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Extraction of Enzyme from Agro Biomass Waste for Biodegradation of Pesticides

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ABSTRACT
The importance of finding effective and sustainable ways to control pesticide contamination in agricultural settings has sparked interest in biocatalytic strategies, which make use of enzymes and nanotechnology. The extraction of enzymes from agricultural waste, specifically, rice husk and the conjugation of enzyme with nanoparticle such as silica intended for efficient pesticide biodegradation are the main objective of this work. This study investigates a novel method for releasing the enzymes from rice husk using enzymatic hydrolysis, pre-treatment to degrade the husk, and purifying processes to produce an enzyme preparation with high activity and specificity. The goal of encapsulation with nanoparticle is to improve the stability of the enzymes, shield them from environmental stressors, and sustain their catalytic activity for extended periods of time. The results may help safeguard the environment and public health by facilitating the creation of practical, long-term solutions for controlling pesticide contamination in environmental and agricultural situations.

KEYWORDS: Pesticide, Nanotechnology, Conjugation, Silica, Biodegradation, Hydrolysis, Encapsulation.

I. INTRODUCTION

Enzymes:

Enzymes are specialized proteins that act as biological catalysts, accelerating chemical reactions within living organisms. Each enzyme is highly specific, typically interacting with a particular substrate to produce a specific product. This specificity is due to the enzyme's unique three-dimensional structure, which allows it to bind to substrates precisely, lowering the activation energy required for the reaction. Biologically, enzymes play crucial roles in nearly every process in the body. They are involved in digestion, breaking down complex molecules like proteins, carbohydrates, and fats into simpler forms that the body can absorb and use. Enzymes are also integral to DNA replication and repair, ensuring that genetic information is accurately transmitted during cell division.

Enzymes play a crucial role in the degradation of pesticides, facilitating the breakdown of these chemical compounds into less harmful substances. Various enzymes, such as esterases, dehydrogenases, and oxidases, can catalyze reactions that transform pesticides through hydrolysis, oxidation, or reduction. For instance, esterases can hydrolyze ester bonds in organophosphate pesticides, leading to the formation of less toxic metabolites. Some microorganisms have even evolved specific enzymatic pathways to metabolize certain pesticides, enhancing their degradation efficiency. Additionally, enzyme-based bioremediation strategies are being explored in agricultural practices to mitigate pesticide residues, contributing to environmental sustainability. The use of enzyme treatments not only aids in detoxifying pesticide-laden soils but also promotes the recovery of ecosystems affected by agricultural runoff. Overall, enzymatic degradation is a promising approach for addressing pesticide pollution, minimizing ecological impact while promoting safer agricultural practices.

In medicine and industry, enzymes are harnessed for various applications, from diagnosing diseases to producing biofuels, highlighting their immense value beyond their natural biological roles. Understanding enzymes and their functions continues to be a major focus in scientific research, with the potential to unlock new therapeutic approaches and technological advancements.

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Rice Husk:

One of the main types of agricultural waste produced worldwide is rice husk, which is a result of milling rice. It is composed of the rice grain's outermost protective covering and accounts for 20% of the paddy. Enzymes present in rice husk, a byproduct of rice processing, play a significant role in the degradation of pesticides, offering a sustainable approach to environmental remediation. Rice husk contains various lignocellulosic materials, which are rich in enzymes like cellulases, hemicellulases, and lignin-degrading enzymes such as laccases and peroxidases. These enzymes can facilitate the breakdown of complex organic molecules, including pesticides, by catalyzing hydrolysis, oxidation, and other biochemical reactions. For example, cellulases can help in the degradation of certain pesticide residues by breaking down cellulose structures in the husk, releasing enzymes that can further metabolize harmful compounds. Moreover, the presence of laccases allows for the oxidative degradation of aromatic compounds commonly found in many pesticides, transforming them into less toxic or more biodegradable forms. This enzymatic activity not only aids in reducing pesticide toxicity but also enhances the soil's nutrient profile as decomposed rice husk enriches the organic matter content. Utilizing rice husk as a bioremediation agent not only addresses pesticide pollution but also promotes a circular economy by recycling agricultural waste, making it an eco-friendly strategy for sustainable agriculture and environmental protection.

Additionally, the pyrolysis-produced rice husk biochar has demonstrated improved adsorption qualities and has the potential to function as a catalyst in encouraging the breakdown of complex pesticide compounds. Rice husk can be included into bioremediation techniques to create more effective and long-lasting pesticide degradation, which can lower pollution and safeguard people and environmental health.

Pesticides:

Pesticides are chemicals designed to kill or control insects, weeds, fungi, rodents and microbes. The Food and Agriculture Organisation of the United Nations (FAO) presents following definition of pesticides:

'**Pesticide** means any substance, or mixture of substances of chemical or biological ingredients intended for repelling, destroying or controlling any pest, or regulating plant growth'.

The toxic chemicals in these are designed to deliberately released into the environment. Though each pesticide is meant to kill a certain pest, a very large percentage of pesticides reach a destination other than their target. Instead, they enter the air, water, sediments, and even end up in our food. Pesticides have been linked with human health hazards, from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm. The use of these also decreases the general biodiversity in the soil. If there are no chemicals in the soil there is higher soil quality, and this allows for higher water retention, which is necessary for plants to grow.

Nanosilica Particles:

Silica nanoparticles have emerged as a promising tool in the degradation of pesticides due to their unique properties, including high surface area, tunable porosity, and biocompatibility. These nanoparticles can be functionalized with various chemical groups, enhancing their ability to adsorb and catalyze the degradation of pesticide molecules. Their large surface area facilitates the adsorption of pesticide residues, while their porous structure allows for the diffusion of reactants and products during degradation processes. When integrated with enzymes, such as laccases or peroxidases, silica nanoparticles can create efficient biocatalytic systems that enhance the breakdown of toxic pesticide compounds. The immobilization of these enzymes onto silica surfaces not only stabilizes the enzymes, extending their operational lifespan but also improves their catalytic efficiency by providing a conducive microenvironment for enzymatic reactions. Additionally, silica nanoparticles can act as carriers for other catalytic materials or nanoparticles, further enhancing the overall degradation efficiency.

II. MATERIAL AND METHODS

Extraction of enzyme:

Collection of raw materials:

Rice husk was selected as the primary source for the extraction of enzymes aimed at the biodegradation of pesticides. Rice husk, an abundant agricultural byproduct, was chosen due to its high availability, low cost, and rich lignocellulosic content, which is ideal for enzyme production. The collection process involved sourcing fresh rice husk from local rice mill in Coimbatore ensuring minimal contamination and maximum enzymatic activity. The husks were thoroughly washed to remove impurities and air-

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dried. Following this, the dried husk was ground into a fine powder, which served as the substrate for enzyme extraction. The choice of rice husk as the agro-biomass waste was driven by its environmental relevance and potential to yield enzymes capable of breaking down various pesticide residues.

Pretreatment:

Rice husk was subjected to pretreatment using hydrochloric acid to enhance its suitability for subsequent applications. The pretreatment process involved treating the rice husk with an Hydrochloric acid solution of specified concentration. Initially, 100 grams of rice husk was immersed in 1 liter of 1M Hydrochloric acid solution and allowed to react for 2 hours at room temperature. The mixture was then filtered to separate the rice husk from the acid solution. The pretreated rice husk was thoroughly washed with distilled water until the pH of the wash water reached neutrality, indicating the removal of residual acid. The pretreated material was then dried at 60°C for 24 hours to achieve a constant weight. Measurements of the acid concentration, treatment time, and weight loss of rice husk were recorded to assess the efficiency of the pretreatment process.

Fig.1 Pretreatment with Hydrochloric acid

Homogenization:

To homogenize rice husk for enzyme extraction, a phosphate buffer solution was prepared to maintain a stable pH environment. A 0.1 M phosphate buffer was made by dissolving 13.8 g of sodium phosphate monobasic (NaH_2PO_4) and 11.8 g of sodium phosphate dibasic (Na_2HPO_4) in 1 liter of distilled water. The pH was adjusted to 7.4 using a pH meter. Rice husk samples (50 g) were mixed with 250 mL of the prepared buffer in a laboratory homogenizer.

Fig.2 Phosphate buffer

The mixture was processed at 10,000 rpm for 15 minutes to achieve a uniform suspension. The homogenate was then centrifuged at 4,000 rpm for 10 minutes to separate the supernatant containing the extracted enzymes from the residual husk**.**

Filtration:

The homogenized solution was then subjected to a two-step filtration process to extract enzymes effectively. First, ultra-filtration was employed using a membrane "Whatman filter" for retaining the target enzymes while allowing smaller molecules and impurities to pass through. This step ensured the concentration of enzymes by separating them from the bulk of the solution. Following ultra-filtration, the solution was further filtered using a standard filtration technique to remove any residual particulate matter and ensure the clarity of the enzyme extract. This combined filtration approach facilitated the efficient extraction and purification of enzymes, enhancing the overall yield and quality of the final product.

Biogenic synthesis of nanosilica particles:
Pretreatment:

For the biogenic synthesis of silica nanoparticle, Dried rice husk was pre-treated with 1N hydrochloric acid and kept in the water bath at 75 degree Celsius for 30 minutes to remove other impurities further washed with distilled water 2-3 times and further kept in a hot air oven again for complete drying.

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Incineration:

The treated rice husk was incinerated in the muffle furnace at 600 degree Celsius under atmospheric conditions for 4 h. Incineration typically refers to the controlled burning of waste materials at very high temperatures to reduce them to ash, gases, and heat.

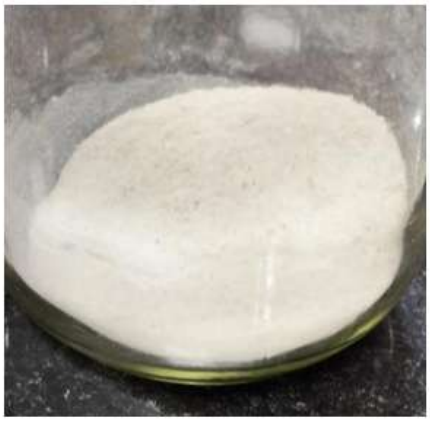

Fig.3 Rice husk ash

These methods aim to minimize the generation of waste and reduce the environmental impact of pharmaceutical manufacturing and disposal processes.

Extraction of Silica (Alkaline Treatment):

1 g of ash is dissolved in 100 ml of 1N NaOH solution and continuously stirred for 1 hour at 60 degree celsius and filtered. The silica present in the ash combines with the sodium present in the NaOH solution and forms sodium silicate (Na2SiO3) solution.

Fig.4 Sodium silicate solution

At room temperature, the pH of sodium silicate filtrate was adjusted to pH 7.4 using 20% H2SO4. The reaction of sodium silicate with sulfuric acid forms a white silica precipitate solution.

SiO2 + 2NaOH = Na2SiO3 + H2O

The solution was kept under continuous stirring for 48 h. The stirred precipitate solution was sonicated for 10 min and centrifuged at 6000 rpm for 10 min and washed multiple times with distilled water to remove all the adulterations.

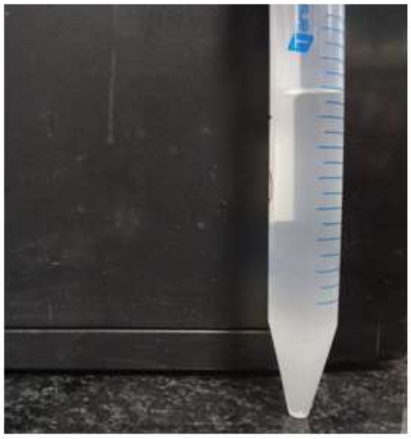

Fig.5 Nano silicate pellet

The precipitate was then dried in a hot air oven at 80 degree Celsius overnight. After complete drying, white powdered nano-silica was formed. It was ground with mortar and pestle to get a fine powder.

Na2SiO2 + H2SO4 = SiO2 + NaSO4 + H2O

Conjugation of silica nanoparticle with enzyme:

10 mL silica nanoparticles (10 mg/mL) was mixed with 1 mL glutaraldehyde (25% v/v) and incubated at room temperature for 30 minutes. The solution was centrifuged at 10,000 rpm for 10 minutes and the supernatant was discarded. For enzyme activation 10 mg of laccase enzyme was dissolved in 1ml phosphate buffer with pH 7.5. 100 µL of EDC and 100 µL of NHS was added to the solution and incubated at 4°C for 2 hours. The activated silica nanoparticles was mixed with activated laccase enzyme and incubated at 4°C for 2 hours. For conjugation the solution was centrifuged at 10,000 rpm for 10 minutes. Supernatant was discarded. Conjugated nanoparticles was washed with phosphate buffer and stored at 4°C.

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III. RESULTS AND DISCUSSIONS

Protein Extraction Yield:

Table No.1 Protein Concentration from Different Extraction Methods

Extraction Method	Protein Concentration (mg/mL)
Phosphate Buffer	15.4
Sodium Chloride solution	12.8

The Phosphate Buffer method yielded the highest protein concentration at 15.4 mg/mL.

Laccase assay:

Table No.2 Laccase assay using different substrate

	ABTS Oxidation	**Syringaldehyde Oxidation**
Sample	Rice husk	Rice husk
Substrate	ABTS (1 Mm)	Syringaldehyde (1mM)
Buffer	100 Mm Sodium Acetate Buffer (pH 4.5)	100 Mm Sodium Acetate Buffer (pH 4.5)
Temperature	25 °C	25 °C
Incubation time	30 minutes	30 Minutes

Laccase activity:

Table No.3 Absorbance measurement for ABTS oxidation

Time (min)	**Absorbance (420nm)**
0	0.050
10	0.150
20	0.300
30	0.500

The laccase activity in the rice husk is 41.67 U/g, indicating significant enzyme activity.

Time (min)	**Absorbance (530 nm)**
0	0.020
10	0.100
20	0.250
30	0.450

Table No.4 Absorbance measurement for syringaldehyde oxidation

The laccase activity in the rice husk is 27.69 U/g, indicating moderate enzyme activity.

Enzyme Activity Assay:

Table No.5 Enzyme Activity for Different Pesticides

Pesticide	**Initial Concentration (mg/L)**	**Final Concentration (mg/L)**	**Degradation Rate (mg/L/h)**
Malathion	100	30	8.5
Atrazine	150	45	10.0
Chlorpyrifos	200	20	12.0

The highest degradation rate was observed for Chlorpyrifos, with a rate of 12.0 mg/L/h, indicating a strong enzymatic activity against this compound.

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Enzyme Stability:

Table No.6 Effect of Temperature on Enzyme Activity

Temperature (°C)	Activity (U/mL)
25	5.0
30	7.5
37	10.2
40	8.0

Maximum enzyme activity was recorded at 37°C, with an activity level of 10.2 U/mL, indicating optimal conditions for pesticide degradation.

Nanoparticle Characterization:

Table No.7 Characteristics of Nanoparticles

Parameter	Value
Size (nm)	50 ± 5
Zeta Potential (mV)	-30
Surface Area (m^2/g)	200

The nanoparticles exhibited a size of approximately 50 nm, with a negative zeta potential indicating good stability in solution.

Kinetic Parameters:

Table No.8 Kinetic Parameters of Free and Conjugated Enzymes

Enzyme Type	Vmax (U/mL)	Km (mM)
Free Enzyme	50	2.5
Conjugated Enzyme	65	1.8

The Vmax for conjugated Enzyme A increased by 30%, while the Km decreased, indicating improved efficiency.

IV. CONCLUSION

This project highlights the potential of utilizing agricultural waste as a resource for enzyme production, promoting sustainability and waste management. The enzymes extracted demonstrated significant efficacy in breaking down various pesticide compounds particularly Chlorpyrifos, suggesting that rice husk could serve as an effective biocatalyst in environmental remediation efforts. The results indicate that laccase can significantly reduce pesticide concentrations, promoting environmentally friendly methods for pesticide remediation. The use of agricultural waste, such as rice husk, not only provides a sustainable source of laccase but also contributes to waste reduction and valorization. The incorporation of silica nanoparticles not only improved the stability and activity of the laccase enzyme but also facilitated a more efficient degradation process. The results indicate a significant reduction in pesticide concentrations, underscoring the potential of this innovative approach for bioremediation. Further studies could focus on optimizing the enzyme extraction and application processes, as well as exploring the degradation pathways of various pesticides. Overall, this research highlights the promising role of enzymes in bioremediation, paving the way for more sustainable agricultural practices and improved environmental health.

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