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Optimization and Characterization of Urease Immobilization from Red Lentil Seeds (*Lens culinaris*) Using Chitosan

Zusfahair[1]*, Dian Riana Ningsih[1], Bilalodin[2], Amin Fatoni[1], Adilla Luthfia[2], Purwati[1], Niken Istikhari Muslihah[1], Inessa Putri Apriliadina[1]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Jenderal Soedirman University, Purwokerto 53123, Indonesia
[2]Department of Physics, Faculty of Mathematics and Natural Sciences, Jenderal Soedirman University, Purwokerto 53123, Indonesia

*Corresponding author email: zusfahair@gmail.com

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ABSTRACT. Urease is an enzyme that plays a vital role in catalyzing the hydrolysis of urea into ammonia (NH_3) and carbon dioxide (CO_2). This study focuses on the isolation of urease from red lentil seeds, followed by its immobilization. The objective of this research is to optimize and characterize urease that has been immobilized using chitosan and activated with glutaraldehyde. Red lentil seeds were processed with a mortar and pestle at low temperatures (4 °C) to obtain a crude enzyme extract, which was then concentrated using 50% acetone (P50) prior to immobilization. The optimization process for P50 urease immobilization involved assessing various factors, including chitosan concentration, glutaraldehyde concentration, temperature, and the immersion duration in glutaraldehyde. The findings revealed that the optimal conditions for immobilizing P50 urease were achieved at a chitosan concentration of 0.75%, with a 2% glutaraldehyde soak at 25 °C for 2 hours, resulting in an enzyme activity of 7.042 U/g. The immobilized P50 urease demonstrated the ability to be reused up to 7 times while maintaining 51% of its initial activity. Scanning Electron Microscopy (SEM) analysis indicated morphological changes in the beads after the addition of glutaraldehyde and the enzyme, shifting from a rounded to an irregular shape. Additionally, Fourier Transform Infrared Spectroscopy (FTIR) analysis identified C-N and C=N peaks, confirming the successful incorporation of glutaraldehyde.

Keywords: immobilization, red lentil seeds, glutaraldehyde, chitosan, urease

INTRODUCTION

Urease is an enzyme that plays a crucial role in catalyzing the hydrolysis of urea into ammonia (NH_3) and carbon dioxide (CO_2) (Singh et al., 2017). This process is a key component of the nitrogen cycle, with significant implications in various biological, agricultural, and industrial applications. Urease plays some important role in the decomposition of organic matter and the nitrogen cycle in the soil for plant growth (Koçak, 2020). The results of urease enzyme hydrolysis can help the biocementation process by forming calcium carbonate (Xu et al., 2021). Urease has been isolated from multiple sources, including legumes such as pea seeds (*Pisum sativum* L.), broad beans (*Vicia faba* L.) (Bedan, 2020), winged bean seeds (*Psophocarpus tetragonolobus* (L.) DC.) (Zusfahair et al., 2023), and mung beans (*Vigna radiata* L.) (Muslihah et al., 2024). Previous research has successfully isolated urease from red lentils, resulting in the production of free enzymes.

Free enzymes are generally more fragile than inorganic catalysts. Their application in industrial processes is often limited by high costs and inherent instability. Urease, for example, is particularly unstable due to its sensitivity to temperature and pH, as well as its inability to be reused. To overcome these limitations, enzyme immobilization—using solid-phase biocatalysts—has emerged as a promising solution. Consequently, there is increasing interest in this approach within bio-based industries (Verma et al., 2020).

Free enzymes tend to be more fragile compared to inorganic catalysts. Their use in industrial applications presents challenges, such as high costs and instability, which can limit their practicality. Urease, for example, is particularly unstable due to its sensitivity to temperature, pH, and inability to be reused. To overcome these limitations, enzyme immobilization using solid-phase has emerged as a promising solution. Consequently, there is increasing interest in this approach within bio-based industries (Verma et al., 2020).

Immobilization generally involves attaching enzymes to or within a solid matrix. Immobilized enzymes display greater robustness and resistance to environmental changes than their free counterparts for

health applications (Al-Garawi et al., 2022). Although immobilized enzymes may exhibit lower catalytic activity than free enzymes, they provide enhanced stability and reusability, making them more cost-effective and efficient for large-scale applications (Kamburov & Lalov, 2014).

In practical applications, urease is typically employed in its immobilized form. Immobilized urease has been utilized in various scenarios, including kidney machines for blood detoxification (Lahiri, 2015), degradation of herbicides (Jamwal et al., 2020), processes in the food industry to remove urea from beverages and food products, and for reducing urea content in agricultural waste (Lv et al., 2018). The immobilization of urease has been investigated using several matrices for clinical analytical applications, including chitosan (Al-Garawi et al., 2022), sodium alginate (Tetiker & Ertan, 2017), cellulose (Lv et al., 2018), and polyether sulfone (Zhang et al., 2019).

The supporting matrix employed in this study is chitosan. Chitosan has several advantages, including good biocompatibility and biodegradability (Kurniasih et al., 2022). In addition, its reactive amino groups make chitosan an excellent choice for immobilization. To improve the mechanical and thermal stability of chitosan, crosslinking agents are necessary to create crosslinks that strengthen its structure. Glutaraldehyde is the most widely used crosslinking reagent (Malhotra & Basir, 2020). It is chosen for its two aldehyde groups, which can interact with free amino acids present in chitosan and proteins. The immobilization of enzymes onto chitosan occurs mainly through the reaction of glutaraldehyde with the free amino groups in both chitosan and the enzyme molecules, resulting in the formation of covalent bonds. Specifically, glutaraldehyde creates covalent imine bonds with the amino groups of chitosan as a consequence of Schiff base rearrangement. Its bifunctional properties, reliability, and ease of use make glutaraldehyde a preferred crosslinker and activating agent (Kamburov & Lalov, 2014).

The P50 urease is subsequently immobilized using chitosan that has been activated with glutaraldehyde. The optimization process for the immobilized P50 urease involves determining the appropriate concentrations of chitosan and glutaraldehyde, as well as the immersion temperature and duration. The resulting immobilized enzyme will be characterized in terms of its reusability, and analyses will be conducted using SEM-EDX and FTIR.

This research focuses on extracting urease from red lentil seeds, followed by purification through precipitation using 50% acetone to obtain P50 urease. The P50 urease is then immobilized using chitosan that has been activated with glutaraldehyde. The optimization process for the immobilized P50 urease involves determining the appropriate concentrations of chitosan and glutaraldehyde, as well as the immersion temperature and duration. The resulting immobilized enzyme will be characterized in terms of its reusability, and analyses will be conducted using SEM-EDX and FTIR. The enzyme is then assessed by detecting the ammonium produced in the reaction using Nessler's reagent.

EXPERIMENTAL SECTION

Materials and Methods

The equipment utilized in this study includes standard glassware typically found in biochemistry laboratories, an incubator, a pH meter (Hanna Instrument), a centrifuge (Ohaus), a UV-Vis spectrophotometer (Bel Photonic, V-M5), Fourier-transform infrared spectroscopy (FTIR), and Scanning Electron Microscopy (JSM-6510LA). The materials used in the experiments consist of red lentil seeds, urea, Nessler's reagent, ammonium sulfate, distilled water, $Na_2HPO_4.2H_2O$, $NaH_2PO_4.2H_2O$, $CuSO_4$, Na_2CO_3, acetic acid, NaOH, and C_3H_6O. All chemical materials were sourced from Merck Chemical Company (Merck, Germany), except for chitosan (DDA ≥ 75%) and glutaraldehyde, which were obtained from Sigma.

Research Procedure

Extraction of urease from red lentils

Urease was isolated using the method previously described by (Zusfahair et al., 2018). The resulting crude extract was subsequently concentrated with acetone.

Concentration using acetone

The concentration process adhered to the method outlined by (Sabilla & Susanti, 2019). A total of 300 mL of crude urease extract was gradually combined with absolute acetone (at 5 ºC) to achieve a 50% saturation level (using a 1:1 volume ratio of 300 mL of crude extract to 300 mL of acetone). This mixture was gently stirred in an ice bath with a magnetic stirrer and left to sit in a refrigerator for 12 hours to enhance precipitation. The precipitated enzyme was then separated by centrifugation at 10,000 rpm for 15 minutes at 4 ºC. The supernatant was carefully discarded, and any remaining acetone on the precipitate was removed by evaporation or absorbed with sterilized filter paper. The precipitate obtained from this concentration process was dissolved in 150 mL of 0.2 M phosphate buffer at pH 7 (NaH_2PO_4/$NaHPO_4$,1:1 *v/v*), resulting in a suspension referred to as P50 urease.

Preparation of chitosan beads

Chitosan beads were prepared following the method described by (Malhotra & Basir, 2020). A chitosan solution was created at a concentration of 0.5% by dissolving 0.025 g of chitosan powder in 5 mL of 2% acetic acid solution. This mixture was homogenized using a stirrer at 60 ºC for 10 minutes. The solution was then transferred into a syringe and slowly pressed to form droplets in a container containing 25 mL of 1 N NaOH solution, resulting in

bead formation. These beads were allowed to soak for 24 hours. Chitosan beads immersed in NaOH solution undergoes an ionotropic gelation process, where the amino groups ($–NH_3^+$) react with OH^- ions, stabilizing the physical structure of the beads to prevent them from redissolving. After immersion, the beads were washed twice and suspended in 0.05 M phosphate buffer at pH 7 prior to activation. The same procedure was applied to chitosan concentrations of 0.25, 0.75, and 1%.

Immobilization of urease on chitosan beads using glutaraldehyde crosslinker

The immobilization of urease onto chitosan beads was conducted using glutaraldehyde as a crosslinker, following the methodology outlined by Malhotra & Basir (2020). In this procedure, the chitosan solution was treated with 2% glutaraldehyde until fully submerged, then allowed to stand at room temperature for 2 hours. The beads were subsequently washed five times using a repeated rinsing method with 0.05 M phosphate buffer at pH 7 to eliminate any residual glutaraldehyde. After washing, the beads were incubated in a solution containing 4 mL of P50 urease at 4 °C for 24 hours, leading to the formation of immobilized P50 urease. The immobilization process was optimized by varying the concentration of glutaraldehyde, immersion temperature, and immersion duration. The glutaraldehyde concentrations tested were 0.5, 1, 3, and 4%, with immersion temperatures of 4, 35, and 40 °C, and immersion times of 0.5, 1, 1.5, and 2.5 hours.

Activity testing of free and immobilized enzymes

The activity of urease was evaluated by adding 1 mL of a 15,000-ppm urea solution to a reaction tube, followed by the addition of 1.95 mL of 0.2 M phosphate buffer at pH 7 and 0.05 mL of P50 urease solution. The control tube contained 1 mL of 15,000 ppm urea and 2 mL of 0.2 M phosphate buffer at pH 7. Both the sample and control tubes were incubated for 15 minutes at 35 ºC. After incubation, the tubes were cooled in a refrigerator for 5 minutes and then heated to 80 ºC for 2 minutes. Both tubes were centrifuged for 15 minutes at room temperature at 5,000 rpm.

For the activity test of the immobilized enzyme, 5 mL of 15,000 ppm urea solution in phosphate buffer at pH 7 was added to the immobilized P50 urease beads, corresponding to the number of beads produced in the previous experiment. This mixture was incubated for 15 minutes at 35 °C. The beads were then separated from the solution by decantation, and the activity was measured using Nessler's reagent. A control solution was prepared in the same manner, using beads without urease.

A sample volume of 1.5 mL from the resulting solution was combined with 0.25 mL of Nessler's reagent. The absorbance of this solution was measured using a UV-Vis spectrophotometer at a wavelength of 500 nm. To quantify the amount of ammonium produced by urease, a standard curve was prepared using ammonium sulfate solutions at concentrations of 6, 9, 12, 15, and 18 ppm, each treated with Nessler's reagent. The urease activity in the red lentil sample was then calculated by comparing its absorbance to the standard curve, allowing determination of the amount of ammonia released by urea hydrolysis. One unit of activity is defined as the amount of ammonia generated from the hydrolysis of urea by urease in the sample, expressed as 1 μmol per minute.

Characterization of immobilized urease

The characterization of immobilized P50 urease was performed on the beads under optimal conditions. This characterization included assessments of reusability and analyses using SEM-EDX and FTIR techniques on the chitosan beads.

RESULTS AND DISCUSSION

Extraction of Urease from Red Lentil Seeds

The extraction process is designed to liberate enzymes from the cells within plant tissues (Pramono et al., 2018). Urease was extracted by immersion red lentil seeds in distilled water, a process that promotes imbibition, or the absorption of water by the dried seeds (Pratantie et al., 2021). After immersion, the lentils were ground and dissolved in a buffer solution pH 7 (NaH_2PO_4/$NaHPO_4$,1:1 *v/v*) followed by centrifugation (10,000 rpm, 4 °C, 15 minutes). The supernatant obtained from this centrifugation was utilized as the crude extract, which was then concentrated using 50% acetone (Zusfahair et al., 2025).

Concentration Using Acetone

The concentration of the crude enzyme extract was achieved by adding 50% acetone (designated as P50). The results indicated that the activity of the free P50 enzyme was measured at 3.443 U/mL. This P50 was subsequently immobilized using chitosan and activated with glutaraldehyde.

Optimization of Immobilized P50 Urease from Red Lentil Seeds Using Chitosan and Glutaraldehyde Activation

Optimization of chitosan concentration

In this study, urease was immobilized using chitosan. The presence of reactive amino groups in chitosan makes it a suitable carrier for the enzyme. Chitosan beads were prepared by dissolving chitosan powder in 2% acetic acid. This dissolution resulted in the formation of a chitosan-carboxylic acid complex, characterized by hydrogen bonding between the hydroxyl groups and the $–NH_3^+$ groups in chitosan, and the carboxyl groups in acetic acid (**Figure 1**).

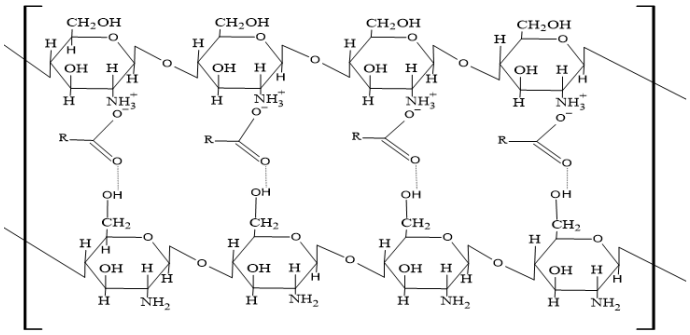

Figure 1. Chitosan complex with carboxylic acid (Basir et al., 2017)

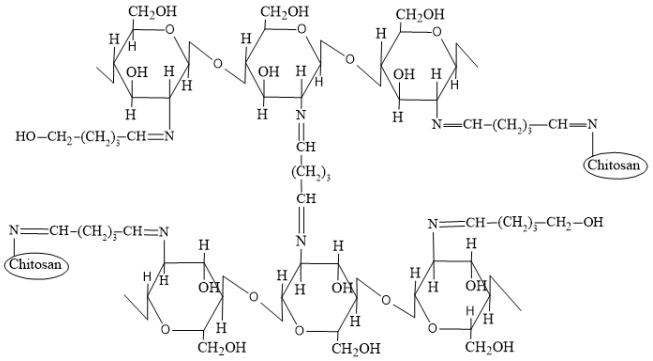

Figure 2. Reaction between chitosan and glutaraldehyde (Kamburov & Lalov, 2014)

The resulting chitosan solution was then transferred to a syringe and gently pressed to form droplets in a container filled with NaOH solution, where they were allowed to soak for 24 hours to form immobilized beads. These immobilized beads were then immersed in a 2% glutaraldehyde solution and left at room temperature. The reaction between chitosan and glutaraldehyde is depicted in **Figure 2**. Glutaraldehyde forms covalent imine bonds with the amino groups of chitosan through Schiff base rearrangement, and it is more likely to react with amino groups than with hydroxyl groups (Qu & Luo, 2020).

The immobilized beads were then incubated with the enzyme solution to promote crosslinking between chitosan and the enzyme, leading to the formation of immobilized P50 urease. **Figure 3** illustrates the reaction involving chitosan, glutaraldehyde, and the enzyme. Glutaraldehyde (-CHO) interacts with various functional protein groups, including amines, thiols, phenols, and imidazoles, due to the high reactivity of nucleophilic amino acid residues, primarily forming covalent -CH=N- (schiff base) linkages with primary amines during immobilization condition (Kamburov & Lalov, 2014).

The protein content of immobilized P50 urease was subsequently analyzed using the Lowry method, and its activity was assessed at pH 7 and an incubation temperature of 35 °C. The resulting data, which illustrates the relationship between chitosan concentration (%) and the relative activity of immobilized P50 urease, is presented in **Figure 4**.

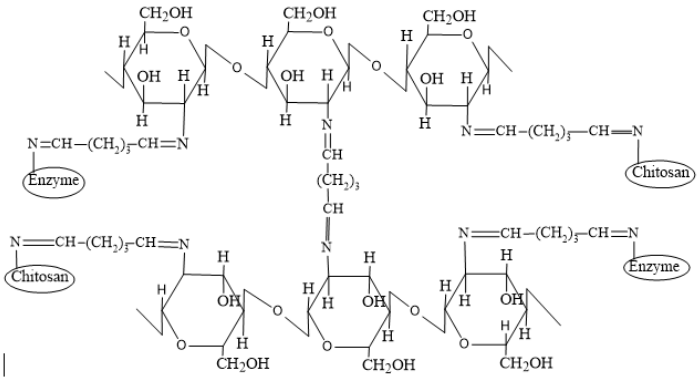

Figure 3. Reaction between chitosan, glutaraldehyde, and the enzyme *(Kamburov & Lalov, 2014)*

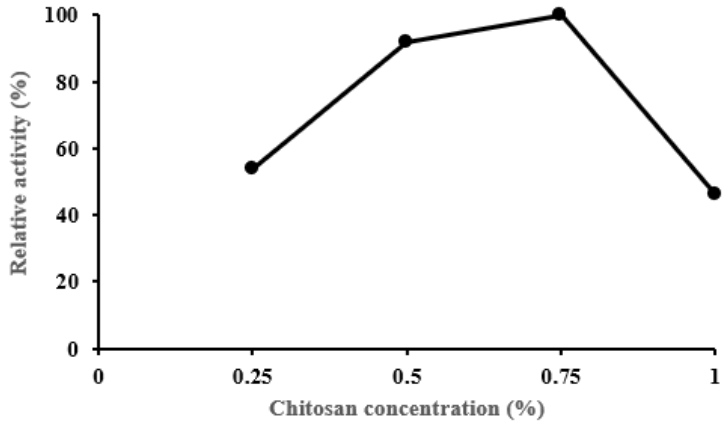

Figure 4. Curve showing the effect of chitosan concentration on the activity of immobilized P50 urease

The curve in **Figure 4** reveals that the activity of immobilized P50 urease increased as chitosan concentrations rose from 0.25 to 0.75%. However, at chitosan concentrations exceeding 0.75%, the activity of the immobilized urease declined. At lower chitosan concentrations, the beads were found to be fragile and soft, resulting in poor mechanical stability and enzyme leaching, which can reduce catalytic activity. At higher concentrations, the beads became harder, potentially hindering substrate access (Malhotra & Basir, 2020). Crosslinking can occur at various reactive sites on chitosan, such as the $–NH_2$ and –OH groups. Therefore, at the optimal chitosan concentration (0.75%), there is a potential for stable binding that does not interfere with enzyme-substrate interactions, resulting in the highest enzyme activity.

Optimization of glutaraldehyde concentration

The impact of glutaraldehyde concentration on the relative activity of immobilized P50 urease is depicted in **Figure 5**. The curve in **Figure 5** indicates that the activity of immobilized P50 urease increased with glutaraldehyde concentrations between 0.5% and 2%, but decreased at concentrations above 2%. At lower glutaraldehyde concentrations, there were also fewer active groups on chitosan, leading to limited crosslinking between chitosan, glutaraldehyde, and the enzyme, which resulted in lower enzyme activity (Hegde & Veeranki, 2014). As the concentration of glutaraldehyde increased, the activation of chitosan's amino groups also increased, enhancing enzyme activity (Chen et al., 2013). At the optimal concentration of 2%, glutaraldehyde provides sufficient crosslinking to securely bind the enzyme without excessive structural rigidity, which helps maintain both catalytic activity. However, excessively high concentrations of glutaraldehyde resulted in binding at multiple sites, which reduced the enzyme's flexibility and increased its brittleness (Malhotra & Basir, 2020).

Effect of glutaraldehyde immersion temperature on enzyme activity

The immersion temperature of glutaraldehyde significantly affects chitosan, influencing both the efficiency of the cross-linking process and the ultimate properties of the chitosan beads. Excessively high temperatures can cause the chitosan beads to become brittle, which can impede effective cross-linking with the enzyme. **Figure 6** presents data on how varying glutaraldehyde immersion temperatures impact the relative activity of immobilized P50 urease.

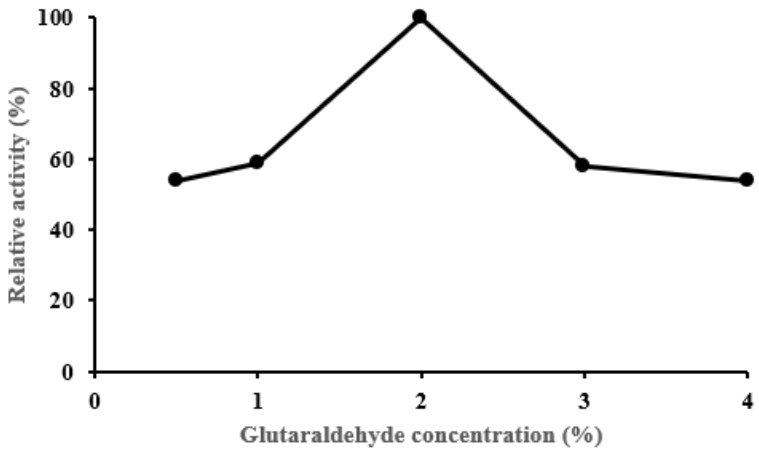

Figure 5. Curve showing the effect of glutaraldehyde concentration on the activity of immobilized P50 urease

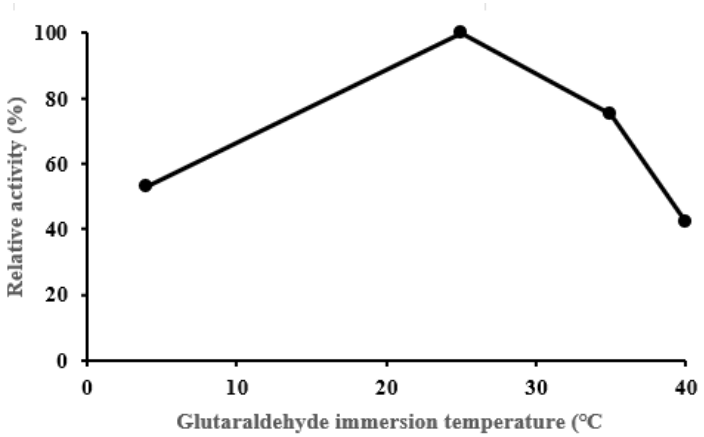

Figure 6. Curve illustrating the effect of glutaraldehyde immersion temperature on the activity of immobilized P50 urease

The curve in **Figure 6** demonstrates that the highest activity of immobilized P50 urease occurred in beads immersed at a temperature of 25 °C. Enzyme activity increased from 4 to 25 °C but experienced a sharp decline at temperatures exceeding 25 °C. This decrease is likely due to the rapid binding of glutaraldehyde's aldehyde groups to the enzyme's amino groups at elevated temperatures, which increases steric hindrance and subsequently reduces enzyme activity (Chen et al., 2013). This finding contrasts with the results of Çetinus & Öztop (2003), who reported an optimal temperature of approximately 35 °C for catalase activity when immobilized on chitosan beads. This suggests that different enzymes may have unique optimal glutaraldehyde immersion temperatures for effective immobilization.

Optimization of glutaraldehyde immersion time

The impact of varying glutaraldehyde immersion time on the relative activity of immobilized P50 urease is illustrated in **Figure 7**. The curve in **Figure 7** indicates that the highest activity of immobilized P50 urease was achieved when the beads were immersed in glutaraldehyde for 2 hours. As the immersion time increased, the enzyme activity also rose. However, enzyme activity declined sharply when the immersion time exceeded 2 hours. This reduction is likely due to excessive cross-linking that occurs with prolonged immersion, which can lead to significant alterations in the enzyme's configuration and result in protein denaturation (Chen et al., 2013).

Effects of Repeated Use of Immobilized P50 Urease

Repeated use is a crucial factor in enzyme immobilization, especially for cost-effective and economical applications. The performance of this immobilized enzyme was evaluated through activity tests until its activity decreased to 50%. **Figure 8** illustrates the impact of repeated usage on the relative activity of immobilized P50 urease. The data in **Figure 8** shows that immobilized P50 urease can be utilized up to seven times, retaining 51% of its initial activity. However, with each successive use, the enzyme's activity declined. This reduction is likely due to the weakening of the bond between the enzyme and its supporting matrix. The decrease in enzyme activity upon reuse may result from distortions caused by frequent interactions between the active site and the substrate, which can diminish catalytic efficiency (Zhou et al., 2013).

The findings in **Figure 8** are further supported by the results of the SEM analysis presented in **Figure 9**. The SEM analysis of chitosan beads (**a**) reveals a spherical morphology with an average pore size of 0.22 μm. In contrast, the chitosan-enzyme beads (**b**) show a reduced spherical shape due to the enzyme coating the chitosan surface. This change in morphology may suggest enzyme attachment. The chitosan-glutaraldehyde-enzyme beads (**c**) exhibit a transformation in surface shape from round to irregular as a result of coverage by the glutaraldehyde-enzyme solution, indicating enhanced surface cross-linking that could help maintain enzyme activity under operational conditions. In the chitosan-glutaraldehyde-enzyme beads used five times (**d**), the loss of enzyme due to repeated use allows the spherical shape to re-emerge more clearly, with an average pore size of 0.31 μm, although the beads still retain enzyme activity, indicating that the immobilized enzyme maintains stability despite repeated cycles. (Gür et al., 2018) noted changes in the morphology of activated and immobilized enzyme beads, reporting that the surface roughness increased following glutaraldehyde treatment and enzyme immobilization. Additionally, surface porosity was found to increase after the application of glutaraldehyde (Gilani et al., 2016).

SEM analysis is utilized to investigate the surface morphology of materials at high magnification, while EDX analysis is employed to assess the elemental composition of the samples (Kustomo, 2022). The results of the EDX analysis can be found in **Figure 10** and **Table 1**.

The elements identified in immobilized P50 urease include carbon (C) and sodium (Na). The carbon

content in chitosan increases with the addition of the enzyme and glutaraldehyde, rising from 21.91% to 59.67%, and then decreases to 56.11% after the beads have been used five times. This decline is attributed to the enzyme being released from the surface of the immobilized beads. The increase in carbon content reflects the presence of enzyme protein and glutaraldehyde, both rich in carbon, nitrogen, and oxygen, indicating successful enzyme immobilization (Gorissen et al., 2018). The subsequent decrease after repeated use suggests gradual enzyme release, correlating with stability and retained activity. In contrast, the sodium content in chitosan decreases following the addition of the enzyme and glutaraldehyde, as sodium dissolves in water during the washing process.

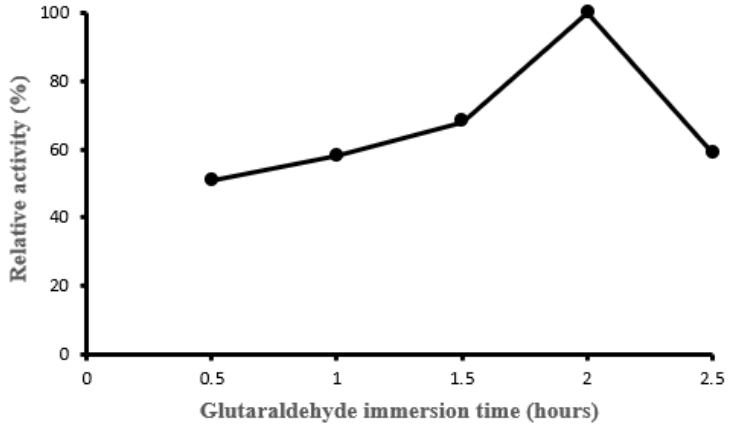

Figure 7. Curve demonstrating the effect of glutaraldehyde immersion time on the activity of immobilized P50 urease

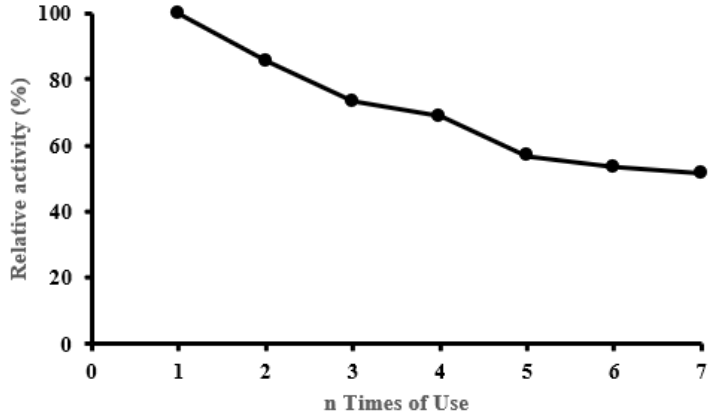

Figure 8. Effect curve of repeated use on immobilized urease P50 activity.

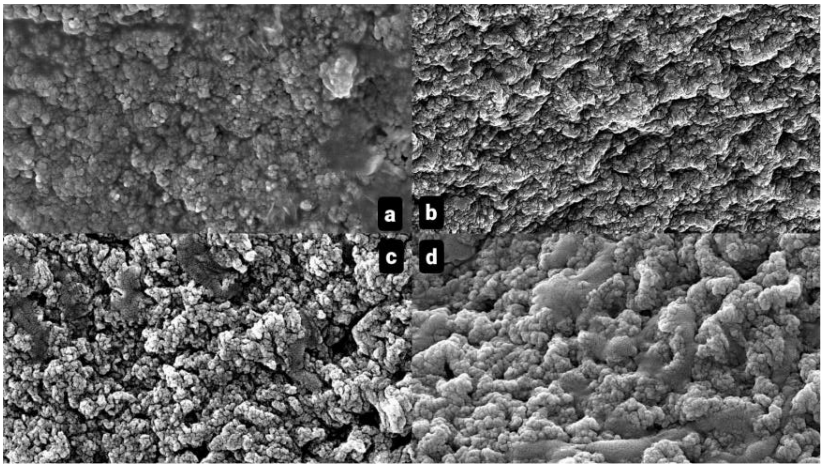

Figure 9. SEM analysis of immobilized P50 beads at 10,000x magnification [(a) chitosan, (b) chitosan-enzyme, (c) chitosan-glutaraldehyde-enzyme, (d) chitosan-glutaraldehyde-

enzyme used 5 times]

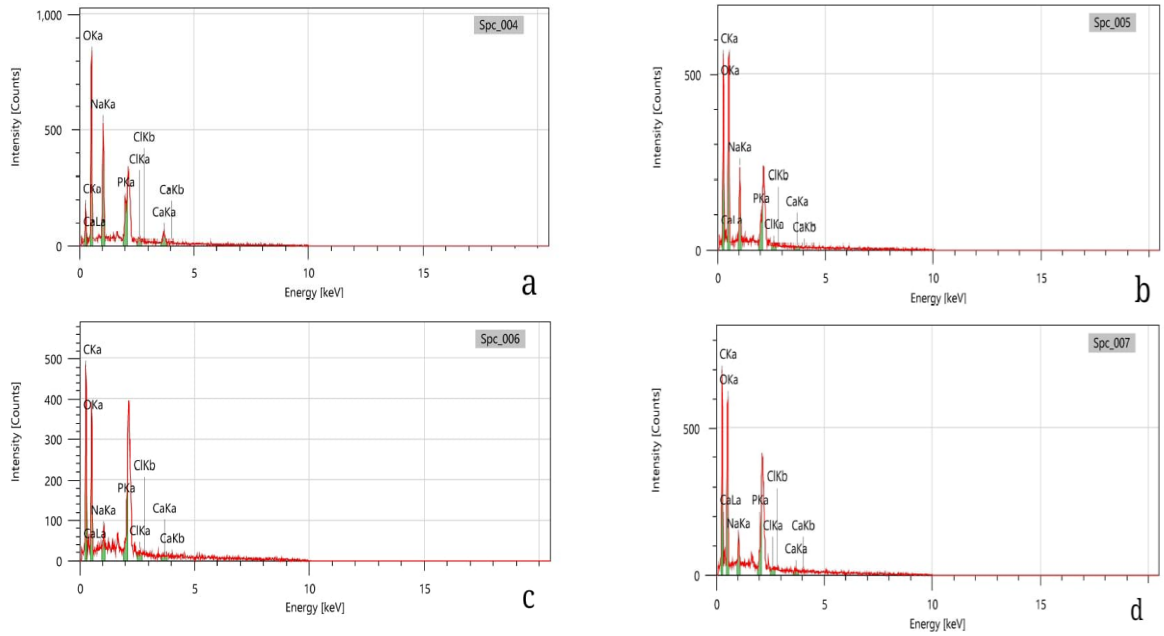

Figure 10. EDX analysis of immobilized P50 beads [(**a**) chitosan, (**b**) chitosan-enzyme, (**c**) chitosan-glutaraldehyde-enzyme, (**d**) chitosan-glutaraldehyde-enzyme used 5 times]

Table 1. Elements of Immobilized Beads in EDX Analysis. EDX analysis was conducted using the standardless ZAF quantification method.

Element	Chitosan (%)	Chitosan-enzyme (%)	Chitosan-glutaraldehyde-enzyme (%)	Chitosan-glutaraldehyde-enzyme (5x use, %)
C	21.91	53.91	59.67	56.11
Na	15.85	5.8	1.6	2.28

FTIR Analysis of Immobilized P50 Urease

FTIR analysis was conducted to identify the functional groups present in the immobilized beads. The data presented in **Figure 11** show that the IR spectra for samples A, B, and C exhibit absorbance at the same wavelengths, indicating the presence of similar functional groups. However, the IR spectra of A, B, and C display different structures, resulting in varying absorption intensities. According to Figure 11, the percentage of transmittance follows the order C < B < A. The increasing absorption intensity from sample A (chitosan) to B (chitosan-enzyme) and highest in C (chitosan-glutaraldehyde-enzyme) corresponds to the progressive addition of enzyme and glutaraldehyde, indicating stronger enzyme immobilization and enhanced structural stability that correlate with improved enzyme activity, this decrease in transmittance can also be seen in the results obtained by Taha et al (2020).

Upon examining the FTIR spectra, consistent bands were identified in A, B, and C at wavelengths of 2870-2936, 1071-1262, and 1316-1322 cm^{-1}. (Galan et al., 2021) associate the bands at 2875, 1022, and 1370 cm^{-1} with the stretching vibrations of C–H, C–O, and C–N, respectively, indicating the polysaccharide structure of chitosan. Furthermore, bands at 3424 and 1651–1654 cm^{-1} were observed in spectra A, B, and C, corresponding to the presence of –OH and –NH groups in chitosan.

The FTIR spectrum revealed distinct bands labeled A, B, and C. A notable band around 1451 cm^{-1} was observed in the glutaraldehyde-activated beads (C). In the research conducted by (Hegde & Veeranki, 2014), a band at 1495 cm^{-1} was identified, which is thought to originate from the C=N bond formed through the Schiff base reaction between the amino groups of chitosan and the aldehyde groups of glutaraldehyde. Additionally, in spectrum C, a band at 1548 cm^{-1} was detected. (Altun & Cetinus, 2007) indicated that the peak at 1574 cm^{-1} signifies the presence of C-N bonds created through crosslinking between glutaraldehyde and chitosan, the different demonstrating structural modifications associated with enzyme attachment. A decrease in transmittance at the C–N and C=N regions in sample C compared to sample B further indicates that the enzyme is bound to the glutaraldehyde crosslinked matrix. Furthermore, spectrum C displayed a band at 2876 cm^{-1}. (Galan et al., 2021) linked the band at 2875 cm^{-1} to C-H crosslinking that overlaps with the -CH_2- groups in the glutaraldehyde structure.

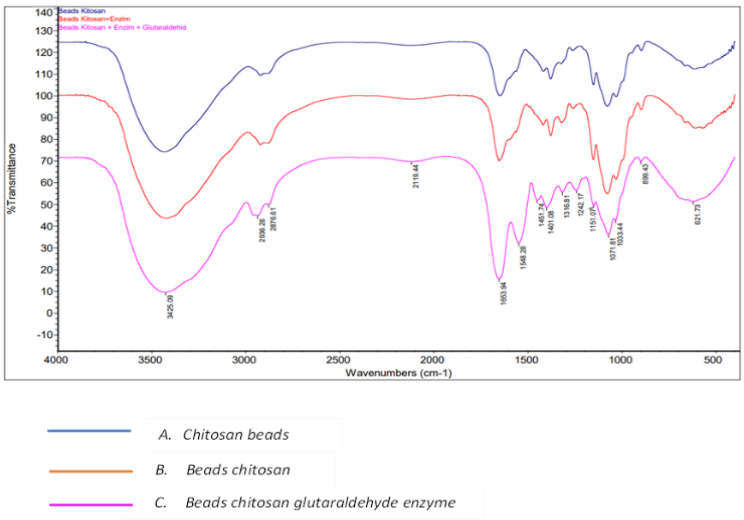

Figure 11. FTIR analysis of immobilized beads

CONCLUSIONS

Based on the research conducted, the following conclusions can be drawn: The production of chitosan beads activated with glutaraldehyde for the immobilization of urease from red lentil seeds, concentrated with 50% acetone, demonstrates optimal activity at a chitosan concentration of 0.75%. This occurs with glutaraldehyde immersion at 2% and a temperature of 25 °C for 2 hours, yielding an activity value of 7.042 U/g. The immobilized P50 urease exhibited a residual activity of 51% after seven uses. SEM-EDX analysis revealed an average pore size of 0.22 μm in the chitosan beads, characterized by a smooth, round surface texture. In contrast, the chitosan-glutaraldehyde-enzyme beads used seven times showed a pore size of 0.31 μm, with a distinctly visible round surface texture. FTIR analysis confirmed the presence of C-N and C=N peaks, indicating the incorporation of glutaraldehyde.

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