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Polyphenol Oxidase from Agricultural Wastes for Dye Removal from Wastewater

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Abstract This research addresses the urgent global need for the safe and economical removal of dyes from wastewater. We assessed the efficacy of polyphenol oxidase (PPO) extracted from different agricultural residues in removing reactive blue dye, marking the initial endeavor to do so. PPOs isolated and refined from rice straw, corn stover, peanut peels, olive husk, date core, and potato peels were examined. Optimal conditions for dye removal, comprising an enzyme concentration of 0.5 U/mL and a 10-min incubation period, were identified for all PPOs. Potato peels emerged as the most abundant source of PPO, displaying the highest dye removal efficiency at around 93.89%. Remarkably, this efficiency was consistent across a wide temperature range (20–80 °C) and a broad pH range (4–9), with no adverse effects noted in the presence of heavy metals. Comparative analysis highlighted potato peels as the most promising waste reservoir, highlighting resilience under diverse conditions. These findings provide valuable insights into utilizing PPO from agricultural waste for effective dye removal, offering potential applications in addressing environmental concerns within industrial settings.

Keywords Polyphenol Oxidase · Reactive blue 4 · Optimum temperature · Optimum pH · Potato peel polyphenol oxidase · Dye removal efficiency

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1 Introduction

Water pollution represents a substantial environmental issue influencing the quality and availability of water resources, jeopardizing the sustainability of ecosystems and human well-being. It involves the introduction of harmful contaminants into various water bodies, including ponds, canals, oceans, and groundwater. This contamination arises from diverse human activities, such as industrial processes, agricultural practices, and improper waste disposal. These pollutants may be chemical, biological, or physical, resulting in detrimental consequences such as the loss of aquatic life, the transmission of waterborne diseases, and the contamination of drinking water supplies (Gaur et al., 2024; Rabeie & Mahmoodi, 2024; Sahoo & Goswami, 2024). Dyes are considered emerging water pollutants as they represent a major threat to aquatic life and human health since they have the potential to be teratogenic, mutagenic, carcinogenic, and highly toxic (Bal & Thakur, 2022; Gallegos-Cerda et al., 2024; Hemashenpagam & Selvajeyanthi, 2023; Tsaffo Mbogno et al., 2024). While easily noticeable, removing these colors from surface water habitats proves to be a formidable task. The intricate molecular structure, which is characterized by robust chemical bonds and aromatic rings, makes the breakdown process challenging. To ensure environmental safety and mitigate toxicity concerns, it becomes imperative to eliminate these hazardous substances efficiently and safely (Bal & Thakur, 2022; Bellaj et al., 2024; Bulgariu et al., 2019; Hynes et al., 2020; Kumar et al., 2024; Yu et al., 2024). Numerous techniques for dye removal exist, encompassing chemical, physical, and biological approaches. Biological methods involve aerobic and anaerobic degradation, and bioremediation using bacteria, fungi, and algae. Chemical methods encompass coagulation, electrocoagulation, and conventional oxidation using agents like ozone, irradiation, or electrochemical processes. Physical treatment methods include adsorption, ion exchange, filtration, and coagulation. Despite these options, many are considered inefficient and economically unfeasible. Therefore, there is a crucial need to shift attention towards emerging strategies for dye decolorization, such as through the enzymatic activities of oxidoreductases, which have proven effective in various bioremediation applications (Routoula & Patwardhan, 2020; Valli Nachiyar et al., 2023). Oxidoreductive enzymes, including peroxidases and polyphenol oxidases, play a crucial role in breaking down aromatic pollutants found in different contaminated sites. These enzymes exhibit versatility in acting on a wide range of substrates, enabling them to catalyze the degradation or removal of organic pollutants even at low concentrations in contaminated areas (Salehi et al., 2021).

Peroxidases have been considered for dye treatment due to their efficacy in decolorization across a broad spectrum, but none have been widely utilized on a large scale. This limitation is attributed to the need for expensive H_2O_2 as a co-substrate in the case of peroxidases (Al-Sakkaf et al., 2023; Sellami et al., 2022). Recently, using polyphenol oxidase (PPO) instead of peroxidase in dye removal by harnessing its oxidative power has taken great attention (Kakkar & Wadhwa, 2023). The importance of PPO in dye removal came from its oxidative power without the need for any other compounds such as H_2O_2 (Verma et al., 2024). Besides this, PPO can degrade a wide range of synthetic dyes and organic pollutants efficiently, converting them into non-toxic compounds without the need for H_2O_2, thus making polyphenol oxidase a promising agent for dye and organic pollutant removal from wastewater (Chmelová et al., 2024). Naseem and colleagues (2023) documented that utilizing polyphenol oxidase for dye biodegradation presents a competitive method for removing dyes from wastewater. This approach capitalizes on the green catalyst properties of PPO, boasting high catalytic efficiency while producing minimal toxic by-products. Similarly, Zouari-Mechichi et al. (2024) reported that polyphenol oxidase is a promising alternative method for dye removal from wastewater with no toxic by-products. Optimization of polyphenol oxidase production from various sources is one of the most important points that should be considered when we apply PPO in dye removal from wastewater (Selvam et al., 2024). Also, Magalhães et al. (2024) reported that one of the challenges of using PPO in dye biodegradation is finding natural, safe sources with low costs. A crucial aspect of applying enzymes for dye removal is the cost-effectiveness of the enzyme source, coupled with high enzyme yield and efficiency. It is worth noting that ongoing research is focused on identifying enzymes from various sources with improved characteristics for specific applications, including dye removal (Kyomuhimbo et al., 2023; Thamke et al., 2023). From this point of view, the present study tries to find cheap sources for PPO. It is well reported that agricultural wastes are promising sources for various value-added products such as antimicrobial and anticancer agents and enzymes (Naguib & Tantawy, 2019; Naguib & Badawy, 2020; Al-Hazmi and Naguib, 2023a, b; Bankeeree et al., 2024). These agricultural wastes are cheap and found in massive quantities, which can contribute to many environmental concerns if these wastes are not usefully used (Sridhar et al., 2022). From this point of view, given the need to find new, safe, and cheap sources for enzymes used for industrial applications such as dye removal and the availability of agricultural wastes rich in valuable compounds, we tried to evaluate polyphenol oxidase from different agricultural wastes for dye removal from water. To our knowledge, this study is the first attempt to extract and purify polyphenol oxidase from agricultural wastes and use it for dye removal, which will offer potential applications in addressing environmental concerns within industrial settings.

2 Material and Methods

2.1 Polyphenol Oxidase Extract from Agricultural Wastes

Polyphenol oxidase (PPO) was extracted and purified from agricultural wastes following the methodology outlined by Lin et al. (2016).

2.1.1 Agricultural Wastes

We used six different agricultural wastes, namely: rice straw, corn stover, peanut peels, potato peels, olive husk, and date core. We obtained these agricultural wastes from various sources. The rice straw, corn stover, peanut peels, and potato peels were sourced from a farm in Sharqia governorate, Egypt. Olive husk and date core were obtained from the New Salhiya olive press in Sharqia governorate and the Al Tahan Dates Factory in Banha, Egypt, respectively.

2.1.2 Preparation of the Crude Enzyme

To initiate the extraction process, the dried agricultural wastes were finely pulverized using an electric blender, resulting in a powder. This powder (100 g) was then homogenized with a mixture comprising 4 g of polyvinylpyrrolidone (Sigma-Aldrich, St. Louis, MO, USA) and 400 mL of 0.1 M phosphate buffer at pH 6.8. After homogenization, the mixture underwent centrifugation at 6000 g for 30 min at 4°C, leading to the collection of the supernatant as the crude enzyme extracts.

2.1.3 Polyphenol Oxidase Purification

The crude protein extracts were subjected to precipitation by adding 50% ammonium sulfate, followed by an overnight incubation at 4°C. The resulting precipitate was separated through centrifugation at 12,000g for 15 min at 4°C, and the resulting pellets were dissolved in 5 mL of 0.1 M phosphate buffer at pH 6.8. This dissolved protein underwent dialysis against 0.1 M phosphate buffer at pH 6.8 at 4°C for an extensive period of 48 h, with the buffer solution being changed every half hour throughout the incubation period. Following dialysis, the purified protein underwent an additional refinement step utilizing a Sephadex G-100 glass chromatography column (Sigma-Aldrich). The column was initially equilibrated with a 0.1 M phosphate buffer at pH 6.8, and the protein sample was loaded onto the column. Elution was conducted using a 0.1 M phosphate buffer at pH 6.8, and fractions of 5 mL each were collected until the column volume was exhausted. Fractions demonstrating PPO activity were selectively collected and subsequently employed as the primary source of PPO for the decolorization of dyes. The enzyme units in the extracted fractions were calculated through a PPO assay test to demonstrate the concentration of the enzyme.

2.2 PPO Assay

Polyphenol oxidase (PPO) activity was assessed using a method outlined by Da Cruz Vieira and Fatibello-Filho (1998). The procedure involved combining 0.5 mL of the enzyme solution, 0.5 mL of a 0.1 M catechol solution, and 4.0 mL of a 0.1 M phosphate buffer at pH 6.8. This mixture was left to incubate at room temperature for 5 min, and the reaction was stopped by adding 1 mL of 10% H2SO4.

To measure PPO activity, the color produced during the reaction was analyzed by measuring absorbance at 400 nm. PPO activity was then quantified as one unit, which represented the amount of enzyme causing a 0.001 absorbance change per minute.

2.3 Application of PPO to dye Removal

The efficacy of the extracted PPO from various agricultural wastes in removing dyes from the solution was evaluated using solutions containing individual dyes. The decolorization capability of the PPO was examined under different conditions to determine the optimum conditions for each PPO needed for maximum dye removal. Enzyme concentration, incubation time, temperature, pH, and the presence of different metals are the key parameters examined in the application of PPO for dye removal.

2.3.1 Dye Solution Preparation

Reactive Blue 4 obtained from Sigma Aldrich was utilized in this study. Single-dye solutions were prepared by dissolving the necessary amount of the dye in distilled water. Specifically, 50 g of the dye powder were dissolved in one liter of distilled water. This preparation yielded a dye solution with a concentration of 50 mg/mL.

2.3.2 Effect of Enzyme Concentration on dye Removal

A dye solution with a concentration of 50 mg/mL was subjected to incubation with varying concentrations of the prepared PPO (ranging from 0.1 to 1 U/mL) in 0.1 M phosphate buffer at pH 6.8, maintained at 37°C for 10 min. After incubation, the absorbance of the solution was measured at the defined λmax of the dye solution (λmax = 595 nm). The removal percentage was calculated by comparing it to the untreated dye solution used as a control.

2.3.3 Effect of Incubation Time on dye Removal

The dye solution (50 mg/mL) was incubated with prepared PPO 0.5 U/ml in 0.1 M phosphate buffer pH 6.8 at 37°C at various times. After incubation, the absorbance of the solution was read at the defined λ_{max} of each dye solution (reactive blue λ_{max} = 595nm). The removal percentage was calculated by taking an untreated dye solution as a control.

2.3.4 Effect of Different Temperatures on dye Removal

A dye solution with a concentration of 50 mg/mL was incubated with prepared PPO at a concentration of 0.5 U/mL in 0.1 M phosphate buffer at pH 6.8, maintained at 37°C for 10 min. After incubation, the absorbance of the solution was measured at the defined λmax of each dye solution (λmax for reactive blue). The removal percentage was calculated by comparing it to the untreated dye solution, which served as the control.

2.3.5 Effect of pH on dye Removal

A dye solution with a concentration of 50 mg/mL was incubated with prepared PPO at a concentration of 0.5 U/mL, and the mixture's pH was adjusted at different points ranging from 3.0 to 10.0. The incubation was carried out at 37°C for 10 min. Following incubation, the absorbance of the solution was measured at the λmax of the dye solution (λmax = 595 nm). The removal percentage was calculated by comparing it to the untreated dye solution, which served as the control.

2.3.6 Effect of Different Metals on dye Removal

The impact of various metals (Fe, Cu, Pb, Ni, Co, Mo, and Cd) on the dye removal capacity of the extracted PPO from different sources was assessed using the following procedure: A dye solution with a concentration of 50 mg/mL was incubated with prepared PPO at a concentration of 0.5 U/mL in the presence of metal ions in the assay mixture (as 50 mM chloride solutions). The incubation occurred in 0.1 M phosphate buffer at pH 6.8 at 37°C for 10 min. After incubation, the absorbance of the solution was measured at the λmax of the dye solution (λmax = 595 nm). The removal percentage was calculated by comparing it to the untreated dye solution, which served as the control.

2.3.7 Calculation of dye Removal (%)

The dye removal percent (DR%) for each case was calculated according to the following equation:

$$DR\% = \frac{ControlAbsorbance - FinalAbsorbance}{ControlAbsorbance} \times 100$$

2.4 Statistical A006Ealysis

All experiments were conducted in triplicate, and the data were presented as the mean of the three replicates ± standard deviation. Statistical analysis was performed using one-way ANOVA, and the mean values were compared using the SPSS program.

3 Results and Discussion

3.1 Extraction of Polyphenol Oxidase from Agricultural Wastes

Agricultural wastes are abundant in protein content, presenting a potential source for animal feed protein (Rasool et al., 2023). These wastes exhibit variability in protein content, as highlighted in a study by Yusree et al. (2021). Figure 1A illustrates a significant difference in protein content among the examined agricultural wastes. Notably, peanut peels demonstrate the highest protein content, while rice straw, date cores, and potato peels exhibit the lowest. This trend aligns with expectations based on the composition of these agricultural wastes, with legumes such as peanuts containing a higher protein content than some other plant types. The outer layer or peel of peanuts, in particular, may harbor a relatively higher protein concentration compared to the peels of other crops (Mingrou et al., 2022).

The observed variations in protein content prompted further investigation into the feasibility of utilizing these agricultural wastes as a source of enzymes to reduce production costs and meet industrial needs (Mesbah, 2022). Polyphenol oxidase (PPO), a protein enzyme associated with senescence and plant protection against environmental conditions, is found in agricultural wastes (Ebrahimi & Lante, 2022). Different agricultural wastes contain varying amounts of PPO (Oleszek et al., 2023). Figure 1B indicates a significant difference in PPO content among agricultural wastes, with potato peels exhibiting the highest, followed by peanut peels, olive husks, and date cores. In contrast, rice straw and corn stover show the lowest PPO content. An essential factor determining the efficacy of agricultural wastes as an enzyme source is the percentage of this enzyme relative to the total protein, reflecting the richness of the source in this enzyme. Our results, as depicted in Fig. 1C, highlight potato peels as the most abundant waste in PPO. The high presence of PPO in potato peels corresponds with its role in the enzymatic browning reaction due to the high phenol content of the potato peels when plant tissues are damaged or exposed to air (Nguyen et al., 2024). Venturi et al. (2019) reported potato peels as an effective source of natural antioxidants, consistent with Panadare and Rathod's (2018) findings, identifying potato peels as the primary source of PPO, followed by other agricultural wastes as cost-effective sources of PPO. Recently, Uddin et al. (2024) reported that potato peels showed higher dye removal than mango and luffa peels due to their higher PPO content.

3.2 Application of Polyphenol Oxidase for dye Removal

The textile industry often discharges effluents containing dyes into water bodies. Azo dyes, widely utilized across industries, raise environmental concerns due to their persistence and potential toxicity (Ahmed et al., 2023; Islam et al., 2023; Ravindiran et al., 2023; Vaiano & De Marco, 2023). Polyphenol oxidase (PPO) can play a role in bioremediation processes by breaking down and eliminating these dyes. The enzyme facilitates the oxidation of phenolic compounds within the dyes, leading to their degradation (Afreen & Mishra, 2023; Wang et al., 2020). It is crucial to acknowledge that the use of PPO for dye removal is an ongoing area of research. The efficiency of dye removal using PPO can be influenced by several factors, including enzyme concentration, incubation time, temperature, pH, and the presence of different metals (Ayodeji et al., 2023).

Determining the optimal enzyme concentration for maximal dye removal is imperative for cost-effectiveness (Elmetwalli et al., 2023). Elevating the concentration of polyphenol oxidase (PPO) typically results in an augmented rate of dye removal. However, an optimal concentration threshold exists; beyond that, further increments do not yield a substantial improvement (Dahiya et al., 2023). Results presented in Fig. 2 indicate that all PPO derived from diverse agricultural wastes exhibit enhanced dye removal as PPO concentration increases up to 0.5 U/mL, beyond which further increases do not result in a significant improvement in dye removal efficiency. Similarly, Svetozarević et al. (2021) observed an augmentation in dye removal with escalating peroxidase concentration until a specific point, after which no significant change in removal efficiency was observed. The absence of a discernible effect on dye removal following the optimum PPO concentration is attributed to the escalating resistance of the dye against enzyme action (Majumdar & Bhowal, 2022). Similarly, Çifçi et al. (2019) reported that the decolorization efficiency of oxidative enzymes increased

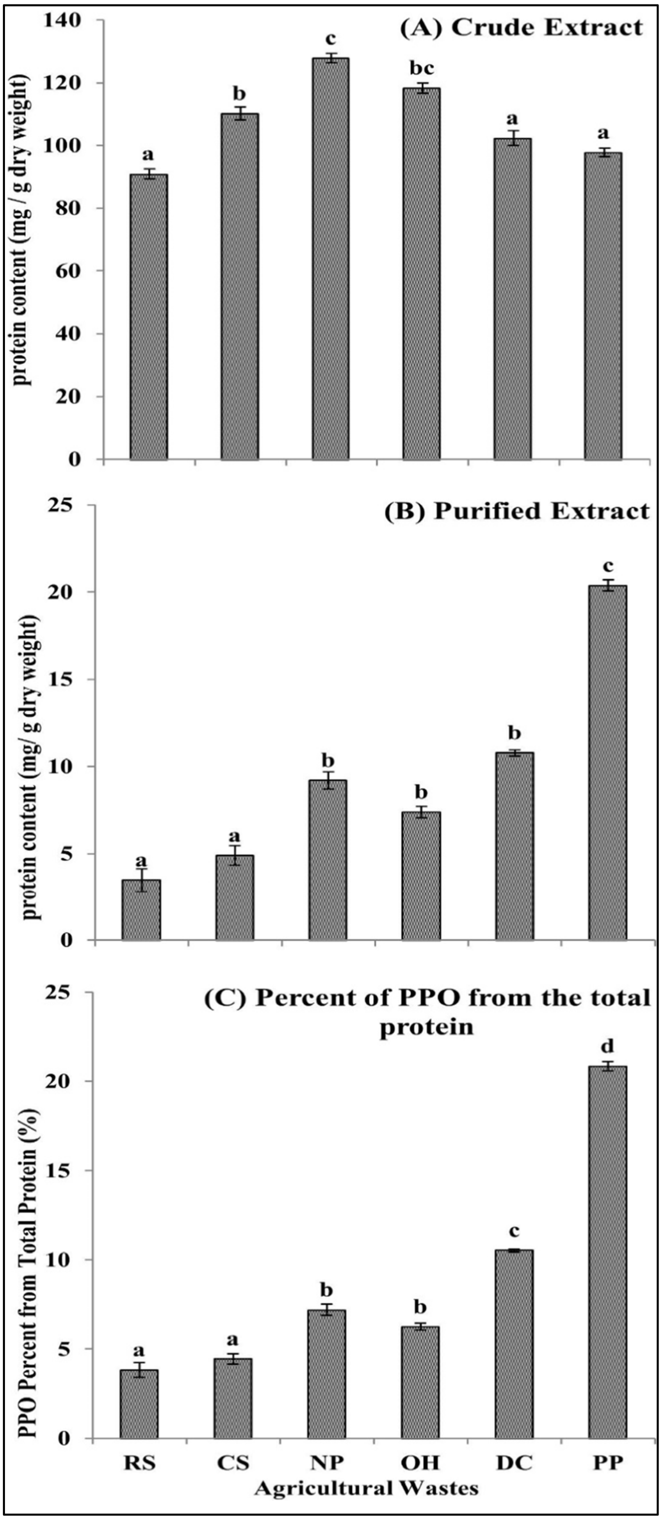

Fig. 1 The protein content in (**A**) crude extract, (**B**) purified extract of agricultural wastes, and (**C**) percent of PPO from the total protein. RS: rice straw, CS: corn stover, NP: peanut peels, OH: olive husk, DC: date core, and PP: potato peels. Columns represent the mean of three replicates, and the error bars represent the standard deviation. Columns followed by different letters are significantly different according to the ANOVA test

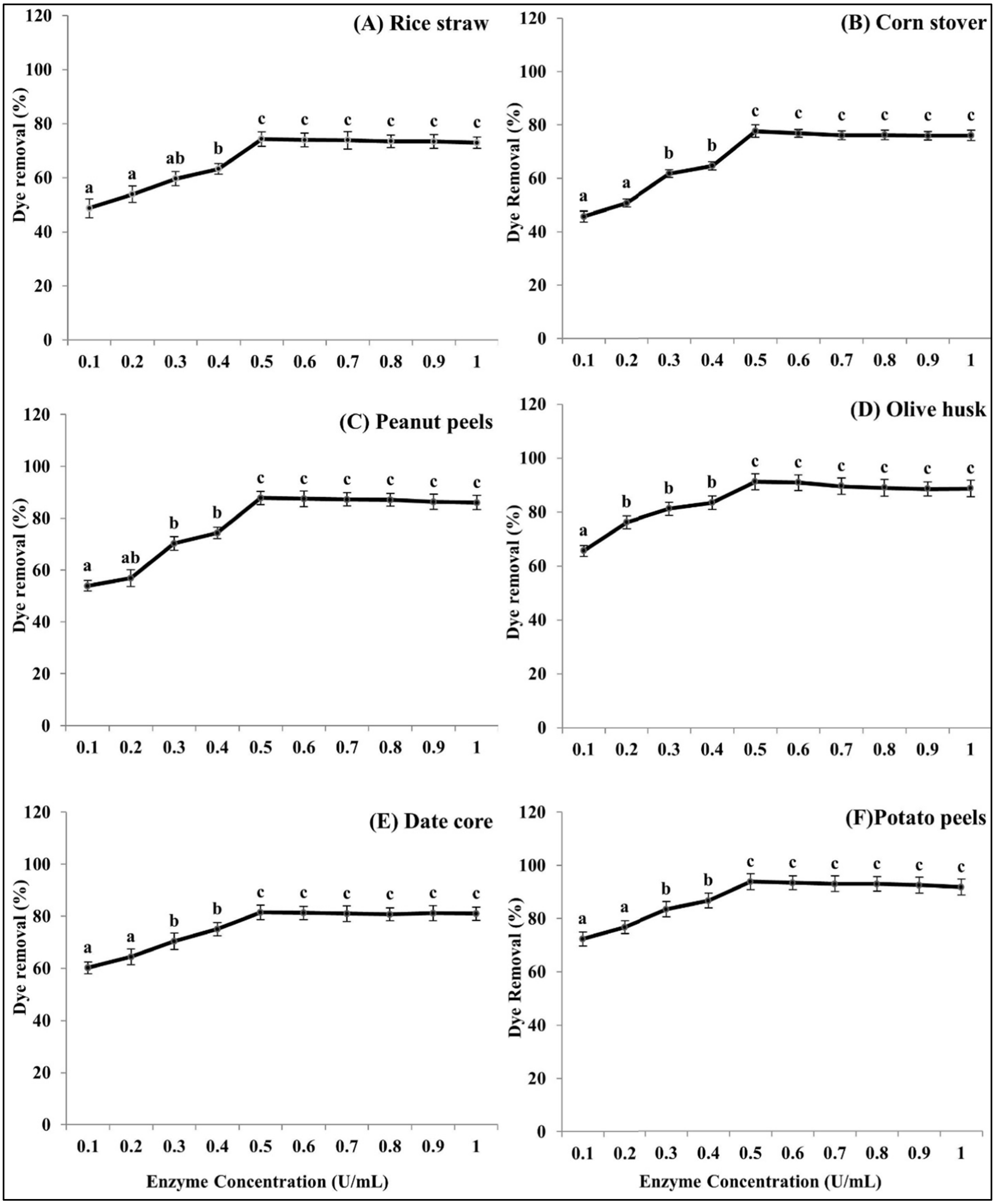

Fig. 2 Effect of different polyphenol oxidase concentrations from different agricultural wastes on dye removal. Points represent the mean of three replicates, and the error bars represent the standard deviation. Points followed by different letters are significantly different according to the ANOVA test

with increasing the enzyme concentration to 0.1 mg/mL and then decreased with increasing the enzyme concentration due to competition between enzymes on the substrate. Also, Shan and Hassan (2022) reported that dye removal decreased with increasing the enzyme concentration more than the optimum enzyme concentration due to the crowded environment leading to completion between enzyme molecules, so the dye removal delays. The lower the optimum enzyme concentration needed for maximum dye removal, the more efficient the enzyme (Kaur et al., 2024).

An additional consideration for the economic efficiency of the removal process is the time required for maximum dye removal (Ayodeji et al., 2023). Figure 3 demonstrates that polyphenol oxidase from all studied agricultural wastes achieves its maximum efficiency after a 10-min incubation period, with no significant change in dye removal efficiency thereafter. The first increase in dye removal with time is often due to the initial availability of enzyme-active sites and the quick binding of the enzyme to the dye molecules (El-Bendary et al., 2023). As the reaction progresses, the enzyme may saturate the substrate (dye molecules). This saturation phase is characterized by a slower rate of dye removal. At this point, most enzyme active sites are occupied, and the reaction approaches equilibrium. Eventually, the reaction may reach equilibrium, where the rate of dye removal levels off. This equilibrium indicates that most of the available substrate has been transformed, and the system has reached a dynamic equilibrium between the formation and breakdown of dye-enzyme complexes (Liu et al., 2024). The short time needed to reach maximum removal efficiency underscores the promising potential of polyphenol oxidase from agricultural wastes as an effective agent for dye removal from wastewater. The efficiency is enhanced by the shorter time required for the enzyme to achieve maximum removal efficiency (Jafari et al., 2023). The value of the short time required for maximum removal is related to the fact that over time, enzymes can undergo denaturation or deactivation, which can affect their catalytic activity. The efficiency of dye removal may decrease at longer incubation times due to enzyme instability (Xu et al., 2024a). PPO from different agricultural wastes in the present study showed superiority to other similar enzymes from various sources in the incubation time needed for maximum dye removal. Laccase from *Cerrena unicolor* decolor accounted for 98.7% of the dye after a 6-h incubation period, and the removal efficiency decreased with increasing the incubation period (Çifçi et al., 2019). Laccase of *Streptomyces sviceus* showed its maximum Congo red removal efficiency after 24 h (Chakravarthi et al., 2021). The crude oxidative enzyme from *Pleurotus ostreatus* could remove 84.23% of the dye in the solution after a 120-min incubation period (Shan & Hassan, 2022). Recently, Uddin et al. (2024) showed that potato peels, mango peels, and luffa peels decolorized dyes after a 120-min incubation period.

Temperature and pH represent pivotal factors influencing enzyme activity, with each enzyme manifesting an optimal range for activation and maximal activity (Pandey et al., 2023). Results in Figs. 4 and 5 show that the removal efficiency of polyphenol oxidase (PPO) from agricultural wastes exhibited an increasing trend with rising temperature and pH until reaching a specific optimal point, resulting in maximal dye removal efficiency. All examined PPO variants demonstrated an optimum pH of 6.5 (Fig. 4) and an optimum temperature of 40°C (Fig. 5). This aligns with existing studies reporting optimal pH at 6.5 and optimum temperature at 40°C for plant-derived PPOs (Česko et al., 2023; Demir et al., 2024; Hong et al., 2024; Liu et al., 2023; Zhang, 2023). The decrease in the PPO beyond the optimum temperature and pH, PPO activity diminished, as elevated temperatures could induce enzyme denaturation, leading to a loss of catalytic activity. The pH factor influences the ionization state of amino acid residues in the enzyme's active site, impacting its structure and activity (Ashok et al., 2024; Ateş et al., 2024; De Oliveira et al., 2021; Sajjad et al., 2023).

Interestingly, PPO enzymes from different agricultural wastes exhibited distinct pH and temperature ranges conducive to high dye removal. Potato peel PPO displayed the broadest temperature and pH range, achieving a dye removal efficiency of approximately 93% across temperatures ranging from 20 to 80°C and maintaining consistent removal efficiency (93%) within a pH range of 4 to 9. In contrast, dried rice straw, corn stover, and peanut peels exhibited a narrower temperature range (30 to 50°C) whereas removal efficiency showed non-significant differences from that at the optimum temperature. The extensive pH and temperature range enhances the

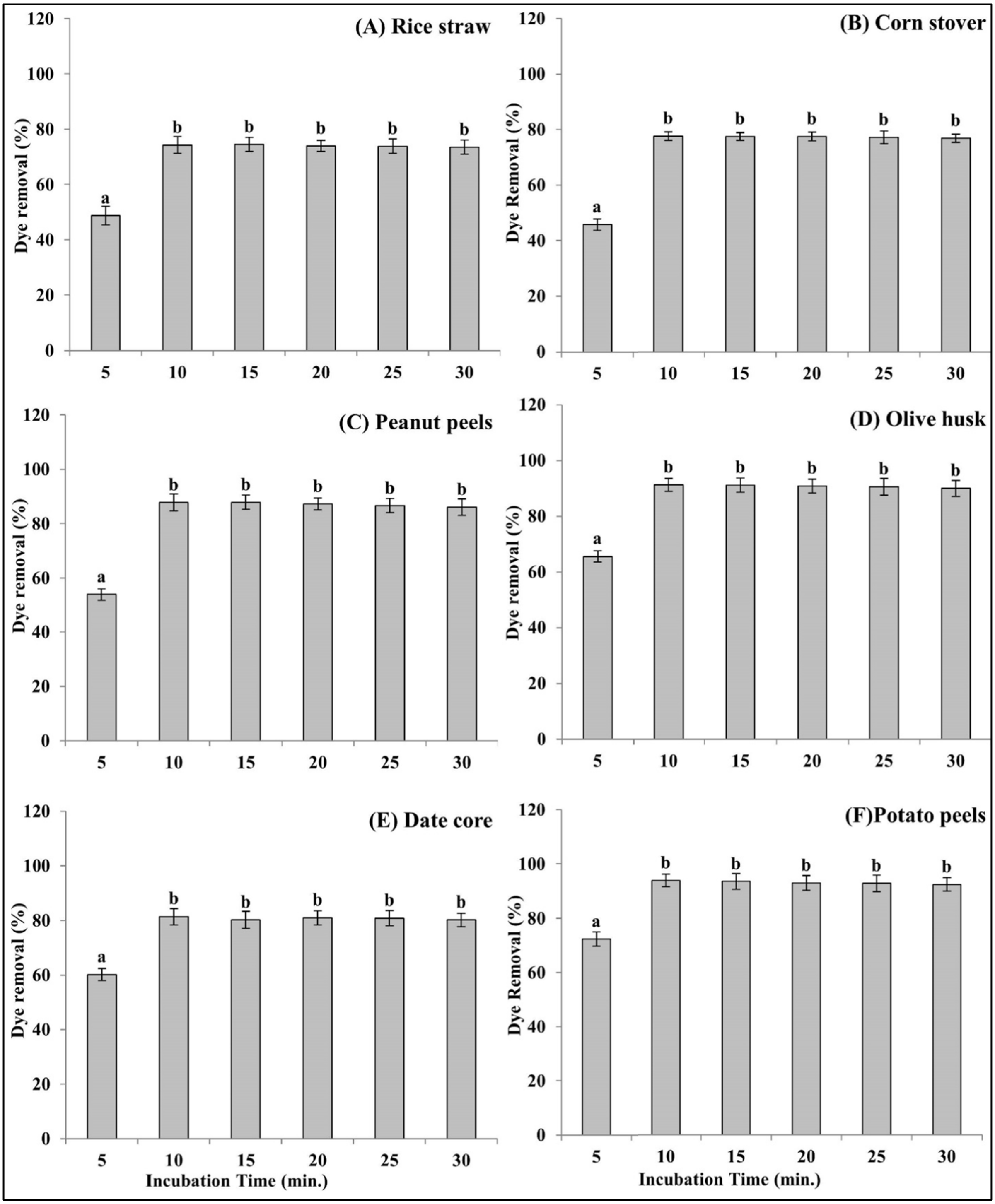

Fig. 3 Effect of different incubation periods on the dye removal efficiency of polyphenol oxidase from different agricultural wastes. Columns represent the mean of three replicates, and the error bars represent the standard deviation. Columns followed by different letters are significantly different according to the ANOVA test

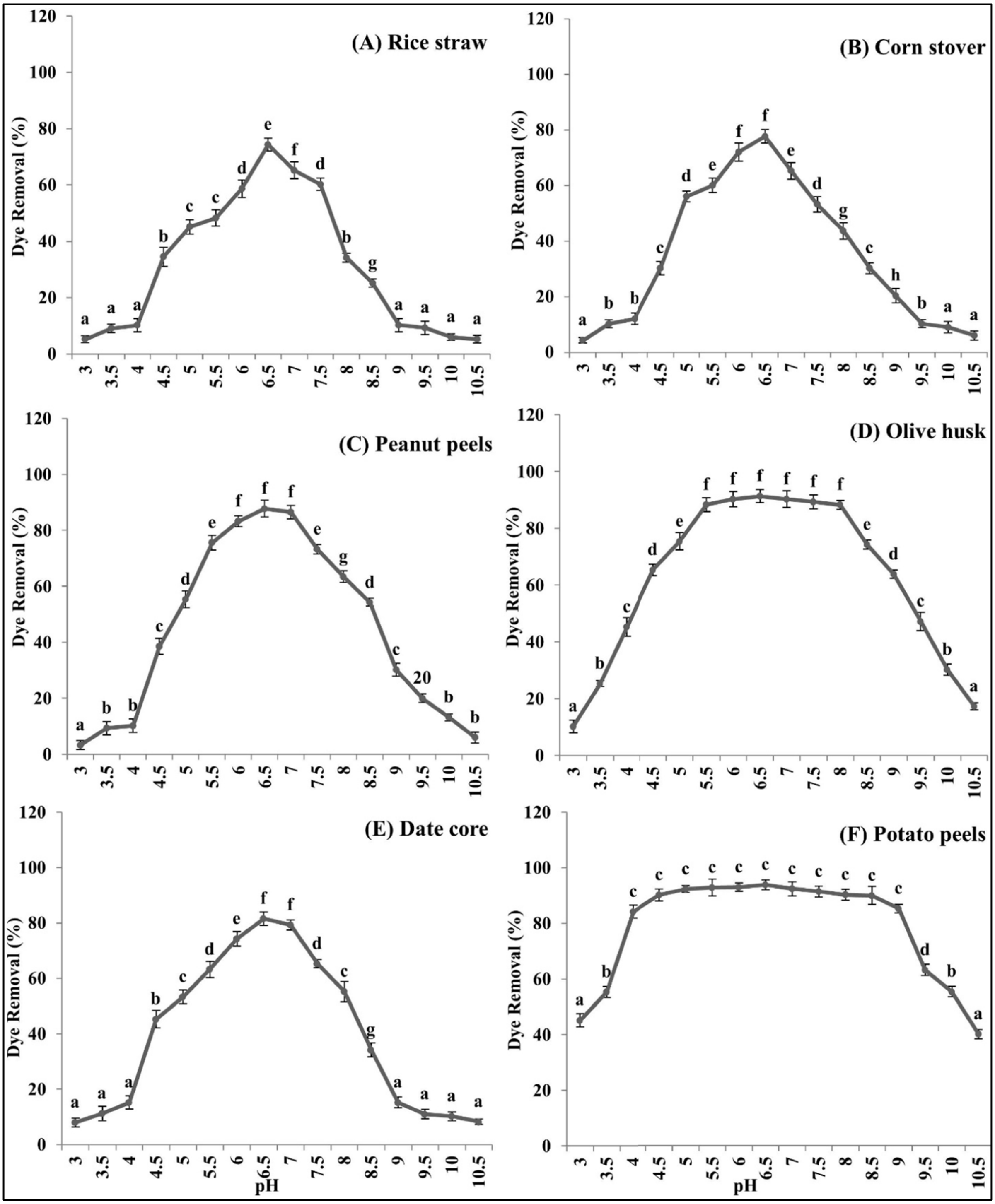

Fig. 4 Effect of different pHs on the dye removal efficiency of polyphenol oxidase from different agricultural wastes. Columns represent the mean of three replicates, and the error bars represent the standard deviation. Columns followed by different letters are significantly different according to the ANOVA test

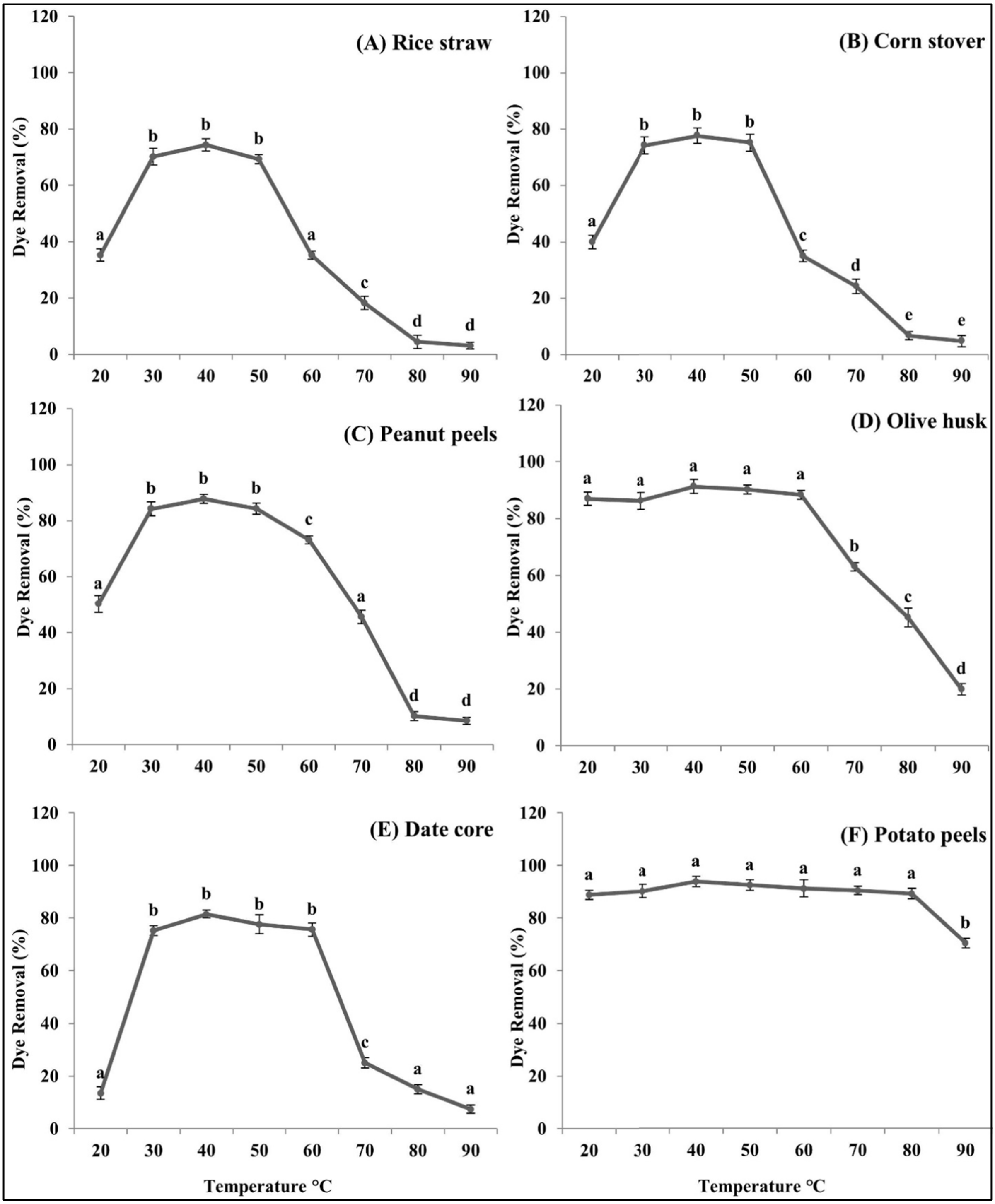

Fig. 5 Effect of different temperatures on the dye removal efficiency of polyphenol oxidase from different agricultural wastes. Columns represent the mean of three replicates, and the error bars represent the standard deviation. Columns followed by different letters are significantly different according to the ANOVA test

cost-effectiveness of dye removal with the enzyme, as maximum efficiency can be attained across various temperatures according to industry conditions (Khodakarami et al., 2024; Kumar et al., 2024). The broad pH and temperature range of an enzyme signifies high stability under diverse conditions, augmenting its effectiveness and rendering it favorable for industrial applications (Castillo-Suárez et al., 2023; Kumar et al., 2023). Recently, Uddin et al. (2024) reported that the wide range of pH and temperature is an essential characteristic for efficient PPO in wastewater treatment. Similarly, Çifçi et al. (2019) reported that efficient enzymatic dye removal needs a wide range of pH and temperature, in their work on the use of laccase in dye removal, the maximum dye removal efficiency occurred in the 5 to 7 pH range. In the work of Motamedi and his colleagues, the maximum enzymatic dye removal efficiency was in the pH range from 4 to 6, and temperature range from 30 to 60°C (Motamedi et al., 2021). Trivedi et al. (2022) reported that maximum dye removal occurred in the pH range between 7 and 9. In addition, Pal and Chakraborty (2023) reported that efficient enzymatic dye removal occurred in a wide range of pH (4–8) and temperature (20–60°). Earlier, laccase from *Pleurotus sajor-caju* showed a single point of pH at 3.2 and a single point of temperature at 35°C for maximum dye removal, and away from this point there was a decrease in dye removal efficiency (Bettin et al., 2019).

In most textile wastewater, the presence of pollutants extends beyond dyes, encompassing metals among other contaminants. Understanding the impact of these metals on decolorization enzyme activity is crucial for practical application (Muduli et al., 2023). Different heavy metals have different effects on enzymatic dye removal (Thulasisingh et al., 2024). Similarly, results in Fig. 6 illustrate that copper (Cu) and iron (Fe) did not exhibit any significant alteration in dye removal efficiency across all agricultural waste PPOs. Conversely, the remaining metals markedly reduced the dye removal efficiency of all agricultural waste PPOs, except for potato peel PPO, which demonstrated non-significant changes in its dye removal activity in the presence of all studied metals. The negative effect of heavy metals on the dye removal efficiency as some heavy metals can act as inhibitors, negatively impacting enzyme activity. Inhibitory effects may result from the binding of metal ions to the enzyme's active site, altering its conformation and reducing catalytic efficiency (He et al., 2024; Xu et al., 2024b). High concentrations of certain heavy metals may be toxic to the PPO enzyme, leading to a decrease in overall activity. This can compromise the enzyme's ability to effectively remove dyes (Paul et al., 2024; Rodrigues et al., 2023; Vasilachi et al., 2023). Also, some heavy metals can form complexes with dyes, influencing their chemical properties and making them unavailable for enzymatic degradation. This interaction can affect the overall efficiency of dye removal (Meetam et al., 2024). On the other hand, Cu, and Fe showed a non-significant effect on the dye removal efficiency this can be due to the ability of Cu and Fe ions to initiate the dye oxidation reaction through Fenton`s reaction (Naz et al., 2024; Yang et al., 2024). Similarly, Wang et al. (2024) reported the efficiency of Cu in the catalytic degradation of Congo red. Also, Sudarsan et al. (2024) reported the catalytic efficiency of Cu and Fe for Congo red removal from aqueous solutions. Another key point reported is that Cu and Fe have an activating effect on oxidase enzymes (Adigüzel et al., 2023; Reja et al., 2023).

Comparing the optimum conditions for the maximum dye removal efficiency of different agricultural wastes PPO is indicated in Table 1. Results showed that potato peels have the highest PPO yield of about 20% of the total protein. Potato peels PPO (0.5 U/mL) for 10 min showed a maximum removal efficiency of about 93.89% under the widest range of temperature from 20–80°C, and the widest pH range from 4 to 9, with no effect with the presence of heavy metals. The stability of potato peel PPO's dye removal efficiency under diverse conditions is associated with the numerous isomers for PPO found in potato peels. These isomers share the same molecular weight but possess different configurations, enabling them to remain stable under varying conditions (Rasmussen et al., 2021; Hamami, 2023). Similarly, Hamida et al. (2024) reported the effectiveness of potato peels for dye removal. Also, Goyi et al. (2024) indicated the efficiency of potato peel powder in dye removal. In addition, Uddin et al. (2024) showed that potato peels have higher dye removal than mango and luffa peels. This shows the importance of the importance for the valorization of potato peels (Singh et al., 2024; Yi et al., 2024).

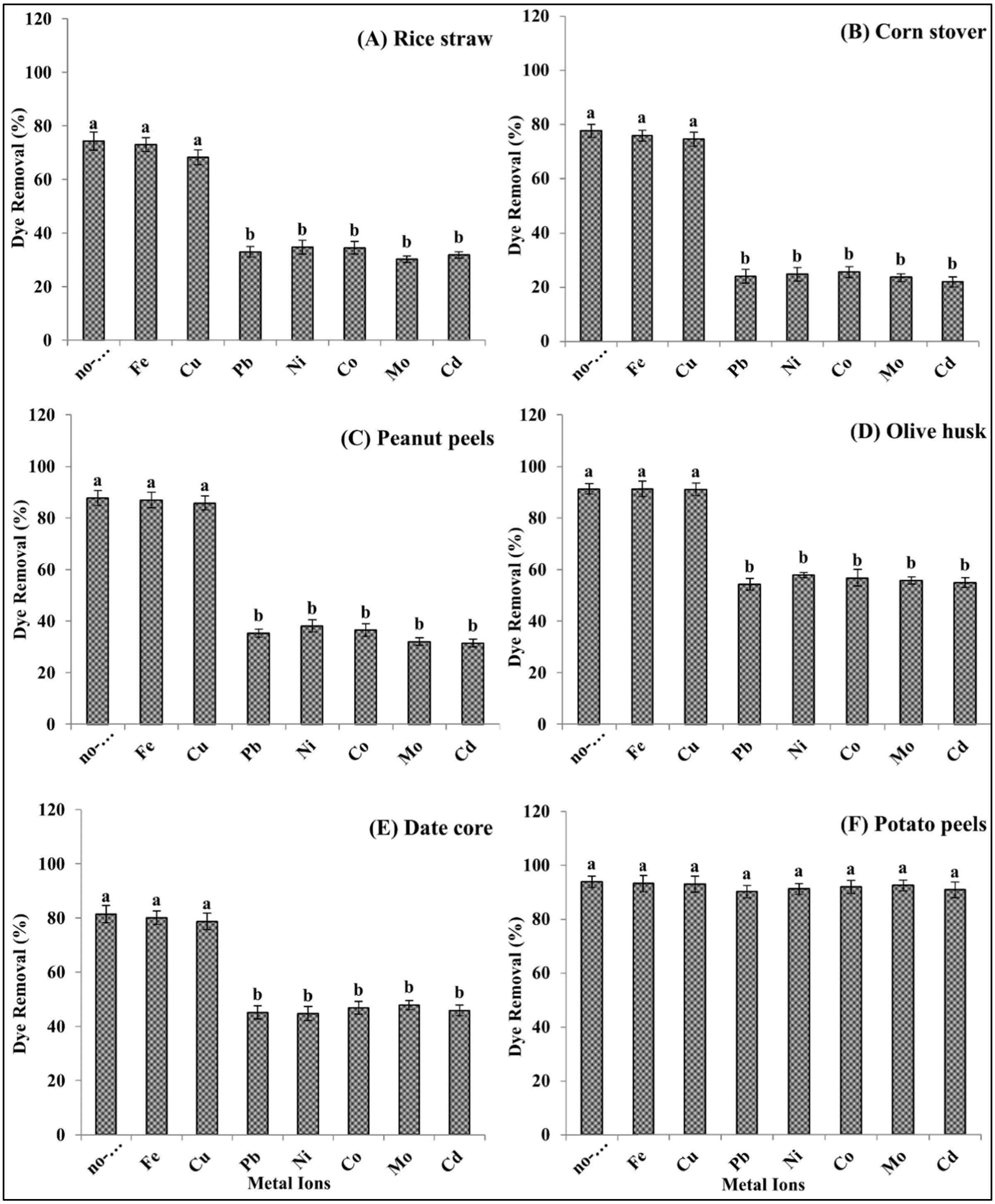

Fig. 6 Effect of different metals on the dye removal efficiency of polyphenol oxidase from different agricultural wastes. Columns represent the mean of three replicates, and the error bars represent the standard deviation. Columns followed by different letters are significantly different according to the ANOVA test

Table 1 Optimum conditions for 0.5 U/mL polyphenol oxidase (PPO) from different agricultural wastes for maximum dye removal for 10min

Agricultural waste	PPO percent from the total protein	Maximum dye removal efficiency	Optimum temperature range	Optimum pH range	Presence of heavy metals (Pb-Ni-Co-Mo-Cd)
Rice Straw	3.83	74.32	30–50	6.5	Decreased
Corn Stover	4.45	77.67	30–50	6.5	Decreased
Peanut peels	7.20	87.78	30–50	6.5	Decreased
Olive husk	6.24	91.25	20–60	5.5–8	Decreased
Date Core	10.53	81.50	30–60	6.5	Decreased
Potato Peels	20.84	93.89	20–80	4–9	No-change

4 Conclusion

This study investigated the extraction of polyphenol oxidase (PPO) from various agricultural wastes and its application for dye removal from wastewater. Results showed significant variations in PPO levels among different agricultural wastes, with potato peels exhibiting the highest PPO content correlated with their role in enzymatic browning reactions due to high phenolic content. Optimal dye removal efficiency was observed with a 10-min incubation period and an optimum pH of 6.5 and temperature of 40°C. Potato peel-derived PPO demonstrated the broadest pH and temperature ranges conducive to high dye removal efficiency. While copper and iron had no significant effect, other heavy metals reduced dye removal efficiency, except for potato peel-derived PPO. The consistency of dye removal efficiency by potato peel PPO across different conditions can be linked to the presence of multiple isomers of PPO in potato peels. Overall, agricultural waste-derived PPOs show promise as cost-effective agents for dye removal, with further research needed for practical application in wastewater treatment processes.

Data Availability All data generated in this work is found in this manuscript and there is no associated data to the manuscript.

Declarations

Ethics Approval This study does not contain any studies on humans or animals.

Consent to Participate Not Applicable.

Consent for Publication Not Applicable.

Conflicts of Interest The authors declare that they have no conflict of interest.

References

Adigüzel, A. O., Könen-Adigüzel, S., Cilmeli, S., Mazmanc, B., Yabalak, E., Üstün-Odabaş, S., Kaya, N. G., & Mazmanc, M. A. (2023). Heterologous expression, purification, and characterization of thermo- and alkali-tolerant laccase-like multicopper oxidase from Bacillus mojavensis TH309 and determination of its antibiotic removal potential. *Archives of Microbiology, 205*, 287. https://doi.org/10.1007/s00203-023-03626-5

Afreen, S., & Mishra, S. (2023). Production of high-value oxidative enzymes by Cyathus bulleri on agricultural and agri-food wastes for application in the textile sector. *World Journal of Microbiology & Biotechnology, 39*, 329. https://doi.org/10.1007/s11274-023-03769-z

Ahmed, I. A., Nosier, S. A., Malash, G. F., Hussein, M., Abdel-Aziz, M. H., Sedahmed, G. H., & Fathalla, A. S. (2023). Removal of Brilliant Yellow Azo Dye from Wastewater by Electrocoagulation in a Cell of Improved Design. *Water, Air, and Soil Pollution, 234*, 237. https://doi.org/10.1007/s11270-023-06241-8

Al-Hazmi, N. E., & Naguib, D. M. (2023a). Agricultural wastes polysaccharides promising soil fertilizer improves plant growth and resistance against soil-borne pathogens. *Plant and Soil*. https://doi.org/10.1007/s11104-023-06358-z

Al-Hazmi, N. E., & Naguib, D. M. (2023b). Control the carcinogenic bacteria with new polysaccharides from agricultural wastes. *Micro Pathogen, 184*, 106343. https://doi.org/10.1016/j.micpath.2023.106343

Al-Sakkaf, M. K., Basfer, I., Iddrisu, M., Bahadi, S. A., Nasser, M. S., Abussaud, B., Drmosh, Q. A., & Onaizi, S. A. (2023). An Up-to-Date Review on the Remediation of Dyes and Phenolic Compounds from Wastewaters Using Enzymes Immobilized on Emerging and Nanostructured Materials: Promises and Challenges. *Nanomater, 13*(15), 2152. https://doi.org/10.3390/nano13152152

Ashok, P. P., Dasgupta, D., Ray, A., & Suman, S. K. (2024). Challenges and prospects of microbial α-amylases for industrial application: A review. *World Journal of Microbiology & Biotechnology, 40*, 44. https://doi.org/10.1007/s11274-023-03821-y

Ateş, B., Ulu, A., Asiltürk, M., Noma, S. A. A., Topel, S. D., Dik, G., Özhan, O., Bakar, B., Yıldız, A., Vardı, N., & Parlakpınar, H. (2024). Enhancement of enzyme activity by laser-induced energy propulsion of upconverting nanoparticles under near-infrared light: A comprehensive methodology for in vitro and in vivo applications. *International Journal of Biological Macromolecules, 260*, 129343. https://doi.org/10.1016/j.ijbiomac.2024.129343

Ayodeji, F. D., Shava, B., Iqbal, H. M. N., Ashraf, S. S., Cui, J., Franco, M., & Bilal, M. (2023). Biocatalytic Versatilities and Biotechnological Prospects of Laccase for a Sustainable Industry. *Catalysis Letters, 153*, 1932–1956. https://doi.org/10.1007/s10562-022-04134-9

Bal, G., & Thakur, A. (2022). Distinct approaches of removal of dyes from wastewater: A review. *Materials Today: Proceedings, 50*, 1575–1579. https://doi.org/10.1016/j.matpr.2021.09.119

Bankeeree, W., Abd-Aziz, S., Prasongsuk, S., Lotrakul, P., Ibrahim, S. N., & Punnapayak, H. (2024). Cellulase as biocatalyst produced from agricultural wastes. In M. Abd-Aziz, M. F. Gozan, S. Ibrahim & L.-Y. Phang (Eds.), *Chemical Substitutes from Agricultural and Industrial By-Products* (pp. 319–336). Wiley online Library. https://doi.org/10.1002/9783527841141.ch16

Bellaj, M., Aziz, K., El Achaby, M., El Haddad, M., Gebrati, L., Kurniawan, T. A., Chen, Z., Yap, P., & Aziz, F. (2024). Cationic and anionic dyes adsorption from wastewater by clay-chitosan composite: An integrated experimental and modeling study. *Chemical Engineering Science, 285*, 119615. https://doi.org/10.1016/j.ces.2023.119615

Bettin, F., Cousseau, F., Martins, K., Zaccaria, S., Girardi, V., Silveira, M. M. D., & Dillon, A. J. P. (2019). Effects of pH, temperature and agitation on the decolourisation of dyes by laccase-containing enzyme preparation from Pleurotus sajor-caju. *Brazilian Archives of Biology and Technology, 62*, e19180338. https://doi.org/10.1590/1678-4324-2019180338

Bulgariu, L., Escudero, L. B., Bello, O. S., Iqbal, M., Nisar, J., Adegoke, K. A., Alakhras, F., Kornaros, M., & Anastopoulos, I. (2019). The utilization of leaf-based adsorbents for dyes removal: A review. *Journal of Molecular Liquids, 276*, 728–747. https://doi.org/10.1016/j.molliq.2018.12.001

Castillo-Suárez, L. A., Sierra-Sánchez, A. G., Linares-Hernández, I., Martínez-Miranda, V., & Teutli-Sequeira, E. A. (2023). A critical review of textile industry wastewater: Green technologies for the removal of indigo dyes. *International Journal of Environmental Science and Technology, 20*, 10553–10590. https://doi.org/10.1007/s13762-023-04810-2

Çesko, C., Gashi, S., Arabaci, G., Paluzar, H., Durmishi, B., Bruci, E., Vllasaliu, F., & Özdemir, N. (2023). Investigation of the effects of pesticides on "Jonagold" apple (Malus x domestica) polyphenol oxidase enzyme activity. *Turkish Journal of Agriculture and Forestry, 47*, 7. https://doi.org/10.55730/1300-011X.3064

Chakravarthi, B., Mathkala, V., & Palempalli, U. M. D. (2021). Degradation and Detoxification of Congo Red Azo Dye by Immobilized Laccase of Streptomyces sviceus. *Journal of Pure and Applied Microbiology, 15*(2), 864–876. https://doi.org/10.22207/JPAM.15.2.41

Chmelová, D., Ondrejovič, M., & Miertuš, S. (2024). Laccases as Effective Tools in the Removal of Pharmaceutical Products from Aquatic Systems. *Life, 14*(2), 230. https://doi.org/10.3390/life14020230

Çifçi, D. İ, Atav, R., Güneş, Y., & Güneş, E. (2019). Determination of the color removal efficiency of laccase enzyme depending on dye class and chromophore. *Water Science and Technology, 80*(1), 134–143. https://doi.org/10.2166/wst.2019.255

Da Cruz, Vieira I., & Fatibello-Filho, O. (1998). Flow injection spectrophotometric determination of total phenols using a crude extract of sweet potato root (Ipomoea batatas (L.) Lam.) as enzymatic source. *Analytica Chimica Acta, 366*(1–3), 111–118. https://doi.org/10.1016/S0003-2670(97)00724-1

Dahiya, M., Islam, D. T., Srivastava, P., Sreekrishnan, T. R., & Mishra, S. (2023). Detoxification and decolorization of complex textile effluent in an enzyme membrane reactor: Batch and continuous studies. *Frontiers in Microbiology, 14*, 1193875. https://doi.org/10.3389/fmicb.2023.1193875

De Oliveira, F. K., Santos, L. O., & Buffon, J. G. (2021). Mechanism of action, sources, and application of peroxidases. *Food Research International, 143*, 110266. https://doi.org/10.1016/j.foodres.2021.110266

Demir, D., Aksu, L., Yılmaz, C., & Eken, C. (2024). Polyphenol oxidase activity in healthy and apple scar skin viroid infected apple (Malus × domestica) fruit. *Biology Bulletin of the Russian Academy of Sciences, 51*, 57–65. https://doi.org/10.1134/S1062359022603135

Ebrahimi, P., & Lante, A. (2022). Environmentally Friendly Techniques for the Recovery of Polyphenols from Food By-Products and Their Impact on Polyphenol Oxidase: A Critical Review. *Applied Sciences, 12*(4), 1923. https://doi.org/10.3390/app12041923

El-Bendary, M. A., Fawzy, M. E., Abdelraof, M., El-Sedik, M., & Allam, M. A. (2023). Efficient malachite green biodegradation by *Pseudomonas plecoglossicide* MG2: Process optimization, application in bioreactors, and degradation pathway. *Microbial Cell Factories, 22*, 192. https://doi.org/10.1186/s12934-023-02194-z

Elmetwalli, A., Allam, N. G., Hassan, M. G., Albalawi, A. N., Shalaby, A., El-Said, K. S., & Salama, A. F. (2023). Evaluation of Bacillus aryabhattai B8W22 peroxidase for phenol removal in wastewater effluents. *BMC Microbiology, 23*, 119. https://doi.org/10.1186/s12866-023-02850-9

Gallegos-Cerda, S. D., Hernández-Varela, J. D., Chanona Pérez, J. J., Huerta-Aguilar, C. A., González Victoriano, L., Arredondo-Tamayo, B., & Reséndiz Hernández, O. (2024). Development of a low-cost photocatalytic aerogel based on cellulose, carbon nanotubes, and TiO_2 nanoparticles for the degradation of organic dyes.

Carbohydrate Polymers, 324, 121476. https://doi.org/10.1016/j.carbpol.2023.121476

Gaur N, Sharma S, Yadav N (2024) Environmental pollution. In: Garg VK, Mohan, Kumari N, Yadav A, S Yadav (eds) Green Chemistry Approaches to Environmental Sustainability Status, Challenges and Prospective. pp: 23–41. https://doi.org/10.1016/B978-0-443-18959-3.00010-0

Goyi, A. A., Sher Mohammad, N. M., & Omer, K. M. (2024). Preparation and characterization of potato peel derived hydrochar and its application for removal of Congo red: A comparative study with potato peel powder. *International Journal of Environmental Science and Technology, 21*(1), 631–642. https://doi.org/10.1007/s13762-023-04965-y

Hamami, Z. (2023). Javanbakht V (2023) Photocatalytic processes using potato peel extract-mediated CuO nanophotocatalyst for fast and efficient direct red 80 dye removal. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-023-05034-7

Hamida, K., Rehali, H., Menasra, H., Bekiri, F., & Aidi, A. (2024). A low-cost bio-composite derived from potato plant waste (PPW-ZnO) for the removal of Rhodamine B. *Reaction Kinetics, Mechanisms, and Catalysis, 2024*, 1–19. https://doi.org/10.1007/s11144-024-02567-4

He, Y., Jiang, Z., Zeng, M., Cao, S., Wu, N., & Liu, X. (2024). Unraveling potential mechanism of different metal ions effect on anammox through big data analysis, molecular docking and molecular dynamics simulation. *Journal of Environmental Management, 352*, 120092. https://doi.org/10.1016/j.jenvman.2024.120092

Hemashenpagam, N., & Selvajeyanthi, S. (2023). Textile dyes and their effect on human beings. In A. Ahmad, M. Jawaid, M. N. Mohamad Ibrahim, A. A. Yaqoob & M. B. Alshammari (Eds.), *Nanohybrid Materials for Treatment of Textiles Dyes. Smart Nanomaterials Technology*. *Springer Nature Singapore Book Series: Smart Nanomaterials Technology Part of: Springer Professional "Wirtschaft+Technik", Springer Professional "Technik"*. Springer. https://doi.org/10.1007/978-981-99-3901-5_3

Hong, Q., Chen, Y., Lin, D., Yang, R., Cao, K., Zhang, L., Liu, Y., Sun, L., & Cao, M. (2024). Expression of polyphenol oxidase of *Litopenaeus vannamei* and its characterization. *Food Chemistry, 432*, 137258. https://doi.org/10.1016/j.foodchem.2023.137258

Hynes, N. R. J., Kumar, J. S., Kamyab, H., Sujana, J. A. J., Al-Khashman, O. A., Kuslu, Y., Ene, A., & Suresh Kumar, B. (2020). Modern enabling techniques and adsorbents based dye removal with sustainability concerns in textile industrial sector – a comprehensive review. *Journal of Cleaner Production, 272*, 122–636. https://doi.org/10.1016/j.jclepro.2020.122636

Islam, T., Repon, M., Islam, T., Sarwar, Z., & Rahman, M. M. (2023). Impact of textile dyes on health and ecosystem: A review of structure, causes, and potential solutions. *Environmental Science and Pollution Research, 30*, 9207–9242. https://doi.org/10.1007/s11356-022-24398-3

Jafari, A., Aghebati, S., & Khayati, G. (2023). Decolorization of azo dyes by produced laccase enzyme in solid-state fermentation using biphenyl as an inducer. *Biocatalysis and Agricultural Biotechnology, 52*, 102814. https://doi.org/10.1016/j.bcab.2023.102814

Kakkar, P., & Wadhwa, N. (2023). In silico and in vitro analysis of polyphenol oxidase: Study in bioremediation of phenol in wastewater. *Environment, Development and Sustainability*. https://doi.org/10.1007/s10668-023-04294-7

Kaur, H., Verma, V., Kaur, M., & Sharma, S. (2024). Advances in polyphenol oxidase mimic as catalyst. In S. J. Ikhmayies (Eds.), *Advances in Catalysts Research*. *Advances in Material Research and Technology* (pp. 99–129). Springer. https://doi.org/10.1007/978-3-031-49108-5_4

Khodakarami, M., Dehghan, G., Rashtbari, S., & Amini, M. (2024). Catalytic removal of malachite green from aqueous solution by a peroxidase-mimicking Cu-Al layered double hydroxide nanoparticle: Synthesis, characterization, and application. *Applied Catalysis A: General, 670*, 119563. https://doi.org/10.1016/j.apcata.2024.119563

Kumar, D., Bhardwaj, R., Jassal, S., Goyal, T., Khullar, A., & Gupta, N. (2023). Application of enzymes for an eco-friendly approach to textile processing. *Environmental Science and Pollution Research, 30*, 71838–71848. https://doi.org/10.1007/s11356-021-16764-4

Kumar, V., Pallavi, P., Sen, S. K., & Raut, S. (2024). Harnessing the potential of white rot fungi and ligninolytic enzymes for efficient textile dye degradation: A comprehensive review. *Water Environment Research, 96*(1), e10959. https://doi.org/10.1002/wer.10959

Kyomuhimbo, H. D., Feleni, U., Haneklaus, N. H., & Brink, H. (2023). Recent Advances in Applications of Oxidases and Peroxidases Polymer-Based Enzyme Biocatalysts in Sensing and Wastewater Treatment: A Review. *Polymers, 15*(16), 3492. https://doi.org/10.3390/polym15163492

Lin, H., Ng, A. W. R., & Wong, C. W. (2016). Partial purification and characterization of polyphenol oxidase from Chinese parsley (*Coriandrum sativum*). *Food Science and Biotechnology, 25*(Suppl 1), 91–96. https://doi.org/10.1007/s10068-016-0103-x

Liu, H., Pan, M., Lu, Y., Wang, M., Huang, S., Li, J., Luo, K., Luo, L., Yao, M., Hua, D., & Wang, H. (2023). Purification and comparison of soluble and membrane-bound polyphenol oxidase from potato (*Solanum tuberosum*) tubers. *Protein Expression and Purification, 202*, 106195. https://doi.org/10.1016/j.pep.2022.106195

Liu, G., Wang, L., Zhao, A., Guo, L., Zhou, L., He, Y., Ma, L., Liu, Y., Gao, J., & Jiang, Y. (2024). Biocatalytic membranes with crosslinked enzyme aggregates for micropollutant removal. *Chemical Engineering Journal, 479*, 147635. https://doi.org/10.1016/j.cej.2023.147635

Magalhães, F. F., Pereira, A. F., Cristóvão, R. O., Barros, R. A. M., Faria, J. L., Silva, C. G., Freire, M. G., & Tavares, A. P. M. (2024). Recent Developments and Challenges in the Application of Fungal Laccase for the Biodegradation of Textile Dye Pollutants. *Source: Mini-Reviews in Organic Chemistry, 21*(6), 609–632. https://doi.org/10.2174/1570193X20666221104140632

Majumdar, S., & Bhowal, J. (2022). Studies on production and evaluation of biopigment and synthetic dye decolorization capacity of laccase produced by *A. oryzae* cultivated on agro-waste. *Bioprocess and Biosystems Engineering, 45*, 45–60. https://doi.org/10.1007/s00449-021-02638-z

Meetam, P., Phonlakan, K., Nijpanich, S., & Budsombat, S. (2024). Chitosan-grafted hydrogels for heavy metal ion adsorption and catalytic reduction of nitroaromatic pollutants and dyes. *International Journal of Biological Macromolecules, 255*, 128261. https://doi.org/10.1016/j.ijbiomac.2023.128261

Mesbah, N. M. (2022). Industrial Biotechnology Based on Enzymes from Extreme Environments. *Frontiers in Bioengineering and Biotechnology, 10*, 870083. https://doi.org/10.3389/fbioe.2022.870083

Mingrou, L., Guo, S., Ho, C. T., & Bai, N. (2022). Review on chemical compositions and biological activities of peanut (Arachis hypogeae L.). *Journal of Food Biochemistry, 46*, 7. https://doi.org/10.1111/jfbc.14119

Motamedi, E., Kavousi, K., Sadeghian Motahar, S. F., Reza Ghaffari, M., Sheykh Abdollahzadeh Mamaghani, A., Hosseini Salekdeh, G., & Ariaeenejad, S. (2021). Efficient removal of various textile dyes from wastewater by novel thermo-halotolerant laccase. *Bioresource Technology, 337*, 125468. https://doi.org/10.1016/j.biortech.2021.125468

Muduli, M., Choudhary, M., Sonpal, V., & Ray, S. (2023). Recent advancements and approaches towards dye industries effluent treatment. *Sustain. Water Resour Manag, 9*, 186. https://doi.org/10.1007/s40899-023-00975-3

Naguib, D. M., & Badawy, N. M. (2020). Phenol removal from wastewater using waste products. *Journal of Environmental Chemical Engineering, 8*, 103592. https://doi.org/10.1016/j.jece.2019.103592

Naguib, D. M., & Tantawy, A. A. (2019). Anticancer effect of some fruits peels aqueous extracts. *Oriental Pharmacy and Experimental Medicine, 19*, 415–420. https://doi.org/10.1007/s13596-019-00398-6

Naseem, S., Rawal, R. S., Pandey, D., & Suman, S. K. (2023). Immobilized laccase: An effective biocatalyst for industrial dye degradation from wastewater. *Environmental Science and Pollution Research, 30*, 84898–84917. https://doi.org/10.1007/s11356-023-28275-5

Naz, A., Bibi, I., Majid, F., Dahshan, A., Jilani, K., Taj, B., Ghafoor, A., Nazeer, Z., Alzahrani, F. M., & Iqbal, M. (2024). Cu and Fe doped NiCo2O4/g-C3N4 nanocomposite ferroelectric, magnetic, dielectric and optical properties: Visible light-driven photocatalytic degradation of RhB and CR dyes. *Diamond and Related Materials, 141*, 110592. https://doi.org/10.1016/j.diamond.2023.110592

Nguyen, T. T., Rossello, C., Mikhaylin, S., & Ratti, C. (2024). Converting potato peel waste into bioactive extracts: Reduction of pesticides by traditional and novel pretreatment technologies. *Sustainable Food Technology, 2*, 386–399. https://doi.org/10.1039/D3FB00173C

Oleszek, M., Kowalska, I., Bertuzzi, T., & Oleszek, W. (2023). Phytochemicals Derived from Agricultural Residues and Their Valuable Properties and Applications. *Molecules, 28*(1), 342. https://doi.org/10.3390/molecules28010342

Pal N, Chakraborty M (2023) Exploring the Biodegradation Potential of Crude Extracelluar Enzyme Mixture from *Anoxybacillus* sp. For Azo Dye Removal: A Sustainable Approach for Industrial Wastewater Treatment. Chelonian Res Found 18(2): 1499–1514. https://www.acgpublishing.com/index.php/CCB/article/view/124.

Panadare, D., & Rathod, V. K. (2018). Extraction and purification of polyphenol oxidase: A review. *Biocatalysis and Agricultural Biotechnology, 14*, 431–437. https://doi.org/10.1016/j.bcab.2018.03.010

Pandey, D., Daverey, A., Dutta, K., & Arunachalam, K. (2023). Dye removal from simulated and real textile effluent using laccase immobilized on pine needle biochar. *Journal of Water Process Engineering, 53*, 103710. https://doi.org/10.1016/j.jwpe.2023.103710

Paul, C., Pal, N., Maitra, M., & Das, N. (2024). Laccase-assisted Bioremediation of Pesticides: Scope and Challenges. *Mini-Reviews in Organic Chemistry, 21*(6), 633–654. https://doi.org/10.2174/1570193X20666221117161033

Rabeie, B., & Mahmoodi, N. M. (2024). Heterogeneous MIL-88A on MIL-88B hybrid: A promising eco-friendly hybrid from green synthesis to dual application (Adsorption and photocatalysis) in tetracycline and dyes removal. *Journal of Colloid and Interface Science, 654*, 495–522. https://doi.org/10.1016/j.jcis.2023.10.060

Rasmussen, C. B., Enghild, J. J., & Scavenius, C. (2021). Identification of polyphenol oxidases in potato tuber (*Solanum tuberosum*) and purification and characterization of the major polyphenol oxidases. *Food Chemistry, 365*, 130454. https://doi.org/10.1016/j.foodchem.2021.130454

Rasool, K., Hussain, S., Shahzad, A., Miran, W., Mahmoud, K. A., Ali, A., & Almomani, F. (2023). Comprehensive insights into sustainable conversion of agricultural and food waste into microbial protein for animal feed production. *Reviews in Environmental Science & Biotechnology, 22*, 527–562. https://doi.org/10.1007/s11157-023-09651-6

Ravindiran, G., Sundaram, H., Rajendran, E. M., Ramasamy, S., Nabil, A., & Ahmed, B. (2023). Removal of azo dyes from synthetic wastewater using biochar derived from sewage sludge to prevent groundwater contamination. *Urban Climate, 49*, 101502. https://doi.org/10.1016/j.uclim.2023.101502

Reja, S., Kejriwal, A., & Das, R. K. (2023). Copper Based Biomimetic Catalysts of Catechol Oxidase: An Overview on Recent Trends. *Catalysis in Industry, 15*, 108–124. https://doi.org/10.1134/S2070050423010063

Rodrigues, A. F., Da Silva, A. F., Da Silva, F. L., Dos Santos, K. M., De Oliveira, M. P., Nobre, M. M., Catumba, B. D., Sales, M. B., Silva, A. R., Braz, A. K. S., Cavalcante, A. L., Alexandre, J. Y., Junior, P. G., Valério, R. B., De Castro, B. V., & Dos Santos, J. C. (2023). A scientometric analysis of research progress and trends in the design of laccase biocatalysts for the decolorization of synthetic dyes. *Process Biochemistry, 126*, 272–291. https://doi.org/10.1016/j.procbio.2023.01.014

Routoula, E., & Patwardhan, S. V. (2020). Degradation of anthraquinone dyes from effluents: A review focusing on enzymatic dye degradation with industrial potential. *Environmental Science and Technology, 54*, 647–664. https://doi.org/10.1021/acs.est.9b03737

Sahoo, S., & Goswami, S. (2024). Theoretical framework for assessing the economic and environmental impact of water pollution: A detailed study on sustainable development of India. *Journal of Future Sustainability, 4*(1), 23–34. https://doi.org/10.5267/j.jfs.2024.1.003

Sajjad, N., Ahmad, M. S., Mahmood, R. T., Tariq, M., Asad, M. J., Irum, S., Andleeb, A., Riaz, A., & Ahmed, D. (2023). Purification and characterization of novel isoforms of the polyphenol oxidase from *Malus domestica* fruit pulp. *PLoS One, 18*(8), e0276041. https://doi.org/10.1371/journal.pone.0276041

Salehi, S., Abdollahi, K., Panahi, R., Rahmanian, N., Shakeri, M., & Mokhtarani, B. (2021). Applications of Biocatalysts for Sustainable Oxidation of Phenolic Pollutants: A Review. *Sustainability, 13*(15), 8620. https://doi.org/10.3390/su13158620

Sellami, K., Couvert, A., Nasrallah, N., Maachi, R., Abouseoud, M., & Amrane, A. (2022). Peroxidase enzymes as green catalysts for bioremediation and biotechnological applications: A review. *Science of The Total Environment, 806*, 150500. https://doi.org/10.1016/j.scitotenv.2021.150500

Selvam, K., Sudhakar, C., & Prasath, A. R. (2024). Optimization of laccase production from Bacillus subtilis strain KSK02 utilizing bi-substrates and their reactive red-120 dye degradation potential. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-024-05365-z

Shan, K. C., & Hassan, S. R. (2022). Extraction of Crude Enzymes from Spent *P. ostreatus* Substrate and its Potential Use in Dye Removal. *AIP Conference Proceedings, 2454*, 030023–1-030023-7. https://doi.org/10.1063/5.0078665

Singh, M., Sharma, V., & Gupta, R. (2024). Biovalorization of potato peel waste: An overview. In R. C. Ray (Eds.), *Roots, Tubers, and Bulb Crop Wastes: Management by Biorefinery Approaches* (pp. 19–41). Springer. https://doi.org/10.1007/978-981-99-8266-0_2

Sridhar, A., Ponnuchamy, M., Kapoor, A., & Prabhakar, S. (2022). Valorization of food waste as adsorbents for toxic dye removal from contaminated waters: A review. *Journal of Hazardous Materials B, 424*, 127432. https://doi.org/10.1016/j.jhazmat.2021.127432

Sudarsan, S., Anandkumar, M., & Trofimov, E. (2024). Synthesis and characterization of copper ferrite nanocomposite from discarded printed circuit boards as an effective photocatalyst for Congo red dye degradation. *Journal of Industrial and Engineering Chemistry, 131*, 208–220. https://doi.org/10.1016/j.jiec.2023.10.020

Svetozarević, M., Šekuljica, N., Knežević-Jugović, Z., & Mijin, D. (2021). Agricultural waste as a source of peroxidase for wastewater treatment: Insight in kinetics and process parameters optimization for anthraquinone dye removal. *Environmental Technology & Innovation, 21*, 101289. https://doi.org/10.1016/j.eti.2020.101289

Thamke V.R., Tapase S.R., Chaudhari A.U., Bapat V.A., Jadhav J.P., & Kodam K.M., (2023) Enzymes responsible for the metabolism of synthetic dyes. In: Govindwar SP, Kurade MB, Jeon B-H, Pandey A (eds) Current Developments in Bioengineering and Biotechnology, 513–538. https://doi.org/10.1016/B978-0-323-91235-8.00008-5

Thulasisingh, A., Ananthakrishnan, K., Raja, A., & Kannaiyan, S. (2024). Bioprospecting of Novel and Industrially Appropriate Enzymes: A Review. *Water, Air, and Soil Pollution, 235*, 12. https://doi.org/10.1007/s11270-023-06831-6

Trivedi, A., Desireddy, S., & Chacko, S. P. (2022). Effect of pH, Salinity, Dye, and Biomass Concentration on Decolourization of Azo Dye Methyl Orange in Denitrifying Conditions. *Water, 14*(22), 3747. https://doi.org/10.3390/w14223747

Tsaffo Mbogno, M. H., Lambert, S. D., Mumbfu, E. M., Caucheteux, J., Farcy, A., Fagel, N., Woumfo, E. D., & Mahy, J. G. (2024). Silane modified clay for enhanced dye pollution adsorption in water. *Results in Surfaces and Interfaces, 14*, 100183. https://doi.org/10.1016/j.rsurfi.2024.100183

Uddin, J., Idrees, M., Ahmed, H., Batool, S., Rahman, T. U., Mehmood, S., Tanoli, A. K., Muhsinah, A. B., Ullah, H., & Musharraf, S. G. (2024). Biodegradation and decolorization of methylene blue, reactive Black-5, and toluidine blue-O from an aqueous solution using the polyphenol oxidase enzyme. *Front Sustain Food System, 7*, 1320855. https://doi.org/10.3389/fsufs.2023.1320855

Vaiano, V., & De Marco, I. (2023). Removal of Azo Dyes from Wastewater through Heterogeneous Photocatalysis and Supercritical Water Oxidation. *Separations, 10*(4), 230. https://doi.org/10.3390/separations10040230

Valli Nachiyar, C., Rakshi, A., Sandhya, S., Britlin Deva Jebasta, N., & Nellore, J. (2023). Developments in treatment technologies of dye-containing effluent: A review. *Case Studies in Chemical and Environmental Engineering, 7*, 100339. https://doi.org/10.1016/j.cscee.2023.100339

Vasilachi, I. C., Stoleru, V., & Gavrilescu, M. (2023). Analysis of Heavy Metal Impacts on Cereal Crop Growth and Development in Contaminated Soils. *Agriculture, 13*(10), 1983. https://doi.org/10.3390/agriculture13101983

Venturi, F., Bartolini, S., Sanmartin, C., Orlando, M., Taglieri, I., Macaluso, M., Lucchesini, M., Trivellini, A., Zinnai, A., & Mensuali, A. (2019). Potato Peels as a Source of Novel Green Extracts Suitable as Antioxidant Additives for Fresh-Cut Fruits. *Applied Sciences, 9*(12), 2431. https://doi.org/10.3390/app9122431

Verma, P., Agrawal, K., & Shah, M. P. (2024). *Polyphenol oxidases: Function, wastewater remediation, and biosensors*. Walter de Gruyter GmbH & Co KG. 389.

Wang, H., Li, S., Li, J., Zhong, L., Cheng, H., & Ma, Q. (2020). Immobilized polyphenol oxidase: Preparation, optimization and oxidation of phenolic compounds. *International Journal of Biological Macromolecules, 160*, 233–244. https://doi.org/10.1016/j.ijbiomac.2020.05.079

Wang, L., Zhao, L., Si, D., Li, Z., An, H., Ye, H., Xin, Q., Li, H., & Zhang, Y. (2024). Polymeric membrane with nanohybrids of Cu nanocomposites and metalloporphyrin-based nanosheets for enzyme-like catalytic degradation of Congo Red. *Separation and Purification Technology, 331*, 125571. https://doi.org/10.1016/j.seppur.2023.125571

Xu, J., Zhang, X., Zhou, Z., Ye, G., & Wu, D. (2024a). Covalent organic framework in-situ immobilized laccase for the covalent polymerization removal of sulfamethoxazole in the presence of natural phenols: Prominent enzyme stability and activity. *Journal of Hazardous Materials,* *462*, 132714. https://doi.org/10.1016/j.jhazmat.2023.132714

Xu, W., Wu, Y., Gu, W., Du, D., Lin, Y., & Zhu, C. (2024b). Atomic-level design of metalloenzyme-like active pockets in metal–organic frameworks for bioinspired catalysis. *Chemical Society Reviews, 53*, 137–162. https://doi.org/10.1039/D3CS00767G

Yang, J., Jia, K., Lu, S., Cao, Y., Boczkaj, G., & Wang, C. (2024). Thermally activated natural chalcopyrite for Fenton-like degradation of Rhodamine B: Catalyst characterization, performance evaluation, and catalytic mechanism. *Journal of Environmental Chemical Engineering, 12*(1), 111469. https://doi.org/10.1016/j.jece.2023.111469

Yi, J., Li, L., Li, X., Duan, X., Wang, J., Han, Y., & Gao, Y. (2024). Valorisation of sweet potato leaves as a potential agri-food resource: Assessing nutritional and nutraceutical values altered by food processing—A review. *International Journal of Food Science & Technology*. https://doi.org/10.1111/ijfs.17014

Yu, Z., Hu, X., & Su, Z. (2024). Activation of peroxymonosulfate by a novel stratiform Co-MOF as catalyst for degradation of dyes in water. *Journal of Molecular Structure, 1296*, 136787. https://doi.org/10.1016/j.molstruc.2023.136787

Yusree, F. I. F. M., Peter, A. P., Mohd Nor, M. Z., Show, P. L., & Mokhtar, M. N. (2021). Latest Advances in Protein-Recovery Technologies from Agricultural Waste. *Foods, 10*(11), 2748. https://doi.org/10.3390/foods10112748

Zhang, S. (2023). Recent Advances of Polyphenol Oxidases in Plants. *Molecules, 28*(5), 2158. https://doi.org/10.3390/molecules28052158

Zouari-Mechichi, H., Benali, J., Alessa, A. H., Hadrich, B., & Mechichi, T. (2024). Efficient Decolorization of the Poly-Azo Dye Sirius Grey by Coriolopsis gallica Laccase-Mediator System: Process Optimization and Toxicity Assessment. *Molecule, 29*(2), 477. https://doi.org/10.3390/molecules29020477