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Extraction of Ligninolytic Enzyme from Industrial Waste

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Abstract

The idea behind the extraction of ligninolytic enzymes originated from the fear of plastic waste; plastic continues to menace the environment despite its ban. Plastic will breakdown chemically but will release toxic by-products that will release microparticles into nature and contribute to global warming. Thus, we designed a greener alternative to use enzymes to biodegrade a plastic accumulation. We remove the ligninolytic enzymes from industrial waste by performing filtration, centrifugation, ammonium sulfate precipitation, dialysis, and ion exchange chromatography. To accelerate the plastic degradation process, we add hydrogen peroxide (H2O2) as a catalyst and use surfactants to biodegrade plastic quickly.

Keywords: *Ligninolytic enzymes, Hydrogen peroxidase (H2O2).*

1. Introduction

With industrial advancement, specifically in newly industrialized countries, comes increased pollution due to waste remediation technologies that are expensive, dangerous, and laborious. Therefore, a viable and sustainable biological solution must be found. This review highlights biological agents in the remediation of industrial wastewater from the pulp, paper and dye industries. (Ojha, N., Karn, R., Abbas, S. and Bhugra, S. 2021). The article discusses microbes related to plastic degradation, including environmental and biotechnological work. It will also provide a systematic review of ligninolytic enzymes as a pathway for decomposing lignocellulosic waste. Laccase, lignin peroxidase and manganese peroxidase were reviewed in terms of environmental detoxification and industrial tolerance. It is estimated that approximately 350 to 400 million metric tons of plastic is produced each year throughout the world, while the nature of recycling and resource recovery is a slow process. As a result, considerable amounts of plastic still seeps into the environment. Microplastics pose a large environmental and human health risk. A recent promising line of research has been the combination of ligninolytic enzymes (natural catalysts produced by fungi, and other life) which have had a number of variants that are showing promise for degrading recalcitrant-type polymers such as lignin and plastic. The natural enzymes (such as lignin peroxidases, manganese peroxidases, laccases) have different mechanisms of action to degrade plastic including genetically modified versions, and as a fun fact, two of the most abundant and persistent plastic litter are polyethylene and polystyrene (Hiscox, J. 2010). The native (ligninolytic) enzymes we are using and modifying for degrading plastic represents a new low-cost and environmentally safe strategy similar in nature to bioremediation where nature helps us remediate our waste (Werkneh, A.A. and Rene, E.R., 2019). This review paper will discuss the new methods proposed to extraction enzymes, how the enzymes degrade plastic polymers, and their usefulness to the problem on plastic pollution. We suggest that the use of native enzymes is a better sustainable method of waste management of plastic and possibly an option for disposal at a low cost.

2. Methods of Extraction

2.1 Selecting Industrial Waste

The first step is to decide on the kind of industrial waste. The waste selected must be lignin-rich, as

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ligninolytic enzymes are designed to function in the presence of high concentrations of lignin. Accordingly, suitable easy sources of industrial waste that are lignin-rich are agricultural wastes, paper mill waste, and sawdust. The waste should also be widespread and available in sufficient amounts to allow for economical extraction of the enzyme [1-3].

2.2 Filtration

Filtration is the first step in the process of ligninolytic enzymes purification, removing larger solids and residual biomass resulting from producing the enzymes. The microorganisms producing ligninolytic enzymes can be allowed to grow in a liquid medium. However, in this case, there would be residual cell debris or broken solids. It has been demonstrated that the two examples here (Whatman filter paper and Membrane filters) have been beneficial for purity (Pinelo, Jonsson and Meyer, 2009), The Use of Whatman Filter Paper in the Isolation of Fungal Enzymes from Culture Media. Filtration will remove all solid residue during the purification process, producing a clear liquid mixture of enzyme to suspend and isolate.

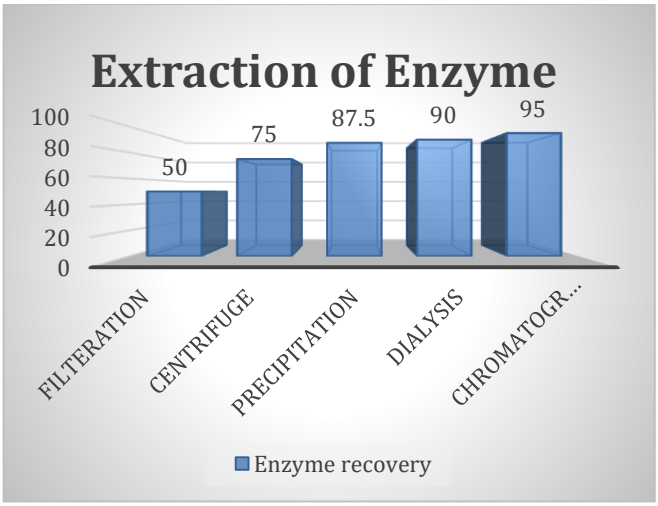

Figure 1 Enzyme Recovery Versus Steps in Extraction Process

2.3 Centrifugation

Industrial wastes must be processed by a stepwise centrifugation method to segregate unwanted solids while acquiring purified supernatants enriched with enzyme content. This technique guarantees a continuous elimination of impurities, increasing yield and quality of solid enzymes at different stages (Liu, Z. and Smith, S.R., 2021) [4-8].

- **Low-Speed Centrifugation (1,000–5,000 g, 10–15 min)**
  The process initiates with low-speed centrifugation which will eliminate the larger particulates of unbroken cells and solid debris. This step aids in clarifying the solution, which contributes to downstream purification.
- **Medium-Speed Centrifugation (10,000–15,000 g, 20–30 min)**
  At this stage, a higher centrifugal force is applied to separate finer particulates and aggregated materials. This improves the purity of the enzyme-containing supernatant while minimizing contamination from residual cellular components (Illuri, R., Kumar, M., Eyini, M., Veeramanikandan, V., Almaary, K.S., Elbadawi, Y.B., Biraqdar, M.A. and Balaji, P., 2021).
- **High-Speed Centrifugation (20,000–50,000 g, 30–60 min)**
  The final stage is the ultracentrifugation step to sediment any remaining cellular debris from the supernatant to yield a highly purified enzyme fraction (Wong, H.H., O'Neill, B.K. and Middelberg, A.P., 1996). This step is important for maximizing enzyme recovery and sufficient purity for downstream applications. Utilising this structured centrifugation pipeline significantly augments the extraction of enzymes from industrial waste, addressing a critical issue in downstream bioprocessing and industrial applications.
- **Ammonium Sulfate Precipitation:** To further concentrate the enzymes, ammonium sulfate precipitation will be performed (Purwanto, M.G.M., 2016). In this method, ammonium sulfate (a salt) is added to the enzyme solution gradually. When the ammonium sulfate concentration is at about 25%, the newly created salt concentration will induce the enzymes to clump together, forming a solid particle. The proteins become less soluble due to the high salt concentration and they will aggregate into either a solid particle or macromolecular aggregate. By centrifugation, the "clumps" of enzymes will be

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collected from the solution. This step also removes unnecessary proteins and aid in concentrating the enzymes we are interested in.

2.4 Dialysis

After converting the enzymes, we then go with dialysis to get rid of any small motes (leftover mariners or derivations) that might be there in the result. The result of the enzyme is put inside of a bag made of a semipermeable membrane (Regenerated Cellulose (RC) Membranes) through which the small motes can go but the larger enzyme motes get stuck on the inside (Weng, Z.H., Nargotra, P., Kuo, C.H. and Liu, Y.C., 2022). The bag was also submerged in a buffer solution to dilute and wash down the lower motes (Mores, W.D., Knutsen, J.S. and Davis, R.H., 2001. Cellulase recovery via membrane filtration. Applied Biochemistry and Biotechnology, 91, pp.297- 309). At the end of this process, the result of the enzyme should no longer contain lower motes that can interfere with the purpose of the enzyme. (Figure 1)

2.5 Ion Exchange Chromatography

Table 1 Sample Proceedings

Process	Weight of Remaining Waste (g)	Time Taken (Approx.) (h)	Enzyme Recovery (%)
Filtration	90	1	50
Centrifugation	85	2	70–80
Ammonium Sulfate Precipitation	70	3	85–90
Dialysis	68	5	90
Ion Exchange Chromatography	67	6	95

This is used in enzyme sanctification. This process uses the charges on the enzymes. An enzyme readout is passed through a column with one-off resin having either a positive or negative charge. The charge of the enzymes determines whether they bind to the resin or pass through the column. By fine-tuning the buffer conditions (ionic strength or pH), the enzymes can also be washed out of the column broadly with incremental wetlands that will dissociate the enzymes at various stages. thus, they will insulating the ligninolytic enzymes from all other proteins according their charges giving rise to a pure final product (Wang, P., Hu, X., Cook, S., Begonia, M., Lee, K.S. and Hwang, H.M., 2008). As shown in Table 1, the purified enzyme excerpt of 100 g sludge retains nearly 95 of the total enzyme exertion, leaving veritably little waste(nearly 67 g). The uprooted enzyme can also be enhanced to further increase the enzyme's efficacity with the addition of surfactants(1 w/ v per 10 g of enzyme) and hydrogen peroxide which act to increase enzyme exertion together [9-13].

3. Mechanisms of Enzymes

Mechanisms show that ligninolytic enzymes may be able to degrade plastics via oxidative processes that target aromatic and ester linkages in polymer structures, suggesting they may be effective agents for the bioremediation and recycling of plastic waste

- **Laccase:** A copper-dependent enzyme has an important role in the oxidative decomposition of phenolic compounds and other substrates by donating electrons to oxygen to generate reactive oxygen species (ROS) such as hydroxyl radicals that assist in the cleavage of complicated polymeric structures like plastics (Yao, C., Xia, W., Dou, M., Du, Y. and Wu, J., 2022). When evaluated with redox mediators like 1-hydroxybenzotriazole (HBt) laccase depicts an improved ability to cleave aromatic rings in polyethylene and polyester
- **Lignin peroxidase (LiP):** Enzymes that contain heme mediate the oxidative degradation of lignin by attacking its aromatic constituents through the sequential formation of highly reactive radical species. White rot fungus laccase (LiP) utilizes hydrogen peroxide (H_2O_2) as a co-substrate to initiate the breakdown of lignin's complex structures, and it has also been shown to effectively hydrolyze polyester (PET) polymers containing ester linkages (Falade, A.O., Nwodo, U.U., Iweriebor, B.C., Green, E., Mabinya, L.V. and Okoh, A.I., 2017). This is promising since laccase has potential as a bio

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recycling agent for plastic degradation and its use of H_2O_2 helps to generate additional reactive oxygen species, ultimately improving the overall efficacy of the enzymatic polymer degradation process.

- **Manganese peroxidase (MnP):** A heme-containing enzyme uses manganese ions (Mn2+) to catalyze the oxidation of phenolic compounds and lignin. During this catalysis, Mn2+ converts to Mn3+, an effective oxidizer that has the ability to cleave and degrade complex polymer structures in lignin or aromatic compounds. Such studies have concluded that MnP can degrade synthetic plastics (eg. PET) by breaking ester bonds releasing monomeric products such as terephthalic acid. Plastic monomers released from this reaction are more biodegradable than plastic polymers and the researchers see MnP as a potential bio recycling tool and opportunity for sustainable plastic waste (Chowdhary, P., Shukla, G., Raj, G., Ferreira, L.F.R. and Bharagava, R.N., 2019).
- **Versatile peroxidase (VP):** A hybrid enzyme with characteristics of lignin peroxidases and manganese peroxidases can oxidize myriad substrates ranging from lignin to other aromatic compounds through the use of hydrogen peroxide (H_2O_2) as a co-substrate. VP is important for the degradation of complex organic molecules through the production of radical intermediates. Notably, VP has been demonstrated to degrade synthetic plastics, including PET, by cleaving ester bonds in the polymer. Its efficiency in the presence of oxidants, such as H_2O_2, demonstrates its potential for bio recycling and represents a new mechanism for sustainable management of plastic waste (Camarero, S., Sarkar, S., Ruiz-Duenas, F.J., Martınez, M.J. and Martınez, A.T., 1999).

4. Results and Discussion

4.1 Results

Ligninolytic enzymes that can degrade plastics have been successfully extracted from industrial waste such as sludge. By the addition of surfactants and hydrogen peroxide, the enzyme activity is greatly improved, increasing the rate of plastic degradation. This process can be, therefore, effectively used to recycle and biodegrade plastic waste and contribute to sustainable development. The process improves waste management, reduces environmental pollution, and aids taking steps to develop a healthier, cleaner environment for all [14-17].

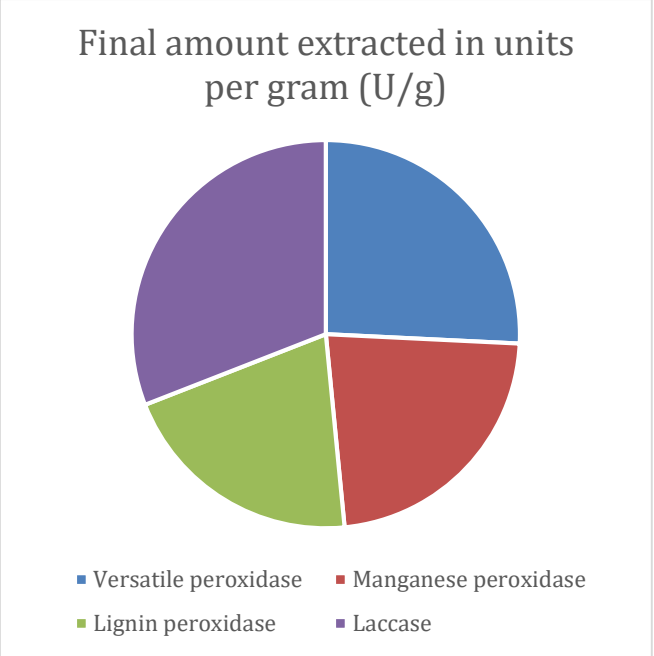

Figure 2 Amount of Extracted Enzyme

Enzymes such as laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP) are effective agents for degradation of plastics, including polyethylene, PET, and polystyrene. These enzymes act by attacking the aromatic structures in the plastics via oxidative processes and degrade them into smaller pieces that can subsequently be degraded by microorganisms. This enzyme-catalyzed degradation process is surely a more environmentally friendly option than conventional chemical pathways that often release toxic products. (Figure 2) [18-22].

4.2 Discussion

Research has indicated that surfactants can considerably accelerate degradation rates when incorporated with laccase-treated plastic films, notably in plastics such as PET that are resistant to enzymatic degradation (Okal, E.J., Heng, G., Magige, E.A., Khan, S., Wu, S., Ge, Z., Zhang, T., Mortimer,

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P.E. and Xu, J., 2023). Researchers also found that when polyethylene and polyester plastics were degraded by laccase-mediated degradation catalyzed with hydrogen peroxide, there was enhanced production rate of reactive oxygen species (ROS), therefore hastening degradation of plastics with aromatic compounds (Temporiti, M.E.E., Nicola, L., Nielsen, E. and Tosi, S., 2022). Additionally, the degradation efficiency with laccase was improved by approximately 70% for polystyrene plastics treated with surfactants and hydrogen peroxide in comparison to laccase alone. Together, these studies demonstrate that surfactants and hydrogen peroxide can markedly enhance laccase-mediated plastic degradation (Fujisawa, M., Hirai, H. and Nishida, T., 2001).

Conclusion

This research has demonstrated the amazing capability of ligninolytic enzymes, such as laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP) to degrade plastics like polyethylene, PET, and polystyrene. The use of surfactants and hydrogen peroxide has been shown to enhance the rate of enzymatic activity due to the stimulating effect hydrogen peroxide has on reactive oxygen species (ROS) to further degrade plastic. This method using ligninolytic enzymes has been found to work well on more complicated aromatic plastics which should provide greater accessibility of plastic for microbial degradation (Ghatge, S., Yang, Y., Ahn, J.H. and Hur, H.G., 2020). This study also demonstrated that enzyme mediated bioremediation offers a natural, environmentally safe method for degrading plastics rather than using conventional methods which may have harmful byproducts. Using a combination of ligninolytic enzymes, surfactants, and hydrogen peroxide, and applying it usually in an enzymatic approach is also much more effective and sustainable to control and tackle the growing problem of plastic waste and reducing Cu (II) into the environment. In conclusion, future studies should establish better efficiency of enzyme formulation, practicality for industry applications, and cost effectiveness for a feasible, scalable and environmentally safe solution to plastic waste on a wider scale.

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