SCIENCE SET
OPEN ACCESS PUBLISHERS

ISSN: 3066-716X
Review Article

Journal of Clinical and Biomedical Advances

Immobilization of Laccase Enzyme and its Application

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Submitted: 02 May 2023 **Accepted:** 11 May 2023 **Published:** 15 May 2023

https://doi.org/10.63620/MKJCBA.2023.1006

Citation: *Dwivedi, E., & Singh, L. K. (2023). Immobilization of Laccase Enzyme and its Application. J of Clin Bio Med Adv, 2(2), 1-9.*

Abstract
Laccases are enzymes with a lot of potential for breaking down toxic chemicals. Fungal laccases, particularly those generated by basidiomycetes, have significant enzymatic activity. A range of isoenzymes and/or enzymatic activity can be obtained depending on the growth conditions and strain employed. Extracellular laccase enzymes generated by Pleurotus ostreatus were discovered in a submerged culture in this investigation (SmF). Zinc nanoparticles were quickly produced from Zn (NO3)2 solution utilizing soluble starch in this study, and nanoparticle production was observed using the in-precipitation method. Zinc that has been produced in a white colour. Different spectroscopic and analytical techniques, such as XRD and SEM, were used to analyze nanoparticles. The generation of well-dispersed zinc nanoparticles with an average particle size was confirmed by X-ray diffraction and SEM examination.

ZnO porous nanoparticles can be employed as a good substrate for surface adsorption stabilization of laccase enzyme, which increases the enzyme's stability properties without compromising its kinetics.

Keywords: Zinc Nanoparticles, Enzyme, Stability, Precipitation, Spectroscopic, Techniques

Introduction

Laccases are multicopper-containing enzymes that reduce molecular oxygen to water and belong to the polyphenol oxidases family [1]. This enzyme is a blue copper protein that uses molecular oxygen as an electron acceptor to catalyze the oxidation of a wide range of organic and inorganic compounds. Laccase exhibit broad substrate specificity towards aromatic compounds containing hydroxyl and amine groups including diphenols, polyphenols, diamines and aromatic amines. The syringaldazine [4- hydroxy-3,5-dimethoxy benzaldehyde azine] is considered as the substrate oxidized only by laccase enzyme [2]. Laccases are formed by bacteria, insects, higher plants, and fungi, among other living organisms. Just a few laccase enzymes from bacteria have been isolated and characterized. The prokaryotic laccase, which was derived from the rizospheric bacterium Azospirillum lipoferum, was the first research on this subject. Bacterial laccases have received increased attention in recent years as a result of their ability to overcome the disadvantages of instability as opposed to fungal laccases. At high temperatures and pH values, they are extremely active and much more stable. Bacterial laccases have evolved into a significant industrial enzyme that is used in a variety of processes, including the detoxification of industrial effluents, primarily from the paper and pulp, textile, and petrochemical industries, as well as a diagnostic method, a cleaning agent for some water purification systems, and a catalyst for the production of anticancer drugs. Lack of adequate enzyme stocks and expense in order to achieve low-cost overproduction of this biocatalyst, as well as chemical modification of enzymes to obtain more robust and active enzymes, were major barriers to commercialization of bacterial laccases [3].

Laccase nomenclature is defined in the Enzyme Commission (EC 1.10.3.2) indicates oxidoreductases EC 1.10 acting on diphenols and related substances as donors E.C.1.10.3 with oxygen as acceptor. Laccase is a multicopper blue oxidase that uses a one-electron transfer mechanism to couple the four-electron reduction of oxygen with the oxidation of a wide variety of organic substrates, including phenols, polyphenols, anilines, and even some inorganic compounds. Laccase was first found in the sap of the Japanese lacquer tree Rhus vernicifera, and Bertrand discovered its property as a metal-containing oxidase in 1985. Laccases have since been discovered in a variety of basidiomycetous and ascomycetous fungi, and fungal laccases have accounted for the largest community of multicopper oxidases in terms of number and extent of characterization. Mayer and Staples (2002) stated that, the large quantity of laccases have been widely reported inside white-rot fungi. While, Claus (2003) mentioned that laccase in nature can be found in eukaryotes as fungi (principally in Basidiomycetes), plants, Oscillatoria, and insects. However, there is a growing body of evidence that it exists in prokaryotes. The presence of corresponding laccase genes in gram-negative and gram-positive bacteria has been reported by a number of researchers [4]. Pratheebaa et al. (2013) reported that laccase is gaining increasing attention due to their possible use in food and textile industries, pulp and paper manufacturing, wastewater treatment, bioremediation and Nano-biotechnology. Laccases have recently attracted a lot of attention as a new biocatalyst in organic synthesis, owing to the fact that environmentally friendly oxidation has become a very important area in green chemistry.

Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are a group of blue multicopper oxidases that are capable of oxidizing mono-, di- and polyphenols, aminophenol's, methoxyphenols, aromatic amines and ascorbic acid. Due to its catalytic properties, laccase is used in several industrial applications, including dye effluent decolorization, in the pulp and paper industry, and in removal of herbicides. Laccase has recently been used as the cathode in enzymatic biofuel cells to reduce oxygen to water. With the use of sustainable and renewable resources, this process will generate a stable current.

One of the great promises that nanoparticles of metal oxides hold in chemical applications is their remarkable ability to chemically absorb a wide variety of molecules, especially organic molecules that are concern as environmental hazards. Because of its unique physical and chemical properties, such as high chemical and mechanical stability, wide range oferadiation absorption, high catalysis activity, electrochemical coupling coefficient, and non-toxic nature, zinc oxide has become a common metal oxide material in recent years. Zinc oxide is classified as a semiconductor in group II-VI in materials science, with a limited energy band of 3.37 eV and a high band energy ofe60meV. It is commonly used in many fields due to its unique properties, including the rubber industry, pharmaceutical and cosmetic industries, textile industries, electronics and electro technology industries, and so on. Nanometric zinc oxide comes in a number of shapes and sizes. It can be found in structures that are one dimensional (1D), two dimensional (2D), and three dimensional (3D). Needles, helixes, nanorods, ribbons, belts, cables, and combs are among the most common one-dimensional structures. Two-dimensional structures of zinc oxide include Nano pellets, nanosheets, and nanoplates. The synthesis method, characterization, and surface modification are all discussed. Micro emulsion synthesis, spray drying, sol-gel method, pyrolysis, controlled precipitation, RF plasma synthesis, vapour transport process, and other methods can all be used to make zinc oxide nanoparticles. Here, the controlled precipitation method was used to make zinc oxide nanoparticles. Zinc oxide (ZnO) is a well-engineered compound that has sparked widespread interest due to its unique properties and applications in a wide range of fields, including pharmaceuticals, cosmetics, photonics, and photocatalysis. Many physiochemical routes can be used to make ZnO nanoparticles (NPs), including sol-gel processes, co-precipitation, laser vaporization, microemulsion, and ball milling.

Review and Literature

Laccase has been immobilized on solid supports to boost its industrial utility by increasing its service, stability, and half-life time. Laccase immobilization was the focus of a systematic review that compiled all of the immobilization methods used, as well as the various aids used and their future applications. Laccase was immobilized using Sepharose, alumina oxide, alginate beads, Silica gel, DEAE cellulose, and glass beads, among other supports. Laccases have been immobilized using a variety of methods, including adsorption, covalent binding, entrapment, encapsulation, and cross-linking [5]. Laccase enzyme from Streptomyces pasmmoticus was partially purified using ammonium sulphate precipitation before being immobilized in alginate beads using a calcium and copper entrapment process. Copper alginate beads were found to be a better support for laccase immobilization than calcium alginate beads, maintaining 61 percent of laccase activity compared to 42.5 percent for calcium alginate beads. The nanotechnology drives from two parts 'Nano' which means tiny sizeable and in other part 'Technology' which spelled in Greek word 'tekhne' it about known about something at tiny scale. It has measured scale at 10-9 m. nanotechnology has even rapidly growing in medical field for delivered therapeutic agent at wounding placed. Nanotechnology is growing up in market as advanced technology so that nanostructure particles have many several properties which are beneficial to the molecular and cellular characteristics functioning. The Nanomaterial carries out size 0.1-100 nm for excellent dispersibility and loading efficiency of surface matrices. Many types of coated material made up through nanoscience which coated the drug by tiny sizeable material used in medical field. However, nanotechnology is widely used in life it is also included cancer treatment and other diagnostics treatment. The Nano constructed materials have magnificent properties to attracted iron oxide coated particles which are being used in several therapy and cancer treatment. The Nanoparticles provided stability in aqueous solution at pH 7.0 which have charge on surfaced that found in 3D structure of Nanoparticles. The 'Nanotechnology' word was initiated introduction by Professor Norio Taniguchi at Tokyo University in 1971. The nanotechnology is spreading in electronic field. It makes low weight and highly capable product. However, the nanotechnology has been faced many problems of our society. The electronic device has been used nanotechnology for manufactured to microchips. Health risks can be treated by nanotechnology therapy that has been applied to a wide spectrum [6]. It is working based on the magnetic properties to which has been used in biosensor, and Nanomedicine. The magnetic nanoparticle has also been screening properties. Furthermore, Nanotechnology used to increase their detection sensivity and reliability. The size and surface are inversely proportional to each other. As the surface area increased and enzyme loading efficiency increased, the size of nanoparticles decreased due to some change in physical and chemical properties in bulk condition. The magnetic nanoparticles would be used in many biological issues. Nanoparticles exist in nature, because the electron device making by magnetic nanoparticles so called storage and which collect the information data and is used in making biosensor [7]. As magnetic nanoparticle can be used for future possibility, which not only acts as an electron carrier but also play important role in spin-orbit coupling to controlled the magnetic characteristics in nanocomposite. The nanoparticles have magnetic properties that are identified through their particle size.

Nanoparticle size range is measured in micro and Nano mater. There are found homogeneous magnetization areas. The superparamagnetic is developed a new configuration structure of magnetic nanoparticle using of their unique properties. Which are most being helpful in application in biological separations and Biomedicine so that magnetic nanoparticles are known as biocompatibility. Additionally, magnetic nanoparticles have become a synthesis in intracellular of magnetostatics bacteria. Further comprising to iron oxide, iron sulfate etc. bacterial magnetic nanoparticle encapsulated by organic membrane which consists of phospholipids and protein that are easily dispersed in aqueous solution. A Magnetostatics bacterium was discovered in 1975. There are classified based on their morphological structure Zinc oxide (ZnO) is a well-engineered compound that has received remarkable interest due to its unique properties and applications in a wide range of fields, including pharmaceuticals, cosmetics, photonics, and photocatalysis. Textile industry utilizes large volume of water and chemicals for wet processing. These chemicals are made up of both inorganic and organic compounds. When dyes are exposed to sun, water, and other chemicals, their chemical composition prevents them from fading. Laccase degrades dye, which is why laccase-based processes with synthetic dyes have been produced and are now used in the industry. Contamination of soil, water, and air occurs as a result of fast industrialization and widespread use of pesticides for improved agricultural output, which is a severe environmental problem today. Polychlorinated biphenyls (PCB), benzene, toluene, ethyl benzene, xylene (BTEX), polycyclic aromatic hydrocarbons (PAH), pentachlorophenol (PCP), 1,1,1-trichloro2,2-bis (4-chlorophenyl) ethane (DDT), and trinitrotoluene (TNT) are carcinogenic and mutagenic Fungi can recycle a wide range of dangerous compounds, which is why they piqued the researcher's interest. Laccase is essential because it is capable of oxidizing both toxic and nontoxic substrates. It's used in the textile, dairy, and wood processing industries, as well as the pharmaceutical and chemical industries. This enzyme is highly specific, environmentally friendly, and an effective catalyst. Laccase can be used in the following ways. Chlorine and oxygen-based chemical oxidants are used in industry to separate and breakdown lignin, which is required for the production of paper. However, there are still certain difficulties to be overcome, such as recycling, cost, and toxicity. Because it results in a partial replacement of ClO2 in pulp mills, LMS might be simply integrated into the current bleaching procedure. Richard Feynman first presented the concept of nanotechnology in 1959, when he gave a talk titled "there is plenty of room at the bottom." He never addressed nanotechnology in his talk, but he implied that it will ultimately be feasible to manipulate atoms and molecules accurately. The discovery of fullerenes in 1985 and the invention of the scanning tunneling microscope in 1981 are believed to be the convergence of experimental discoveries that led to the emergence of nanotechnology in the 1980s. Nanotechnology is described as the control and manipulation of matter at Nano dimensions, and nanoscience is frequently considered as the defining technology for the twenty-first century. As a result of the advancement of nanotechnology, Nano powders have been developed, and they can be employed in a range of applications. Nanomaterials have gotten a lot of interest in recent years because of their unique properties compared to bulk materials. One of the most exciting properties of metal oxide nanoparticles in chemical applications is their extraordinary capacity to chemically absorb a wide range of chemicals, particularly organic compounds that are potentially harmful to the environment. The morphology of zinc oxide nanoparticles is determined by the synthesis method. Nanorods, nanoplates, nanospheres, Nano boxes, hexagonal, tripods, tetrapod’s, nanowires, nanotubes, nanoring’s, nanocages, and nanoflowers are some of the possible structures. Zinc oxide nanoparticles are more effective against gram- positive bacteria than other NPs from the same element community. Salmonella, Staphylococcus aureus, and E. coli are all bacteria that can cause illness. E. coli is more likely to contaminate ready-to-eat foods, posing a significant risk to food safety and quality. Antimicrobial substances are used to preserve packaged goods from spoiling. Antimicrobial packaging contains a nontoxic material which inhibits or slows down the growth of microbes present in food or packaging material . Nanoparticles may be toxic to some microorganisms, but they may be essential nutrients to some of them [8]. Nanotoxicity is primarily caused by microbial cell membrane disruption, which allows nanoparticles to enter the cytoplasm and accumulate. The effect of nanoparticles on bacterial and viral growth is heavily influenced by particle size, shape, concentration, agglomeration, colloidal composition, and media pH [9].

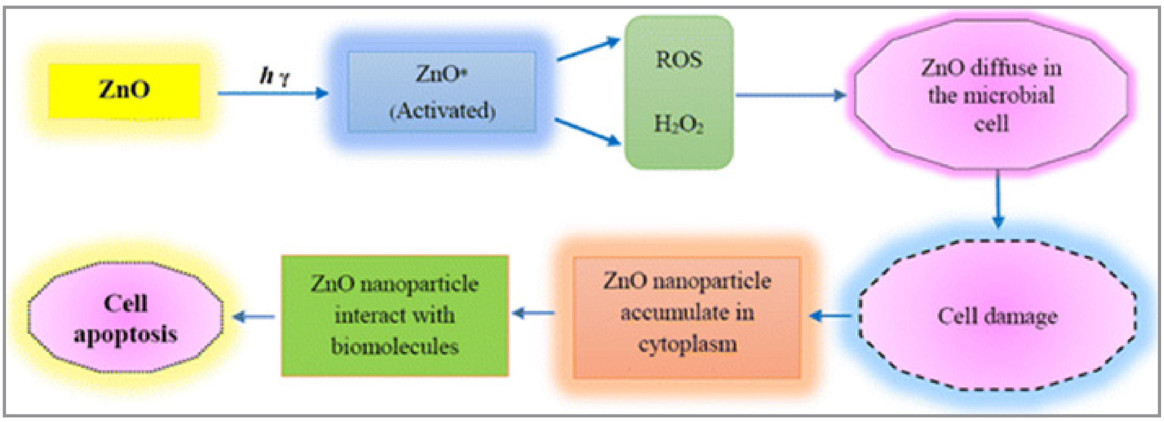


Figure 1: Mechanisms of zinc oxide nanoparticle antimicrobial activity

Material and Methods

Preparation of Zinc Oxide Nanoparticles

Synthesis of Porous Zinc Oxide Nanoparticles

4milliliters (2 M) of NaOH were mixed with 4 milliliters of (0.02 M) CTAB and then maintained at room temperature for 30 minutes. 6.3 grams of ZnCl, was dissolved in 50 milliliters of distilled water and the CTAB solution was added to it simultaneously with stirring. After an hour of stirring, the resultant precipitate was collected using a filter and washed with distilled water and ethanol. After collecting with filter, the precipitate was incubated at 100°C for 24 hours. The dried sample was then placed in a furnace at 600°C for one hour.

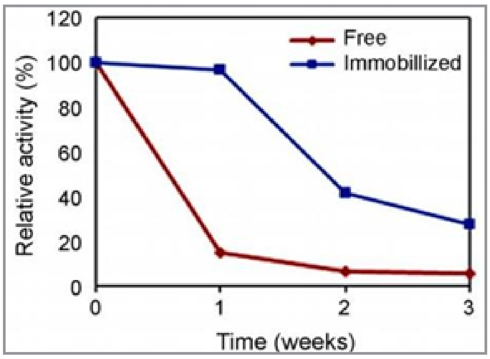
Enzyme Immobilization on Nanoparticle Surface

In this study, the adsorption method was used to immobilization of the laccase enzyme on the surface of synthesized nanoparticles. In summary, 4 milligrams of the synthesized nanoparticle were mixed in 900 milliliters of Mellvane buffer (pH 6.5) and then homogenized using a sonication for 2 minutes. 100μl of laccase enzyme solution (10 mg/ml) was added to the nanoparticle mixture and then was incubated for 4h using a shaker incubator for 24 hours. Subsequently, the mixture was centrifuged at 1200 rpm for 5 minutes. Then the supernatant was removed and the sediment was washed 3 times by adding I milliliter of Mellvane buffer. Finally, the remaining residue was mixed in 1 milliliter buffer and then homogenized for subsequent experiments. The optimized pH for immobilization of the enzyme was evaluated by performing the immobilization process using buffer pH 3, 4, 5, 6, and 7.

Results and Discussions

Investigating the variation of immobilized enzyme activity after successive cycles of use, recycling and re-use of the enzyme indicates that enzyme activity decreases after each use (Fig. 2). The analysis of the obtained line shows that the reduction of enzyme activity is stabilized with a gradient of about 12%. After using the stabilized enzyme, its activity decreases by 12%, which will eventually be deactivated after 8 times. Investigating the storage stability of enzyme indicates that free enzyme activity is reduced by about 85% after a maintenance week at the laboratory temperature, until it actually reaches 5% of the initial activity in the third week. While in the immobilized enzyme in the first week, there is no significant change in enzyme activity. However, in the second and third weeks, 60% and 20% of the initial activity decreased, respectively. Although the activity of the enzyme has decreased in the third week, it is still significantly higher than the free enzyme.

(a)



(b)

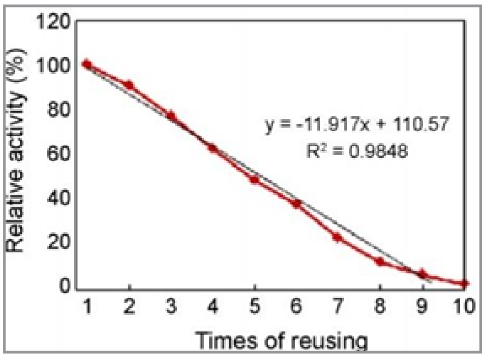


Figure 2: Assessment of the immobilized laccase (a) Stability and (b) Reusability

Conclusion

Stabilization of the laccase enzyme using adsorption method on ZnO nanoparticles can be used as a suitable method for reusing and improving the temperature stability and maintenance of the enzyme without affecting the kinetic parameters.

References

1. Elshafei, A. M., Hassan, M. M., Elsayed, M. A., & Othman, A. (2012). Optimization of laccase production from Penicillium martensii NRC 345. Advances in Life Sciences, 2, 31–37.
2. Thurston, C. F. (1994). The structure and function of fungal laccases. Microbiology, 140, 19–26.
3. Muthukumarasamy, N. P., & Murugan, S. (2014). Production, purification and application of bacterial laccases. Biotechnology, 13, 196–205.
4. Giardina, P., Faraco, V., Pezzella, C., & Sannia, G. (2010). Laccases: A never-ending story. Cellular and Molecular Life Sciences, 67, 369–385.
5. Hullo, M. F., Moszer, I. M., & Danchin, A. L. (2001). CotA of Bacillus subtilis is a copper-dependent laccase. Journal of Bacteriology, 183, 5426–5430.

6. Jadhav, U. U., Dawkar, V. V., & Ghodake, G. S. (2008). Biodegradation of Direct Red 5B, a textile dye by newly isolated Comamonas sp. UVS. Journal of Hazardous Materials, 158, 507–517.

7. Coelho, J. S., Souza, C. G. M., Oliveira, A. L., & Bracht, A. (2010a). Comparative removal of Bentazon by Ganoderma lucidum in liquid and solid-state cultures. Current Microbiology, 60, 350–355.

8. Wang, H. X., & Ng, T. B. (2006). Purification of a laccase from fruiting bodies of the mushroom Pleurotus eryngii. Applied Microbiology and Biotechnology, 69, 521–525.

9. Lee, S. Y., & Chang, J. H. (2011). Inorganic nanomaterial-based biocatalysts. BMB Reports, 44, 77–86.