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Polydopamine Coated Sawdust as a Support for Lipase Immobilization: a Sustainable Strategy for Oily Wastewater Bioremediation

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Abstract: The swift expansion of the edible oil industry has caused a significant increase in oily wastewater production, posing serious environmental challenges. This study explores the immobilization of *Candida rugosa* lipase (CrL) on polydopamine-coated sawdust (PD/SD) through covalent bonding, employing Michael addition reactions or the formation of Schiff base to yield CrL/PD/SD. Both free and immobilized CrL demonstrated optimal catalytic activity at pH 8 and 40°C; however, the immobilized enzyme demonstrated enhanced stability across a broader range of pH and temperature. Notably, immobilized CrL retained 51% of its original activity at 70°C, whereas free CrL dropped to 8% under identical conditions. Kinetic parameters for both enzyme forms were systematically analyzed. Additionally, the immobilized CrL exhibited excellent reusability, preserving 43% of its initial activity after sixteen recurrent catalytic cycles. Both free and immobilized CrL effectively hydrolyzed various edible oils, displaying comparable activity trends, although the immobilized form achieved superior hydrolysis yields. Collectively, these findings underscore the potential of CrL/PD/SD as a sustainable and efficient biocatalyst for the remediation of oily wastewater.

keywords: *Candida rugosa* lipase; sawdust; immobilization; edible oils; wastewater treatment; hydrolysis.

1.Introduction

The growing global output of waste oils, greases, and fats is causing a number of problems for the economy, environment, and public health. It is one of the main causes of water pollution and a significant contributor to expensive pipeline maintenance [1]. Edible oils consist primarily of triacylglycerides of fatty acids varying in saturation nature and chain length, and their nature and concentration are mostly dictated by the respective bio-based source [2, 3]. The production of edible oils involves the processing and refining of oilseeds, which can produce a variety of waste products in large quantities, such as inorganic residues, organic solid waste, and wastewater [4]. The release of untreated wastewater containing edible oil into the environment reduces the oxygen content and light penetration in the water, causing damage of aquatic life in an irreversible way that can lead to negative environmental and health impacts if not properly managed [5]. In general, there are several methods for treating edible oil industry wastewater, including physiochemical, biological, and electrochemical methods [6-9]. However, there aren't enough studies on electrochemical treatment compared to other wastewater treatment methods, which calls for more research [5]. Furthermore, the primary drawbacks of physicochemical treatments are their high chemical costs and challenging sludge management [10]. Hence, the biological

treatment would be the best choice over other currently used techniques due to its many advantages, such as its environmental friendliness, simplicity, affordability in operation and maintenance, and suitability for carbonaceous stabilization. Additionally, biological processes that break down organic materials molecularly produce stable end products [11, 12].

Among the biological techniques, enzymatic treatment with lipase and esterase enzymes has been widely used in the bioprocessing of wastewater rich in oils, fats, and greases [13]. Lipases (EC 3.1.1.3) are known as hydrolases that cleave the carboxyl ester bonds in triacylglycerols in aqueous solutions to liberate free fatty acids and glycerol [14]. Free lipases are limited in their widespread industrial application due to their high cost, instability, sensitivity to variations in pH and temperature in aqueous solutions, and challenges in reuse and recycling [15, 16]. Nevertheless, lipase immobilization has been used to resolve these problems. Preserving the enzymes' structural, functional, and biological properties, allowing them to be separated from the reaction environment, and lowering process costs by enhancing their stability and reusability are the main goals of immobilization [17, 18].

Choosing the appropriate support or matrix for enzyme immobilization is a crucial step that requires careful consideration due to its properties' effect on the immobilized enzyme system's performance [19, 20]. Various materials, from costly synthetic compounds to natural polymers, have been used for enzyme immobilization [21]. In the available literature, there are several studies that achieved good results in oily wastewater treatment using immobilized lipase on different supports, such as hybrid sol-gel/calcium alginate matrix [22], carbon nanotubes [13], chitosan [23], and others. In this context, sawdust is employed as an organic support since it is inexpensive, sustainable, and environmentally friendly [24, 25]. Notable qualities of sawdust include its large surface area, high porosity, low specific gravity, coarse surface, good liquid-holding capacity, and superior mechanical stability [26]. Interestingly, polydopamine (PD) has a high adhesive ability, and owing to quinone and catechol groups present on the PD surface, it can react readily with molecules containing thiols and amino acids through the Schiff-base reaction and/or Michael addition, offering a flexible platform for additional modification [27, 28].

In this work, PD was used to coat sawdust (SD) producing (PD/SD) to allow the covalent immobilization of *Candida rugosa* lipase (CrL) enzyme via covalent bonds, forming (CrL/PD/SD) for its application as a biocatalyst in the hydrolysis of oil. The surface alteration of the SD and the immobilization of CrL were clarified using ATR-FTIR. The characteristics of free and immobilized CrL activity were thoroughly examined under various operational circumstances. Additionally, the kinetic behavior of both free and immobilized lipase was characterized using Michaelis-Menten parameters. Furthermore, the reusability of immobilized CrL and its capacity to hydrolyze edible oils were investigated as well.

2. Materials and methods

2.1. Materials

Candida rugosa lipase, p-nitrophenol, p-nitrophenyl palmitate, isopropanol, Arabic gum, Triton X-100, and dopamine hydrochloride were bought from Sigma-Aldrich Co. Sawdust particles were sourced from waste generated by woodworking operations. Olive, soybean, corn, and sunflower oils were obtained from local markets. All buffer solutions were made with salts of analytical quality.

2.2. Sawdust activation

Sawdust particles (SD) were initially dried in an oven after being carefully cleaned with distilled water. Subsequently, at room temperature with constant stirring, 2 g of SD were incubated in about 25 mL of a dopamine solution, which was created by dissolving 100 mg of dopamine in 25 mL of 0.01 M Tris-HCl buffer (pH 8.6). After 24 hours, the sawdust particles coated with polydopamine (PD/SD) They were rinsed three times with distilled water before being dried for future usage at 50 °C in an oven.

2.3. Covalent immobilization of CrL

Candida rugosa lipase (CrL) was covalently immobilized onto polydopamine-coated sawdust (PD/SD) particles through Michael

addition and Schiff base formation. Here, 4 mL of CrL solution (15 U/mL), made in 0.05 M Tris-HCl buffer (pH 8), was incubated with 0.4 g of PD/SD particles for 18 hours. The immobilized enzyme (CrL/PD/SD) was then washed with the buffer solution to take out any unbound CrL and subsequently stored at 4 °C for further analysis. The following formula was used to determine the immobilization efficiency:

Immobilization efficiency % = (The specific activity of CrL/PD/SD / the initial specific activity of free CrL utilized in the immobilization) * 100

2.4. ATR-FTIR Analysis

Using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy, the chemical structure and surface functional groups of SD, PD/SD, and CrL/PD/SD were investigated. The samples were analyzed at a resolution of 4 cm^{-1} throughout a wavenumber range of 400–4000 cm^{-1}.

2.5. Lipase activity assay

Using *p*-nitrophenyl palmitate (*p*-NPP) as a substrate, the activity of free CrL and CrL/PD/SD was assessed, following the method described by Winkler and Stuckmann (1979) [29] with slight alterations. The substrate solution's preparation was made by mixing solution A (0.015 g of *p*-NPP dissolved in 5 mL of isopropanol) with the solution B (0.05 g of Arabic gum and 0.2 mL of Triton X-100 dissolved in 45 mL of 0.05 M Tris-HCl buffer, pH 8) under continuous stirring until complete dissolution. The reaction mixture (1 mL) was composed of an appropriate amount of free CrL or CrL/PD/SD, 0.05 M Tris-HCl buffer (pH 8), and 0.9 mL of the prepared substrate solution, followed by incubation at 37°C for 15 minutes. The enzymatic reaction was terminated by adding 0.15 mL of 0.1 M Na_2CO_3, and absorbance was measured at 410 nm using a spectrophotometer. Enzyme activity was determined based on a standard curve of *p*-nitrophenol (p-NP), with one unit of enzyme activity defined as the amount of enzyme needed under the test conditions to generate 1 µmol of *p*-NP per minute per milliliter [30].

2.6. Determination of optimal pH and temperature

The optimal pH for the maximum catalytic activity of free CrL and CrL/PD/SD was determined by conducting the above-described enzymatic assay under standard conditions using 50 mM buffer solutions at varying pH levels. The buffers utilized included citrate-phosphate (pH 5.5), sodium phosphate (pH 6), Tris-HCl (pH 7–8), and glycine-NaOH (pH 9–9.5), with all reactions carried out at a constant temperature of 37°C. To assess the optimal temperature, the catalytic activities of free CrL and CrL/PD/SD were evaluated at various temperatures ranging between 20°C and 70°C, while maintaining the pH at 8. All results were expressed as relative activity, with the highest observed activity normalized to 100%.

2.7. Kinetics of free and immobilized CrL

The kinetic parameters of free CrL and CrL/PD/SD were determined by carrying out the enzymatic assay under optimized pH and temperature conditions, using *p*-nitrophenyl palmitate (*p*-NPP) at varying concentrations (0.16–0.71 mM). A Lineweaver-Burk plot was generated to calculate the Michaelis-Menten constant (Km) and the maximum reaction velocity (Vmax) for both enzyme forms (free and immobilized CrL), providing insights into the effect of immobilization on enzyme kinetics.

2.8. Reusability of immobilized CrL

The reusability of CrL/PD/SD was assessed over sixteen consecutive reaction cycles using the enzymatic assay described earlier. Following every cycle, the immobilized CrL/PD/SD was recovered and thoroughly washed with buffer solution to dispose of any leftover substrate or product. The washed immobilized enzyme was then reused in the subsequent reaction cycle utilizing a new substrate solution, allowing for the evaluation of enzyme stability and performance over multiple cycles.

2.9. Hydrolysis of edible oils using free and immobilized CrL

The ability of free CrL and CrL/PDA/SD to hydrolyze edible oils was evaluated using titration analysis to quantify the fatty acids released after treatment of olive, corn, soybean, and sunflower oils. The reaction mixture consisted of 10% (w/v) edible oil emulsified with 10% (w/v) Triton X-100 in 0.05 M Tris-

HCl buffer (pH 8). 10 mL of this emulsion was mixed with free CrL or CrL/PD/SD, and it was then incubated for an hour at 40°C and 130 rpm in a shaker incubator. The enzymatic reaction was stopped by the addition of 1 mL of an acetone:ethanol (1:1) solution along with a few drops of the indicator phenolphthalein. The fatty acids released during the reaction were quantified by titration with 0.05 M NaOH. The amount of free fatty acids produced was expressed as micromoles (µmol) per milliliter of lipase enzyme solution in the reaction medium [18, 31].

3. Results and discussion

3.1. Sawdust modification and covalent immobilization of CrL

ATR-FTIR spectroscopy of unmodified sawdust revealed the characteristic bands of its primary components, as illustrated in Fig. 1. Specifically, the spectrum showed a wide band with O–H stretching vibrations at 3200–3500 cm^{-1} and a band with C–H stretching vibrations of cellulose at 2860 cm^{-1}. Furthermore, the C=O stretching of hemicellulose acetyl ester and lignin aldehyde was represented by the band at 1731 cm^{-1}, whereas the C=C stretching vibrations and aromatic rings of lignin were detected at 1660 cm^{-1} and 1510 cm^{-1}, respectively. The band at 1041 cm^{-1} was associated with the C–O groups [32-35]. Unmodified sawdust is not appropriate for the direct covalent immobilization of CrL, even though it has a lot of hydroxyl groups on its surface. In order to resolve this limitation, a layer of polydopamine was applied to the sawdust surface. Polydopamine, known for its strong adhesion properties, provides an active surface for the covalent immobilization of CrL. This modification was accomplished by immersing the sawdust in an aqueous dopamine solution at pH 6.8, allowing for the self-polymerization of dopamine into polydopamine[36]. The successful adhesion of polydopamine onto the sawdust was indicated by the dark brown color of the coated sawdust (PD/SD). ATR-FTIR analysis further validated the alteration, as seen in Fig. 1, with an enhanced intensity of the band at 3100–3500 cm^{-1} corresponding to the O–H and N–H stretching vibrations of polydopamine. Wide bands appeared at 1500 cm^{-1} to 1600 cm^{-1}, corresponding to N–H bending vibrations and C=C stretching vibrations of polydopamine, respectively [35, 37]. The strong band at 1020 cm^{-1} was owing to the C–O and C–N stretching vibrations arising from polydopamine adhered to the sawdust surface [35, 38]. A new band was observed at 560 cm^{-1} derived from out-of-plane bending vibrations of the C-H bonds in the aromatic structure of polydopamine. The free amine groups of CrL were covalently bonded to PD/SD through the formationtion of a Schiffff base, facilitated by the catechol and quinone groups present in polydopamine. This process resulted in immobilized CrL (CrL/PD/SD) with an immobilization efficiency of approximately 68% [39]. The ATR-FTIR spectra of CrL/PD/SD revealed that detecting the characteristic bands of immobilized CrL, including the Schiff base (C=N, 1610 cm^{-1}), amide I (1641 cm^{-1}), and amide II (1510 cm^{-1}), was challenging due to their overlap with the aromatic ring bands of polydopamine and lignin within the same spectral region.

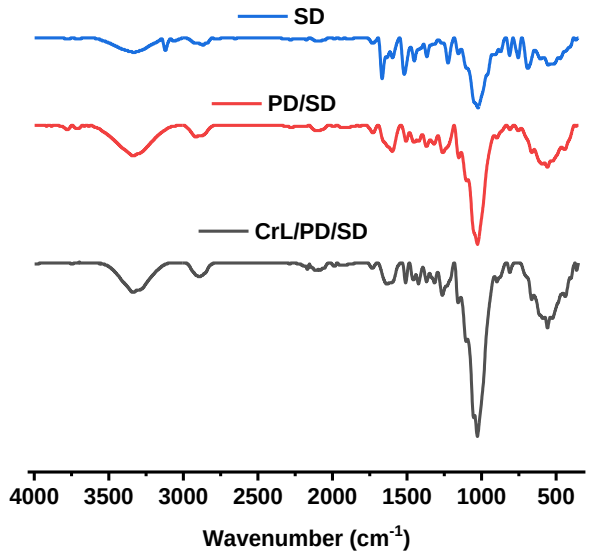

Fig. 1: ATR-FTIR spectra of pure sawdust (SD), polydopamine-coated sawdust (PD/SD), and immobilized CrL (CrL/PD/SD).

3.2. Determination of optimal pH and temperature

Enzyme activity is significantly influenced by its surrounding microenvironment, with pH being a critical factor affecting catalytic performance. Each enzyme exhibits an optimal pH at which its catalytic activity reaches its peak [40]. A number of variables affect the ideal pH of lipases, such as the source of the

enzyme, the immobilization technique, the characteristics of the support material, and the status of amino acid ionization in its active sites [41, 42]. The effect of pH on the activity of free CrL and CrL/PD/SD was investigated across a pH range of 5.5 to 9.5, maintaining a constant temperature of 37°C. As depicted in Fig. 2(a), the maximum catalytic activity for both free CrL and CrL/PD/SD was noted at pH 8. This finding aligns with prior reports indicating that most lipases exhibit optimal activity within a pH range of 7.0 to 9.0, although some lipases demonstrate broader ranges, spanning from pH 4 to 10 [41]. At pH 7, free CrL and CrL/PD/SD retained 67% and 84% of their initial activities, respectively. Increasing the pH to 9.0 resulted in a reduction of initial activity to 34% for free CrL and 52% for CrL/PD/SD. At pH 9.5, the catalytic activity declined further, with immobilized CrL/PD/SD retaining 26% of its original activity, while free CrL retained just 8%. These findings highlight the enhanced stability and activity of CrL/PD/SD at broader pH values compared to free CrL. The results are consistent with studies by Hombalimath et al. [43], which reported similar optimal pH values for lipase immobilized on chitosan magnetic microparticles. The significant impact of pH on enzyme activity can be owing to its influence on the ionization of amino acids, especially in the enzyme's binding site. At acidic pH levels, enzyme stability is reduced, leading to a loss of catalytic performance [44, 45].

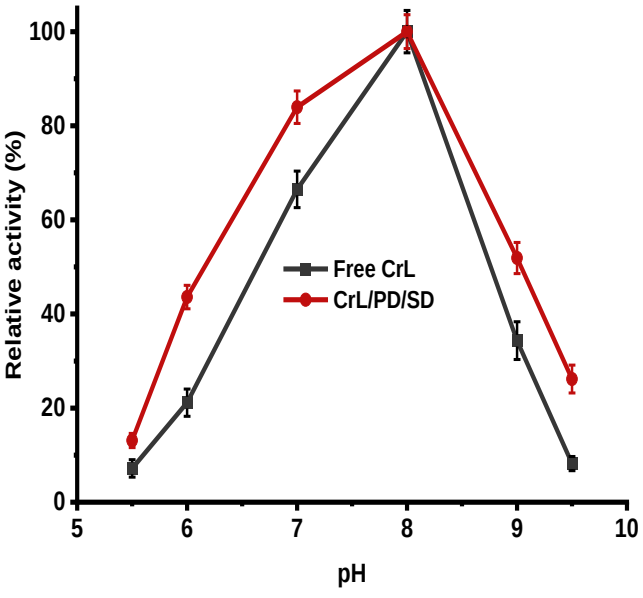

Fig. 2 (a): Effect of pH on the activity profiles of free CrL and CrL/PD/SD at a constant temperature of 37 °C.

Temperature also plays a pivotal role in enzymatic hydrolysis of esters catalyzed by lipases. Investigating the temperature dependence of lipase activity not only identifies its optimal operating conditions but also assesses the enzyme's thermal tolerance range [46]. The temperature effect on free CrL and CrL/PD/SD was analyzed within a range of 20°C to 70°C. As shown in Fig. 2(b), both free CrL and CrL/PD/SD exhibited minimal activity at 20°C, retaining 37% and 48% of their initial activity, respectively. Maximum catalytic activity for both forms was observed at 40°C. Elevating the temperature beyond the optimum resulted in reduced activity. At 70°C, CrL/PD/SD retained 51% of its initial activity, while free CrL retained only 8%. This observation can be explained by the denaturation effect, where deviations from the optimal temperature led to enzyme conformational changes, resulting in activity loss and reduced product formation [47]. The immobilized CrL/PD/SD exhibited greater thermal tolerance than free CrL, likely because of CrL's covalent binding to the support. This enhances the structural stability of CrL, reduces conformational flexibility, and mitigates diffusion limitations imposed by the carrier material [48]. These findings are consistent with reports by Atiroğlu and Hombalimath et al. [43, 49], which demonstrated that the optimal catalytic activities for both free and immobilized lipases were achieved at 40°C.

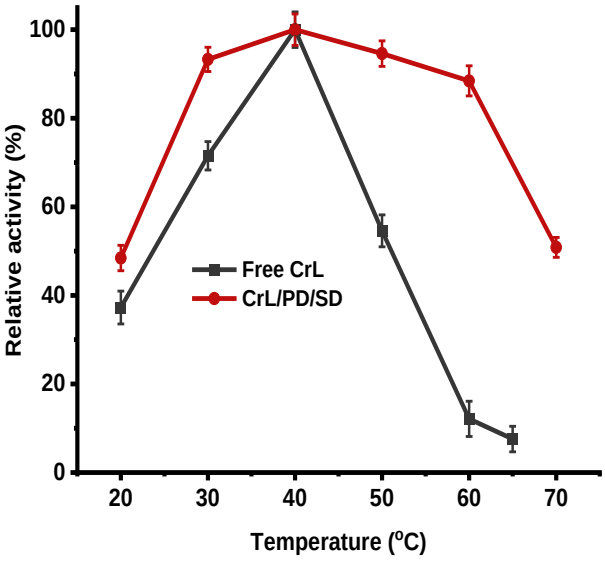

Fig. 2 (b): Temperature effect on the activity profiles of free CrL and CrL/PD/SD at an optimal pH of 8.

3.3. Kinetics of free and immobilized CrL

Kinetic studies were conducted to assess the substrate affinity and kinetic parameters of free CrL and CrL/PD/SD. The determined kinetic parameters of free CrL and CrL/PD/SD revealed Km of 0.15±0.01 and 0.21±0.03 mM, respectively. Their Vmax values were 769.2 ± 8.63 and 714.3 ± 6.58 µmol/min/mg protein, respectively (Fig. 3). The slight variation in the apparent Km and Vmax values can be ascribed to conformational changes induced during the immobilization process [43] and possible steric obstruction of the active site of the enzyme due to the carrier substance [49, 50]. These findings align with earlier studies, such as those by Gao et al. [51], who immobilized lipase on dopamine-functionalized mesoporous onion-like silica, and Işık et al. [18], who immobilized lipase on eggshell membrane.

3.4. Reusability of immobilized CrL

One of immobilized enzymes' many significant benfits is their recyclability and stability, which are essential for industrial applications and cost reduction in operational processes. The reusability of CrL/PD/SD was evaluated over sixteen successive cycles, as illustrated in Fig. 4. The immobilized enzyme kept approximately 86% of its original activity after ten reuse cycles. This enhanced stability can be attributed to the strong covalent bonding between lipase and PD/SD, which minimizes the denaturation and leakage of lipase molecules during repeated use [51]. Beyond ten cycles, the activity of CrL/PD/SD declined gradually, reaching 43% of its original activity after sixteen cycles. This progressive decrease in activity is likely due to factors such as accumulation of products of the reaction on the support surface and desorption of physically adsorbed lipase molecules. These factors collectively reduce the ability of molecules of the substrate to reach the active sites of the enzyme [30]. The observed reusability of CrL/PD/SD is comparable with results from other immobilization studies, as summarized in Table 1.

Table 1: Comparison of reusability performance of immobilized lipase compared with previously reported immobilized systems.

Support carrier	*Lipase*	*Reuse cycles*	*Residual activity*	*Ref.*
Dopamine-functionalized mesoporous onion-like silica	*Candida sp. 99-125* lipase	6	71%	[50]
Polyether sulfone	*Candida rugosa* lipase	12	20 %	[51]
Chitosan magnetic microparticles	*Aspergillus niger* lipase	6	55%	[42]
Ca-alginate gel beads	*Lysinibacillus macroides FS1* lipase	6	46 %	[52]
Glutaraldehyde-activated chitosan beads	*Candida rugosa* lipase	**7**	50 %	[53]
Metal oxide hybrid support	*Candida rugosa* lipase	12	32.7 %	[30]
flexible nanoporous MIL-53(Fe)	*Candida antarctica* lipase	6	< 10 %	[47]
Polydopamine/ saw dust	*Candida rugosa* lipase	6 16	91.5 % 43.2 %	This work

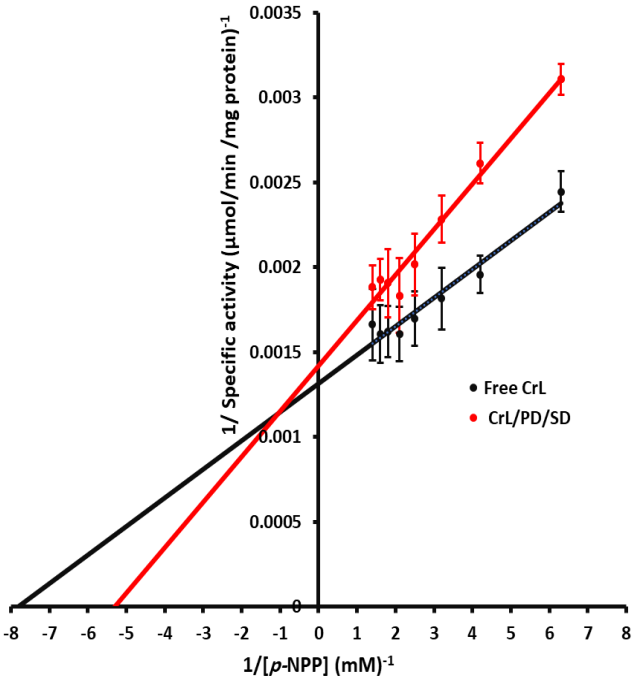

Fig. 3: Lineweaver-Burk plots for determining the kinetic parameters (K_m and V_{max}) of free CrL and CrL/PD/SD.

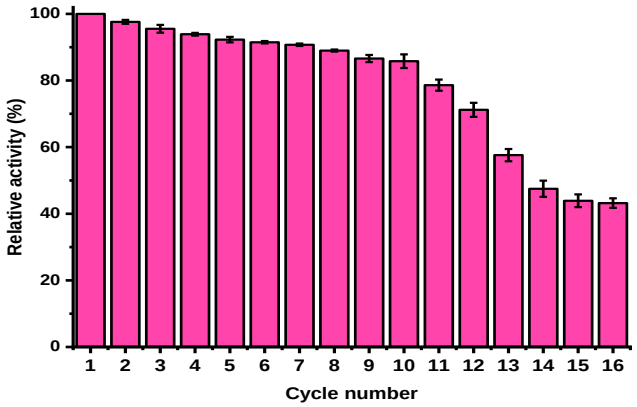

Fig. 4: Reusability of immobilized lipase (CrL/PD/SD).

3.5. Edible oils removal using free and immobilized CrL

As depicted in Fig. 5, free CrL and CrL/PD/SD have successfully hydrolyzed all edible oils under this study. Both free and immobilized lipase showed the same behavior during hydrolysis and both demonstrated the highest hydrolyzing activity against olive oil. The hydrolysis activity of free CrL and CrL/PD/SD was shown in the following order: olive oil > corn oil > sunflower oil > soybean oil. It was reported that the variations in hydrolysis yield for the different oils were due to the physical structure of the oil, oil impurities, oil viscosity, and the dissolving effect [55, 56]. Oleic and linoleic acids were identified as the predominant fatty acids in the oils examined. Notably, olive oil exhibited a high hydrolysis yield due to its high concentration of oleic acid (64%) and a low concentration of linoleic acid (16%) [57, 58]. In contrast, the other three oils tested contained elevated levels of linoleic acid (60-64%) [57]. Taking these observations into account, both CrL and CrL/PD/SD exhibit a strong affinity and specificity for hydrolyzing oils rich in oleic acid, and our findings align with those reported by Işık. et al., 2021[18]. In more detail, the amount of oleic acid (26.7%) in corn oil is slightly higher than that in sunflower and soybean oils, but the content of oleic acid in sunflower (21.4%) and soybean (20.2%) oils is almost equal [57, 59]. This similarity accounts for the nearly equivalent quantities of free fatty acids released due to the hydrolyzing activity of CrL in both sunflower and soybean (52 ± 1.02 and 50 ± 1.0 µmol/ml for free CrL and 59 ± 0.95 and 57 ± 0.85 µmol/ml for immobilized CrL, respectively). Additionally, as illustrated in Fig. 5, CrL/PD/SD demonstrated superior hydrolysis efficiency with the oils examined compared to the free variant. Our results align with the research conducted by Yiğitoğlu and Temoçin (2010)[56]. It is thus feasible to employ both free CrL and CrL/PD/SD in the hydrolysis of different types of edible oils. Notably, CrL/PD/SD exhibits significant potential for the remediation of wastewater that contains edible oils with elevated levels of oleic acid.

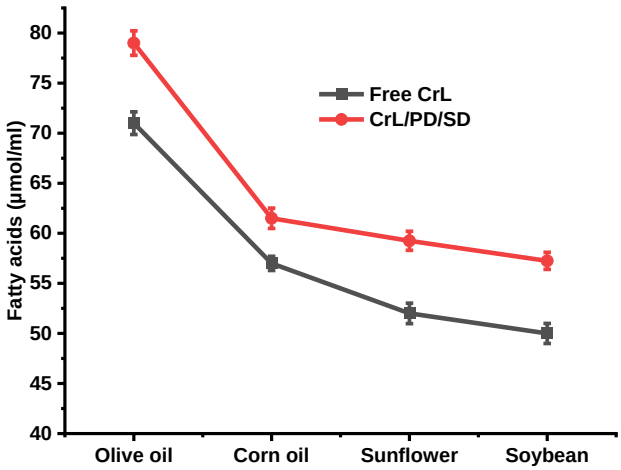

Fig. 5: Edible oil hydrolysis using free CrL and CrL/PD/SD.

4. Conclusion

This study highlights the covalent immobilization of *Candida rugosa* lipase (CrL) utilizing polydopamine-activated sawdust as a support through either a Schiff base or Michael addition mechanism. This process yields the formation of a robust biocatalyst capable of hydrolyzing and eliminating edible oils. The immobilization efficiency achieved was approximately 68%. The immobilized CrL demonstrated enhanced stability when subjected to elevated pH and temperature conditions compared to its free counterpart. In particular, at a pH of 9.5, the immobilized CrL/PD/SD retained 26% of its original activity, whereas the free CrL's catalytic activity diminished to 8%. Furthermore, after raising the temperature to 70°C, the remaining activity of CrL/PD/SD was 51%, in contrast to the free CrL, which maintained only 8% of its initial activity. Additionally, CrL/PD/SD was successfully reused and easily recovered over sixteen cycles, preserving approximately 43% of its original activity. The application of CrL/PD/SD for the hydrolysis of various

vegetable oils was also demonstrated. Consequently, the modified sawdust-immobilized lipase presents itself as a cost-effective, environmentally friendly, and efficient biocatalyst with potential for widespread use in diverse industrial applications, particularly in the treatment of oily wastewater with high oleic acid concentrations.

5. References

1. Adulkar, T.V. and V.K. Rathod, (2014) Ultrasound assisted enzymatic pre-treatment of high fat content dairy wastewater. Ultrasonics Sonochemistry,. **21**(3): p. 1083-1089.
2. Satyarthi, J., D. Srinivas, and P. Ratnasamy, (2011) Hydrolysis of vegetable oils and fats to fatty acids over solid acid catalysts. Applied Catalysis A: General,. **391**(1-2): p. 427-435.
3. Baena, A., et al., (2022).Enzymatic hydrolysis of waste fats, oils and greases (FOGs): Status, prospective, and process intensification alternatives. Chemical Engineering and Processing-Process Intensification, **175**: p. 108930.
4. Ngoie, W.I., et al., (2020) Valorisation of edible oil wastewater sludge: bioethanol and biodiesel production. Waste and biomass valorization,. **11**: p. 2431-2440.
5. Ahmad, T., et al., (2020) Utilization of wastewater from edible oil industry, turning waste into valuable products: A review. Trends in Food Science & Technology,. **99**: p. 21-33.
6. Iskandar, M.J., et al., (2018) Palm oil industry in South East Asia and the effluent treatment technology—A review. Environmental technology & innovation,. **9**: p. 169-185.
7. Louhıchı, G., et al., (2019) Process optimization via response surface methodology in the physico-chemical treatment of vegetable oil refinery wastewater. Environmental Science and Pollution Research,. **26**: p. 18993-19011.
8. Šereš, Z., et al., (2016) Edible oil industry wastewater treatment by microfiltration with ceramic membrane. International *Journal of Chemical and Molecular Engineering,*. **10**(4): p. 410-413.
9. Ma, Z., et al., (2015) Submerged membrane bioreactor for vegetable oil wastewater treatment. Chemical Engineering & Technology,. **38**(1): p. 101-109.
10. Abdollahzadeh Sharghi, E., A. Shorgashti, and B. Bonakdarpour, (2016) *T*he study of organic removal efficiency and membrane fouling in a submerged membrane bioreactor treating vegetable oil wastewater. *International Journal of Engineering,*. **29**(12): p. 1642-1649.
11. Ishak, S., A. Malakahmad, and M.H. Isa, (2012) Refinery wastewater biological treatment: A short review..
12. Sanghamitra, P., D. Mazumder, and S. Mukherjee, (2021) Treatment of wastewater containing oil and grease by biological method-a review. *Journal of Environmental Science and Health, Part A,*. **56**(4): p. 394-412.
13. Jamie, A., et al., (2016) Immobilization and enhanced catalytic activity of lipase on modified MWCNT for oily wastewater treatment. Environmental Progress & Sustainable Energy,. **35**(5): p. 1441-1449.
14. Bellaouchi, R., et al., (2021) Characterization and optimization of extracellular enzymes production by Aspergillus niger strains isolated from date by-products. *Journal of Genetic Engineering and Biotechnology,*. **19**(1): p. 50.
15. Yücel, S., P. Terzioğlu, and D. Özçimen (2012), Lipase applications in biodiesel production. Biodiesel-feedstocks, production and applications. InTech, Croatia,: p. 209-250.
16. Kuang, G., et al., (2023) Immobilization of lipase on hydrophobic MOF synthesized simultaneously with oleic acid and application in hydrolysis of natural oils for improving unsaturated fatty acid production. *International Journal of Biological Macromolecules,*. **242**: p. 124807.
17. Rodriguez, J.A., et al., (2000) Reaction of NO2 with Zn and ZnO: photoemission, XANES, and density functional studies on the formation of NO3. *The Journal of Physical Chemistry B,*. **104**(2): p. 319-328.

18. Işık, C., et al., (2021) A new bioremediation method for removal of wastewater containing oils with high oleic acid composition: Acinetobacter haemolyticus lipase immobilized on eggshell membrane with improved stabilities. New *Journal of Chemistry,*. **45**(4): p. 1984-1992.
19. Brena, B., P. González-Pombo, and F. Batista-Viera, (2013) :Immobilization of enzymes: a literature survey. Immobilization of Enzymes and Cells: Third Edition, p. 15-31.
20. Bashir, N., M. Sood, and J.D. Bandral, (2020) Enzyme immobilization and its applications in food processing: A review. *Int. J. Chem. Stud,*. **8**(2): p. 254-261.
21. Costa-Silva, T.A., et al., (2022) Highly effective Candida rugosa lipase immobilization on renewable carriers: Integrated drying and immobilization process to improve enzyme performance. Chemical Engineering Research and Design,. **183**: p. 41-55.
22. Jeganathan, J., G. Nakhla, and A. Bassi, (2007) Hydrolytic pretreatment of oily wastewater by immobilized lipase. *Journal of hazardous materials,*. **145**(1-2): p. 127-135.
23. Waseem, A., et al., (2024) Exploring chitosan-immobilized Rhizopus oligosporus lipase for olive-mill wastewater treatment. International *Journal of Environmental Science and Technology,*: p. 1-14.
24. Daâssi, D., et al., (2016) Sawdust waste as a low-cost support-substrate for laccases production and adsorbent for azo dyes decolorization. *Journal of Environmental Health Science and Engineering,*. **14**: p. 1-12.
25. Oliveira, R.V.M., et al., (2024) Chitosan-based magnetic bioadsorbent beads from eucalyptus sawdust waste for the Direct Violet-51 dye remediation: Eco-friendly strategy and statistical optimization. *International Journal of Biological Macromolecules,*. **254**: p. 127764.
26. Mallakpour, S., F. Sirous, and C.M. Hussain, (2021) Sawdust, a versatile, inexpensive, readily available bio-waste: From mother earth to valuable materials for sustainable remediation technologies. Advances in Colloid and Interface Science,. **295**: p. 102492.
27. Zhang, H., et al., (2018) Biocatalytic membrane based on polydopamine coating: a platform for studying immobilization mechanisms. Langmuir,. **34**(8): p. 2585-2594.
28. Yassin, M.A. and A.A.M. Gad, (2023) Decolorization of dye effluents via immobilized glycoprotein peroxidase on post-consumer polystyrene foam. *International Journal of Biological Macromolecules,*. **236**: p. 124019.
29. Winkler, U.K. and M. Stuckmann, (1979) Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by Serratia marcescens. *Journal of bacteriology,*. **138**(3): p. 663-670.
30. Ramlee, N.N., et al., (2022) Biochemical and physical characterization of immobilized Candida rugosa lipase on metal oxide hybrid support. Catalysts,. **12**(8): p. 854.
31. Saraç, N. and A. Ugur, (2016) A green alternative for oily wastewater treatment: lipase from Acinetobacter haemolyticus NS02-30. Desalination and Water Treatment,. **57**(42): p. 19750-19759.
32. Akhouairi, S., et al., (2019) Natural sawdust as adsorbent for the eriochrome black T dye removal from aqueous solution. Water, Air, & Soil Pollution,. **230**: p. 1-15.
33. Idrus, M.M., et al., (2011) Treated tropical wood sawdust-polypropylene polymer composite: mechanical and morphological study. *Journal of biomaterials and nanobiotechnology,*. **2**(04): p. 435.
34. Rahman, N.u., et al., (2021) Activated ailanthus altissima sawdust as adsorbent for removal of acid yellow 29 from wastewater: Kinetics approach. Water,. **13**(15): p. 2136.
35. Youssef, R.A., et al., (2025) Sustainable biocatalyst for textile dye effluent treatment using immobilized turnip peroxidase on polydopamine coated sawdust. Bioresource Technology Reports,. **30**: p. 102118.

36. Yassin, M.A. and A.A.M. Gad, (2020) Immobilized enzyme on modified polystyrene foam waste: a biocatalyst for wastewater decolorization. *Journal of Environmental Chemical Engineering*,. **8**(5): p. 104435.
37. Yassin, M.A., et al., (2019) Facile coating of urinary catheter with bio–inspired antibacterial coating. Heliyon,. **5**(12).
38. Huang, T., et al., (2018) Polydopamine coated graphene oxide aerogels and their ultrahigh adsorption ability. Diamond and Related Materials,. **86**: p. 117-127.
39. El Yakhlifi, S. and V. Ball, (2020) Polydopamine as a stable and functional nanomaterial. Colloids and Surfaces B: Biointerfaces,. **186**: p. 110719.
40. Alabdalall, A.H., et al., (2021) Application and characterization of crude fungal lipases used to degrade fat and oil wastes. Scientific Reports,. **11**(1): p. 19670.
41. Öztürk, B., (2001) Immobilization of lipase from Candida rugosa on hydrophobic and hydrophilic supports.: Izmir Institute of Technology (Turkey).
42. Bayramoglu, G., et al., (2022) Immobilization of Candida rugosa lipase on magnetic chitosan beads and application in flavor esters synthesis. Food chemistry,. **366**: p. 130699.
43. Hombalimath, V., S. Desai, and A. Sharanappa (2020), Characterization of lipase immobilized on chitosan Magnetic microparticles for economic biodiesel production. *International Journal of Scientific and Technology Research*,. **9**(3): p. 5111-5116.
44. Enachi, E., et al., (2018) Extraction, purification and processing stability of peroxidase from plums (Prunus domestica). *International Journal of food properties*,. **21**(1): p. 2744-2757.
45. Gad, A.A.M., A. Gora-Sochacka, and A. Sirko, (2024) Recombinant C-terminal catalytic domain of rat L-gulono lactone oxidase produced in bacterial cells is enzymatically active. Current Issues in Molecular Biology,. **46**(8): p. 8958-8968.
46. Patel, V., et al., (2015) Synthesis of ethyl caprylate in organic media using Candida rugosa lipase immobilized on exfoliated graphene oxide: Process parameters and reusability studies. *Biochemical Engineering Journal*,. **95**: p. 62-70.
47. Sri Kaja, B., et al., (2018) Investigating enzyme activity of immobilized Candida rugosa lipase. *Journal of Food Quality*,. **2018**(1): p. 1618085.
48. Ghasemi, S., M. Yousefi, and A. Nikseresht, (2023) Comparison of covalent and in situ immobilization of Candida antarctica lipase A on a flexible nanoporous material. 3 Biotech,. **13**(3): p. 99.
49. Atiroğlu, V., (2020) Lipase immobilization on synthesized hyaluronic acid-coated magnetic nanoparticle-functionalized graphene oxide composites as new biocatalysts: Improved reusability, stability, and activity. *International journal of biological macromolecules*,. **145**: p. 456-465.
50. Aghaei, H., et al., (2020) Utilization of two modified layered doubled hydroxides as supports for immobilization of Candida rugosa lipase. *International Journal of Biological Macromolecules*,. **162**: p. 74-83.
51. Gao, J., et al., (2017) Dopamine-functionalized mesoporous onion-like silica as a new matrix for immobilization of lipase Candida sp. *99-125*. Scientific reports,. **7**(1): p. 40395.
52. Zare, A., et al., (2019) The immobilization of Candida rugosa lipase on the modified polyethersulfone with MOF nanoparticles as an excellent performance bioreactor membrane. *Journal of biotechnology*,. **289**: p. 55-63.
53. Jigajinni, S.K. and B.S. Meti, (2021) Immobilization optimization and characterization of immobilized lipase from Lysinibacillus macroides FS1 for biodiesel production. *Int. J. Curr. Microbiol. App. Sci*,. **10**(04): p. 232-245.
54. Gonawan, F.N., et al., (2022) Immobilization of Candida rugosa lipase on the glutaraldehyde-activated chitosan beads. J*ournal of Chemical Engineering and Industrial Biotechnology*,. **8**(1): p. 33-41.
55. Rathod, V.K. and A.B. Pandit, (2009) Effect of various additives on enzymatic

hydrolysis of castor oil. *Biochemical Engineering Journal,*. **47**(1-3): p. 93-99.
56. Yiğitoğlu, M. and Z. Temoçin, (2010) Immobilization of Candida rugosa lipase on glutaraldehyde-activated polyester fiber and its application for hydrolysis of some vegetable oils. *Journal of Molecular Catalysis B: Enzymatic,*. **66**(1-2): p. 130-135.
57. Annisa, A.N. and W. Widayat. (2018) A review of bio-lubricant production from vegetable oils using esterification transesterification process. in MATEC Web of Conferences.. EDP Sciences.
58. Hernández, M.L., et al., (2021) The oleic/linoleic acid ratio in olive (Olea europaea L.) fruit mesocarp is mainly controlled by OeFAD2-2 and OeFAD2-5 genes together with the different specificity of extraplastidial acyltransferase enzymes. Frontiers in Plant Science,. **12**: p. 653997.
59. Yousefi, M., L. Nateghi, and H. Karimian, (2012) Evaluation and comparison the physicochemical properties and fatty acid profile of shahrodi sunflower and soybean oil. Advances in Environmental Biology,. **6**: p. 2866-2869.