from oxidative coupling of 4-hydroxyphenyl-propanoids". *Phytochem. Rev*, 3, 29–60, 2004.

[5] N. Bansal and S. S. Kanwar, "Purification and characterization of an extracellular peroxidase of a bacterial isolate Bacillus sp. F31," Current Biotechnology, vol. 2, no. 2, pp. 155–161, 2013.

[6] R. Chandra, A. Abhishek, and M. Sankhwar, "Bacterial decolorization and detoxification of black liquor from rayon grade pulp manufacturing paper industry and detection of their metabolic products," Bioresource Technology, vol. 102, no. 11, pp. 6429–6436, 2011.

[7] Sasikumar V., Priya V., Shiv S.C., Sathish S.D. "Isolation and preliminary screening of lignin-degrading microbes". J. Acad. Indus. Res, 3:291–294, 2014.

[8] Falade A.O., Eyisi O.A.L., Mabinya L.V., Nwodo U.U., Okoh A.I. "Peroxidase production and ligninolytic potentials of freshwater bacteria *Raoultella ornithinolytica and Ensifer adhaerens*. Biotechnol". Rep; 16:12–17, 2017.

[9] Rajkumar, R., Yaakob, Z., Takriff, M. S, and Kamarudin, K. F. "Optimization of medium composition for the production of peroxidase by *Bacillus sp"*. Der Pharma Chemica, 5 (2): 167-174, 2011.

[10] Bentely, R. "Mutarotase methods in enzymology". Academic Press. USA, PP. 219-225, 1962.

[11] Foote, N., Thompson, A. C., Barber, D. and Greenwood. "Pesudomonas cytochrome c-551 peroxidase". Biochem. J. 209: 701-707, 1983.

[12] Lowry O, Rosbrough N, Farr A, Randall R "Protein measurement with the folin phenol reagent". J Biol Chem 193:265–275, 1951.

[13] Antonopoulos, V.T., Hernandez, M., Arias, M.E., Mavrakos, E. and Ball, A.S. "The use of extracellular enzymes from Streptomyces albus ATCC 3005 for the bleaching of eucalyptus kraft pulp. Appl Microbiol Biotechno" l 57, 92–97, 2001.

[14] Rao, P. R., and Kavya. P. "Production, Isolation and Purification of Peroxidase Using *Bacillus subtilis"*. International Congress on Environmental, Biotechnonlogy and Chemistr Engineering, 64(5):21-27, 2014.

[15] Falade A.O., Mabinya L.V., Okoh A.I., Nwodo U.U. "Agrowastes utilization by *Raoultella ornithinolytica* for optimal extracellular peroxidase activity". Biotechnol. Appl. Biochem, 66:60–67, 2019.

[16] Dawkar VV, Jadhav UU, Telke AA, Govindwar SP "Peroxidase from *Bacillus sp*. VUS and its role in the decolorization of textile dyes". Biotechnol Bioprocess Eng, 14: 361–368, 2009.

[17] Ghodake GS, Kalme SD, Jadhav JP, Govindwar SP "Purification and partial characterization of lignin peroxides from *Acinetobacter calcoaceticus* NCIM 2890 and its application in decolourization of textile dyes". Appl Biochem Biotechnol, 152:6–14, 2009.

[18] Tuncer M., Rob A., Ball A.S., Wilson M.T. "Optimisation of extracellular lignocellulolytic enzyme production by a thermophilic actinomycete *Thermomonospora fusca* BD25. Enzym. Microb. Technol; 25:38–47, 1999.

[19] Colombo JC, Cabello M, Arambarri AM "Biodegradation of aliphatic and aromatic hydrocarbons by natural soil microflora and pure culture of imperfect and ligninolytic fungi". Environ Pollut, 94:355–362, 1996.

[20] Köksal E, Gülçin. "Purification and characterization of peroxidase from cauliflower *(Brassica oleracea* L.) buds". ProteinPeptide Lett. 15(4): 320-326, 2008.

[21] Lineweaver H, Burk D. "The determination of enzyme dissociation constants". J. Am. Chem. Soc. 56: 658-661, 1934.

[22] Christensen GM, Olson D, Riedel B "Chemical effects on the activity of eight enzymes": A review and a discussion relevant to environmental monitoring. Environ. Res, 29: 247 - 255, 1982.

[23] Nedelina Kostadinova, Ekaterina Krumova, Rumyana Boteva, Radoslav Abrashev, Jeni Miteva-Staleva, Boryana Spassova and Maria Angelova: "Plant Biosystems- An International Journal Dealing with all aspects of Plant Biology", pp 2-3, 2018.

[24] Vrsanska M, Buresova A, Damborsky P, Adam V. "Influence of different inducers on ligninolytic enzyme activities". J Metal Nanotechnol, 3: 64–70, 2015.

[25] Asgher M. "Characterization of a novel manganese peroxidase purified from solid state culture of *Trametes versicolor* IBL-04". Bioresources, 6: 4317–4330, 2011.

[26] Saitou N., Nei M. "The neighbor-joining method: a new method for reconstructing phylogenetic trees". Mol. Biol. Evol, 4:406–425, 1987.

[27] Kumar, S.; Stecher, G.; Tamura, K. MEGA7: "Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets". Mol. Biol. Evol,33. 1870–1874 2016.

[28] Khalid, M. Arshad, and D. E. Crowley, "Biodegradation potential of pure and mixed bacterial cultures for removal of 4- nitroaniline from textile dye wastewater," Water Research, vol. 43, no. 4, pp. 1110–1116, 2009.

[29] R. Grover, S. S. Marwaha, and J. F. Kennedy, "Studies on the use of an anaerobic baffled reactor for the continuous anaerobic digestion of pulp and paper mill black liquors," Process Biochemistry, vol. 34, no. 6-7, pp. 653–657, 1999.

[30] K. E. Hammel and D. Cullen, "Role of fungal peroxidases in biological ligninolysis," Current Opinion in Plant Biology, vol. 11, no. 3, pp. 349–355, 2008.

[31] S. T. Ong, P. S. Keng, W. N. Lee, S. T. Ha, and W. T. Hung, "Dye waste treatment," Water, vol. 3, no. 1, pp. 157–176, 2011

[32] H. T. Tien, Z. Salamon, J. Kutnik et al., "Bilayer Lipid Membranes (BLM): an experimental system for biomolecular electronic device development," Journal of Molecular Electronics, vol. 4, supplement 4, pp. S1–S30, 1988.

[33] V. V. Dawkar, U. U. Jadhav, S. U. Jadhav, and S. P. Govindwar, "Biodegradation of disperse textile dye Brown 3REL by newly isolated Bacillus sp. VUS," Journal of Applied Microbiology, vol. 105, no. 1, pp. 14–24, 2008.

[34] J. Jauregui, B. Valderrama, A. Albores, and R. Vazquez-Duhalt, "Microsomal transformation of organophosphorus pesticides by white rot fungi," Biodegradation, vol. 14, no. 6, pp. 397–406, 2003.

[35] Ayala, M., Pickard, M.A., Vazquez-Duhalt, R. "Fungal enzymes for environmental purposes, a molecular biology challenge". J. Mol. Microbiol. Biotechnol. 15 (2–3), 172–180, 2008.

[36] W. Zheng and L. M. Colosi, "Peroxidase-mediated removal of endocrine disrupting compound mixtures from water," Chemosphere, vol. 85, no. 4, pp. 553–557, 2011.

[37] Bilal, M., Asgher, M. "Sandal reactive dyes decolorization and cytotoxicity reduction using manganese peroxidase immobilized onto polyvinyl alcohol-alginate beads". Chem. Cent. J, 9 (1), 47, 2015.

[38] Torres-Duarte, C., Vazquez-Duhalt, R. "Applications and prospective of peroxidase biocatalysis in the environmental field. Biocatalysis Based on Heme Peroxidases". Springer, Berlin, Heidelberg, pp. 179–206, 2010.

[39] J. Raymond, T. Rogers, D. Shonnard, and A. Kline, "A review of structure-based biodegradation estimation methods," Journal of Hazardous Materials, vol. 84, no. 2-3, pp. 189–215, 2001.

[40] S. Ahammad, "Hydrogen peroxide biosensors based on horseradish peroxidase and haemoglobin," Jounal of Biosensors & Bioelectronics, 2013.

[41] J. Song, J. Xu, P. Zhao, L. Lu, and J. Bao, "A hydrogen peroxide biosensor based on direct electron transfer from hemoglobin to an electrode modified with Nafion and activated nanocarbon," Microchimica Acta, vol. 172, no. 1, pp. 117–123, 2011.

[42] S. Xu, X. Zhang, T. Wan, and C. Zhang, "A third-generation hydrogen peroxide biosensor based on horseradish peroxidase cross-linked to multi-wall carbon nanotubes," Microchimica Acta, vol. 172, no. 1, pp. 199–205, 2011.

[43] M. Dai, S. Jin, and S. R. Nugen, "Water-soluble electrospunnano fibers as a method for on-chip reagent storage," Biosensors, vol. 2, no. 4, pp. 388–395, 2012.

[44] Alneyadi, A.H.; Ashraf, S.S. "Differential enzymatic degradation of thiazole pollutants by two different peroxidases"—A comparative study. Chem. Eng. J. 303, 529–538, 2016.

[45] Liu, H.; Guo, S.; Jiao, K.; Hou, J.; Xie, H.; Xu, H. "Bioremediation of soils co-contaminated with heavy metals and 2,4,5-trichlorophenol by fruiting body of *Clitocybe maxima*". J. Hazard. Mater, 294, 121–127, 2015.

[46] Zhang, J.; Feng, M.; Jiang, Y.; Hu, M.; Li, S.; Zhai, Q. "Efficient decolorization/degradation of aqueous azo dyes using buffered H_2O_2 oxidation catalyzed by a dosage below ppm level of chloroperoxidase". Chem. Eng. J. 191, 236–242, 2012.

[47] Shuler, M.L.; Kargi, F. "Bioprocess Engineering Basic Concepts"; Prentice-Hall: Englewood Cliffs, NJ, USA, 1992. 44. McEldoon, J.P.; Dordick, J.S. "Unusual thermal stability of soybean peroxidase". Biotechnol. Prog. 12, 555–558, 1996.

[48] A. Musengi, N. Khan, M. Le Roes-Hill, "Journal of Applied Microbiology", 2013.

[49] Siseon Lee, Minsik Kang, Jung-Hoon Sohn and Bong Hyun Sung; "Frontiers in Bioengineering and Biotechnology", 2019.