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Development of biopolymer encapsulated enzyme for efficient acetaminophen degradation

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Abstract: The study aimed to investigate the effectiveness of partially purified turnip (*Brassica rapa*) peroxidase immobilized in calcium alginate for degrading paracetamol, also known as acetaminophen (AAP), a commonly used over-the-counter analgesic and antipyretic. The encapsulation of peroxidase was optimized to minimize enzyme leakage and maintain maximum activity by adjusting the sodium alginate content, enzyme loading, and calcium chloride concentration. The optimal conditions for encapsulating peroxidase in calcium alginate matrices with the best retention and efficiency were determined to be 1.3 U/mL enzyme loading, 1.5 % sodium alginate, and 0.05 M calcium chloride concentration. This research focuses on investigating the efficacy and application of immobilized turnip peroxidase in degrading pharmaceutical effluents, specifically targeting paracetamol. The results revealed the maximum degradation of AAP at a pH of 2.0 and a temperature of 30 °C, with paracetamol and hydrogen peroxide concentrations of 1g/L and 1.2 mol/L, respectively, resulting in a 97 % yield using the stabilized peroxidase. The study also determined the kinetic characteristics of the enzymatic reaction, such as the maximum rate and the Michaelis–Menten constant. Furthermore, the stabilized enzyme can be utilized multiple times, specifically up to three occasions, in ideal conditions, while maintaining 80 % of its ability to degrade AAP.

Keywords: calcium alginate; decomposition; peroxidase, pharmaceutical effluent.

INTRODUCTION

Water, the most essential compound on our planet, has supported life for over 4.0 billion years without any other example of such abundant and persistent life conditions.[1] However, water is also the most vulnerable and readily polluted natural resource due to the popularization of synthetic chemical products, domes-

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tic and industrial effluents, inappropriate disposal of waste pesticides, agrochemicals, and especially of many therapeutic drugs, posing a major challenge for our society.[2,3] Acetaminophen (AAP), also known as paracetamol, is a widely used medication employed to treat mild pain, such as headaches, joint or muscle discomfort and to reduce fever.[4,5] Its action spectrum is identical to that of non-steroidal anti-inflammatory drugs.[6,7] Paracetamol is a safe and effective analgesic and antipyretic medication that can relieve headaches and eliminate pain without being expensive, with moderately few adverse effects when used at the recommended therapeutic dosage.[8,9]

The partial biodegradation of acetaminophen and other pharmaceutical compounds in urine lead to their presence in treated water.[4,10,11] These compounds, often referred to as active pharmaceutical ingredients (APIs), are introduced into the aquatic environment through wastewater treatment plants (WWTPs), hospital effluents and improper disposal methods.[12,13] Without adequate treatment, there is a high risk of acetaminophen contamination in waters due to its low removal efficiency in wastewater.[14,15] AAP, as an unregulated trace contaminant, can be found in aquatic environments at concentrations ranging from ng/L to μg/L. When taken in therapeutic doses, 58–68 % of the ingested AAP is excreted from the body unchanged.[16] Traditional biological treatments for water contamination do not fully eliminate AAP, with concentrations of several hundred ng/L still detected in treated effluent.[17,18] The conventional methods used to remove acetaminophen from contaminated water typically involve multiple chemical reactions or intensive energy sources, resulting in increased waste disposal costs.[19,20] As a result, there is a growing interest in exploring new methods for the removal of pharmaceutical compounds from the effluent. Bioremediation using enzymes stands out as one of the most secure and sustainable approaches to decontaminating polluted environments.[21] The peroxidase family of enzymes is a subject of extensive research and is considered essential for life in aerobic conditions.[22,23] Peroxidase, an oxidoreductase enzyme, possesses the unique ability to break down hydrogen peroxides, which is the basis for its name.[15] These enzymes can be found in a wide variety of organisms, including plants, bacteria and humans, in various isoforms.[24,25] The most well-known plant enzymes used for paracetamol degradation, include horseradish peroxidase (HRP), a well-studied plant peroxidase capable of oxidizing paracetamol *via* hydrogen peroxide-dependent radical mechanisms; its use has been explored in cancer enzyme prodrug therapy and environmental applications.[26,27] Laccase enzymes, which have also been demonstrated to transform acetaminophen via oxidative coupling leading to polymerized, biologically inactive products.[28]

Chemical modification has been found to enhance TP's activity, improving its stability and catalytic performance, which is beneficial for wastewater treatment involving pharmaceuticals.[29]

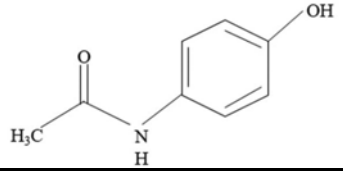
Enzymes are well known for their highly specific catalytic activity, typically at mild reaction conditions, but are highly susceptible to heat and denaturation, resulting in the loss of their catalytic activity.[30] Enzyme immobilization is a process where the enzyme is attached to a surface and trapped in suitable matrices, such as inorganic materials, organic polymers and hydrogels, to enhance their stability, reusability and selectivity.[31,32] Various methods of enzyme immobilization in biopolymers, such as hydrogel beads, have been utilized to encapsulate enzymes in their porous structure.[33] Sodium alginate crosslinking by calcium ions has the properties to form semi-permeable beads with suitable mechanical and chemical stability, as well as good compatibility with enzyme proteins, making it widely used for immobilizing different enzymes.[32] The focus of this study was to investigate the removal of AAP using immobilized turnip peroxidase entrapped in calcium alginate (ITP–Ca-AG) beads. The initial phase involved optimizing the immobilization parameters, including determining the appropriate dose of calcium alginate, the concentration of calcium chloride and the optimal concentration of peroxidase for effective immobilization. Once the optimal conditions for immobilization were established, the study proceeded to examine the effects of various parameters on the removal efficiency, such as pH, temperature, contact time, mass of beads and substrate concentrations of hydrogen peroxide and acetaminophen (AAP).

EXPERIMENTAL

Chemicals

Crude peroxidase was extracted from fresh vegetables of turnip (*Brassica rapa*) collected from local market. Acetone, Sephadex gel G-100, hydrogen peroxide aqueous solution (30 mass %), phenol and 4-amino-antipyrine were of analytical grade and were obtained from Sigma–Aldrich. Paracetamol, also known as acetaminophen, was obtained from Merck. Detailed properties of AAP along with the structure are presented in Table I.

TABLE I. Characteristics of acetaminophen[9]

Name of the drug	Acetaminophen
Chemical name	*N*-Acetyl-*p*-aminophenol
f	$C_8H_9NO_2$
Molecular weight	151.16 g/mol
IUPAC name	*N*-(4-Hydroxyphenyl)acetamide
Stability	Dry, pure paracetamol is stable up to 45 °C
Structure	

Isolation and purification of turnip peroxidase (TP)

The process of separating and purifying TP using acetone at low temperature of 4 °C has been thoroughly explained in prior research.[34,35] Gel filtration serve as the final stage in the purification of proteins, requiring a relatively pristine preparation with a small volume for optimal effectiveness. The stationary phase is composed of Sephadex G-100 gel, which is poured into a column measuring 25 cm×1cm in dimensions. The mobile phase consists of semi-purified TP dissolved in a pH 6.0 buffer solution, which is passed through a 1 cm×60 cm column at a slow flow rate of 15.75 mL/h. The collected solutions were chosen to be in fractions of 1 ml, diluted in 2 ml of the pH 6.0 buffer solution.

Storage stability of TP

Free and partial purified TP were incubated in phosphate buffer at 4 °C (50 mM, pH 6.0). The stability of the peroxidase in both cases, crude and purified, was assessed by measuring the peroxidase activity at regular time intervals every 5 days for a period exceeding three months.

Preparation of alginate beads and optimization of immobilization conditions

The enzyme was purified and then immobilized in sodium alginate using a method similar to one described in a previous study.[35] To achieve this, a volume of semi-purified TP solution was thoroughly mixed with a mass of sodium alginate under continuous agitation until a homogeneous mixture was obtained. Subsequently, this mixture was dispensed dropwise through a 23G syringe needles into a solution of $CaCl_2$ under agitation, resulting in the instantaneous formation of calcium alginate beads with a diameter of approximately 2.8–2.9 mm. These beads were then agitated for about 2 h to harden and stabilize the enzyme within them. Finally, the beads underwent a washing process with distilled water and were stored at 4 °C in a pH 6.0 buffer solution until further use. It is crucial to optimize the parameters related to the support and the enzyme to achieve a higher enzyme retention rate and improved activity. This optimization is necessary to reduce enzyme leakage and overcome diffusional limitations, thereby enhancing process efficiency. The immobilization conditions to be optimized include the concentration of sodium alginate (AG-Na, ranging from 1 to 2.5 %), enzyme loading concentration (measured in UI/ml), and the concentration of the gelating agent $CaCl_2$ (ranging from 0.05 to 0.2 M). The efficiency of immobilization and factor efficiency were calculated using Eqs. (1) and (2), while the activity of free, immobilized, and filtrate TP was measured using the phenol-4 aminoantipyrine method:[36]

$$IE(\%) = 100\frac{A_0V_0 - A_fV_f}{A_0V_0} \quad (1)$$

$$\eta(\%) = 100\frac{\text{Total activity of beads (U)}}{\text{Total free TP activity used for beads production (U)}} \quad (2)$$

where A_0 and A_f are activities of free and filtrate TP, respectively; V_0 and V_f are the volumes of TP of free and filtrate solution respectively.

Enzyme and protein assay

The activity of free and entrapped peroxidase in aqueous solution was determined spectrophotometrically at 517 nm using the 4-aminoantipyrene method,[36] with phenol and hydrogen peroxide as substrates and 4-aminoantipyrene as the chromogen.[37] Protein concentration was determined by the Bradford method,[38] with absorbance measured at 280 nm, and bovine serum albumin was used to generate the standard calibration curve.

Removal of acetaminophen

Removal of acetaminophen from the aqueous phase by immobilized TP was studied using the one-factor-at-a-time (OFAT) experimental method as shown in Table II and the optimal value was selected for the following experiment. Experiments were conducted in a closed tube under constant temperature 20 °C. The reaction mixtures contained 1 mL of hydrogen peroxide 0.1 M, 1 mL of AAP 0.5 g/L, 8 mL of buffer solution and a mass of 0.3 g of alginate beads. Mixtures were kept for a fixed duration for 1h without agitation and the treated solution was centrifuged at 4000 rpm for 5 min to separate the precipitate. Details are presented in Table II.

TABLE II. Ranges of experimental parameters

Parameter	Range
pH	2–10
Temperature, °C	20–80
$[H_2O_2]$ / M	0.1–1.2
[AAP] / g L^{-1}	0.1–1
m_{beads} / g	0.3–1.5
Contact time, min	0–80

The residual drug concentration was measured spectrophotometrically at 288 nm. Optimum values were those that resulted in the highest degradation yield. Control (blank) experiments were conducted without immobilized TP to assess abiotic (nonenzymatic) degradation *via* photocatalytic or chemical processes. The decomposition yield for acetaminophen was calculated, with the percent degradation parameter defined as:

$$\text{Decomposition yield}(\%) = 100\frac{A_0 - A_t}{A_0} \tag{3}$$

where A_0 and A_t represent the absorbance before and after enzyme treatment, respectively.

Reusability of immobilized TP

The reusability of immobilized TP was investigated by conducting multiple experiments using the same beads for the removal of drug AAP under consistent optimal conditions. The study involved performing consecutive experiments over five batches, with the beads containing immobilized TP being separated from the mixture, rinsed with distilled water, and reused in a fresh degradation medium after each reaction. To monitor the drug biotransformation, absorbance at λ_{max} (288 nm) was measured using UV–Vis spectroscopy at the conclusion of each batch. All enzymatic degradation experiments were conducted in triplicate to ensure the reliability of the results.

RESULTS AND DISCUSSION

Extraction, purification and stability test of TP

Purification of peroxidase is necessary to maintain its maximum catalytic power by removing protein and non-protein impurities. The test results for both the crude and purified extract are summarized in Table III.

Sephadex G-100 gel filtration was used to achieve purification by separating molecules based on their size and shape. The elution profile of the chromatogram

(Fig. 1) demonstrates a complete separation of the compounds, with larger molecules being eluted first. The larger molecules with higher peroxidase activity are eluted between fractions 15 and 20, as indicated by the curve of enzymatic activity variation. The second peak in the elution profile represents proteins that do not possess peroxidase activity.

TABLE III. Characteristics of enzymatic solutions before and after purification

Purification step	Total protein, mg	Total activity, U	Specific activity, U/mg	Purification factor	Activity yield %
Crude enzyme	46.6	2.474	0.053	–	100
Purification with acetone	17.5	1.557	0.089	1.689	63
Sephadex gel G-100 purification	0.045	0.8	17.84	336.60	32

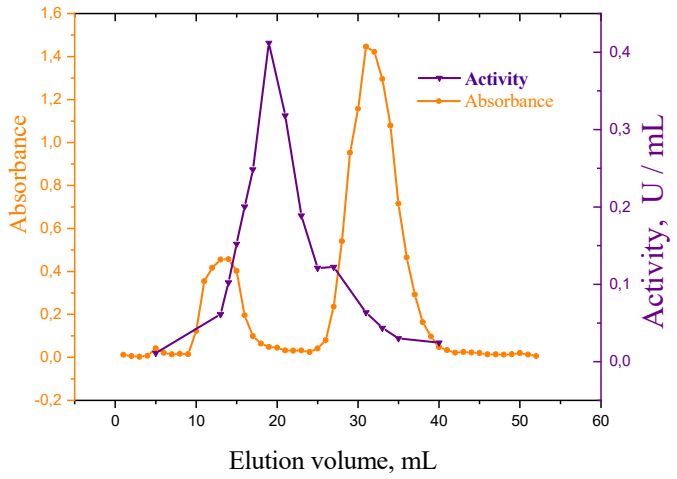


Fig. 1. Elution profile of TP on a Sephadex G-100 resins.

Table III presents the purification process of peroxidase extracted from turnip (*Brassica rapa*), which can be analyzed and explained based on the results provided. A decrease in purification yield indicates the loss of enzyme activity during the purification process, as observed when the purification yield decreases to 63 % with acetone purification and further to 32 % with Sephadex gel filtration. This suggests that significant enzyme activity is lost due to incomplete recovery or enzyme denaturation during these purification steps. The purification factor measures the concentration and purification of the enzyme in each step, with an increase indicating successful concentration and purification. When purified with acetone, the purification factor increases to 1.7, suggesting some initial concentration but not significant purification. In contrast, the purification factor dramatically increases to 336 with Sephadex gel filtration, indicating successful

concentration and purification of the peroxidase enzyme into a smaller volume. In summary, acetone precipitation concentrates the enzyme to some extent with moderate purification, while Sephadex gel filtration greatly concentrates and purifies the peroxidase enzyme, despite a lower final yield. When peroxidase from *Peganum harmala* seeds is purified using gel filtration, a purification fold of 4.4 and an enzyme recovery of 15 % are achieved.[39] On the other hand, the purification of peroxidase from the leaf of oil palm (*Elaeis guineensis*) using the same gel filtration method results in an overall purification fold of 4 with 51.9 % enzyme recovery.[40]

Storage stability of crude and semi-purified TP

Acetone precipitation is a method used to concentrate and stabilize enzymes during the precipitation process. The activity of the crude turnip peroxidase (CTP) can be observed to decrease over time, as shown in Fig. 2. After two weeks, the activity begins to drop and after one month it decreases significantly from 2.3 to 0.4 U/mL. This indicates that the crude enzyme is not stable over time and loses most of its activity within a month. In contrast, the semi-purified enzyme exhibits much better stability, with its activity remaining constant at 1.2 U/mL for three months. This suggests that the purification process with acetone has significantly improved the enzyme's stability. Despite the crude enzyme's higher initial activity, it is less stable and loses most of its activity within a month. On the other hand, the semi-purified enzyme, starting with a lower initial activity of 1.2 U/mL, remains stable and retains its activity for at least three months.

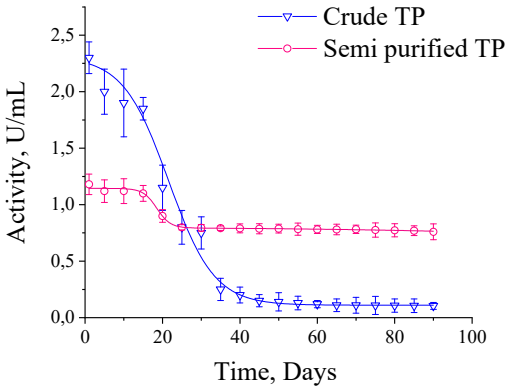


Fig. 2. Storage stability of crude and semi purified TP.

Optimization of immobilization parameters for paracetamol degradation

Alginate is a commonly used biopolymer for immobilization by inclusion due to its gelling properties, non-toxicity, biodegradability, ease of use and cost--effectiveness. The efficiency of immobilization increases with the concentration of the support, specifically with the mass of alginate, as shown in Table IV. The

presence of more gel in the sodium alginate/peroxidase mixture increases viscosity and leads to the formation of firmer beads, enhancing enzyme retention and reducing leakage into the surrounding medium. The optimum immobilization efficiency, reaching 76.59 %, is achieved at an AG-Na concentration of 1.5 %. The concentration of the enzyme to be immobilized significantly impacts enzyme retention and activity, with higher concentrations resulting in better retention but decreased enzymatic activity due to steric hindrance. An enzyme concentration of 1.3 U/mL is found to be optimal for good enzyme retention and maximal efficiency factor, as reported in Table IV. Calcium chloride serves as a gelling agent, and Table IV illustrates that the highest immobilization and efficiency factor of the ITP–Ca-AG are achieved at a concentration of 0.05 M $CaCl_2$. Using 1.5 % sodium alginate, an enzyme loading concentration of 1.3 U/mL, and a calcium chloride concentration of 0.05 M are the optimal conditions for entrapping peroxidase within calcium alginate beads, achieving the highest efficiency and retention rate. In contrast to the study of Gao *et al*., employing a higher sodium alginate and calcium chloride concentration of 2 % and 0.3 M, respectively, is aimed at reinforcing bead rigidity while maintaining diffusion efficiency.[41]

TABLE IV. Immobilization and factor efficiency (*IE*, η) of alginate beads obtained at different gelation conditions

Essay 1	Sodium alginate concentration, %; [$CaCl_2$] = 0.1 M; [E] = 0.7 U/mL			
	1	1.5	2	2.5
IE / %	71.94	76.59	76.01	83.32
η / %	26.27	45.73	37.96	32.00
Essay 2	Enzyme loading, U/mL; [AG-Na] = 1.5 %; [$CaCl_2$] = 0.05 M			
	0.7	1.0	1.3	1.8
IE / %	77.82	60.08	63.45	54.00
η / %	13.98	30.10	40.82	41.94
Essay 3	Chloride calcium dose, M; [AG-Na] = 1.5 %; [E] = 1.3 U/mL			
	0.05	0.1	0.15	0.2
IE / %	52.30	35.78	50.01	46.23
η / %	30.86	28.39	27.90	27.40

Morphology of ITP–Ca-AG beads

Alginate beads were observed using scanning electron microscopy (SEM). In Fig. 3, the distribution of peroxidase on the outer layer of the alginate bead can be seen, where the enzyme appears as multiple white dots. This can be confirmed after enlarging the image 160 times, where the presence of the enzyme is clear in the bead containing peroxidase, as illustrated in Fig. 3, compared to the empty bead.

Effect of pH

Fig. 4A illustrates the impact of pH on ITP–Ca-AG activity. The study investigates the degradation rates of AAP at various pH levels to identify the

most favorable conditions for maximum degradation efficiency. The optimal pH for this biodegradation process is determined to be 2, resulting in a 24 % biodegradation yield. Under neutral and basic pH conditions, the biodegradation efficiency is observed to be comparatively low. The immobilization of the enzyme in calcium alginate serves to stabilize it and potentially improve its catalytic performance, particularly in extreme pH environments.

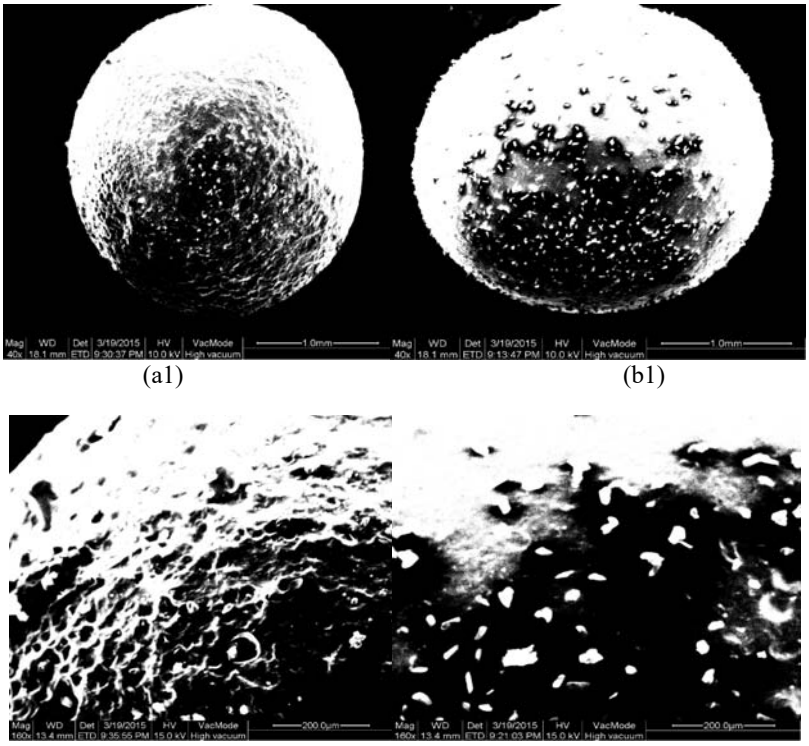


Fig. 3. SEM photographies of the outer layer of calcium alginate beads: a) without enzyme, b) with enzyme at different magnification (1, 40×, 2, 160×).

Effect of temperature

When peroxidase is immobilized in calcium alginate, its thermal stability is enhanced. The alginate matrix offers some protection against temperature-induced denaturation of the enzyme. The degradation of AAP by ITP–Ca-AG is affected by temperature, with a temperature range of 30 °C being optimal for maximum enzyme activity, as illustrated in Fig. 4B, both excessively high and low temperatures can have negative effects on the process, either by denaturing the enzyme or by slowing down the reaction rate. At temperatures beyond the optimal range (T_{opt}), the decomposition efficiency decreases to 23 % at 50 °C

and 20 % at 70 °C. For temperatures lower than the optimal range, the efficiency decreases by 17 %.

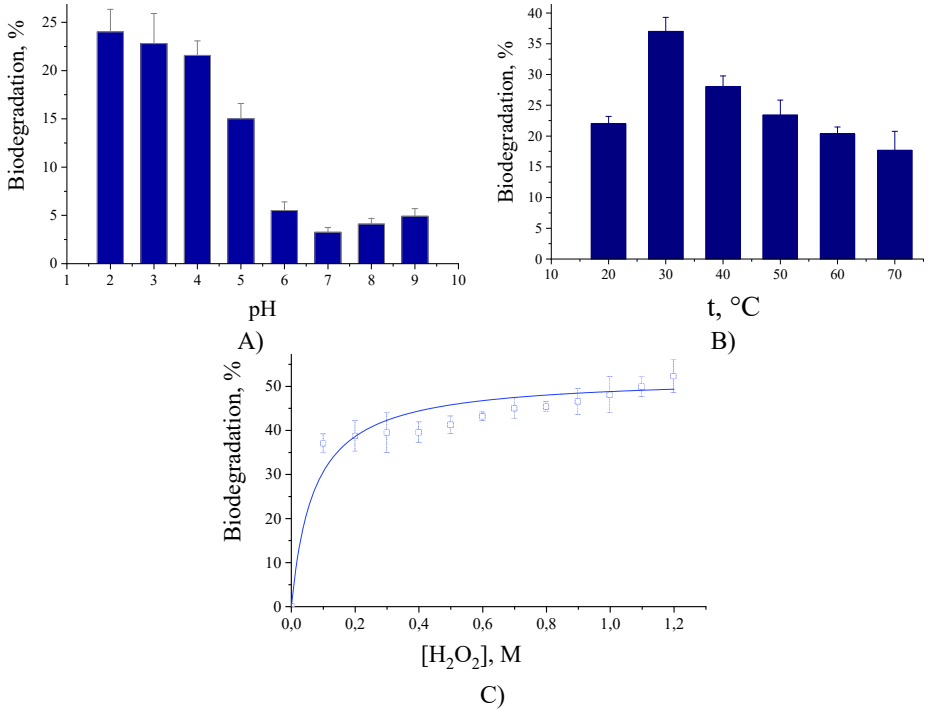


Fig. 4. Effect of: A) pH, B) temperature and C) H_2O_2 concentration on the degradation of AAP by ITP–Ca-AG.

Effect of hydrogen peroxide concentration

Fig. 4C demonstrates the impact of hydrogen peroxide on the degradation of AAP, revealing a notable increase in efficiency. With the increase in H_2O_2 concentration from 0.1 to 1.2 M, there is a substantial enhancement in the degradation yield, rising from 38 to 58 %. In the reaction, hydrogen peroxide functions as an electron acceptor, while peroxidase enzymes play a role in facilitating the transformation of AAP molecules into less harmful substances. Turnip peroxidase isoenzymes have demonstrated high thermostability and resistance to hydrogen peroxide inactivation compared to horseradish peroxidase, suggesting advantages in oxidative applications.[42] TP has also shown a great potential in degrading phenols, volatile organic compounds (VOCs) and dyes.[34,35,43]

Effect of AAP concentration

Fig. 5A illustrates that as the concentration of AAP increases, both the degradation and removal rate of AAP also increase. When the paracetamol dose was

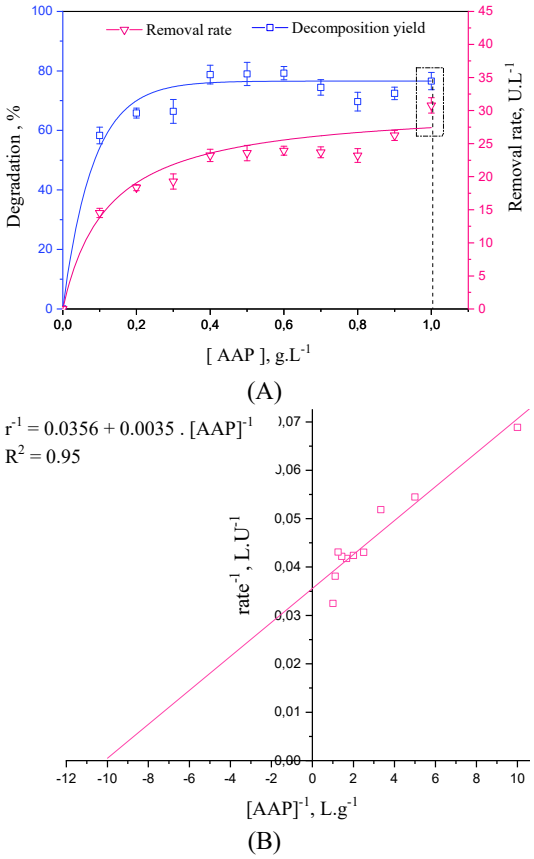


Fig. 5. Effect of: A) AAP concentration on the degradation yield and rate with ITP–Ca-AG and B) their double reciprocal plot or Lineweaver–Burk plot for the substrate paracetamol.

varied from 0.1 to 0.4 g/L, the decomposition yield, which represents the efficiency of the breakdown process, increased by 20 %. This indicates that the catalytic efficiency of turnip peroxidase increases when it is immobilized at higher concentrations of AAP, likely due to the enzyme having more substrate (AAP) available to interact with, thus increasing its catalytic activity. The results demonstrate that the maximum decomposition yield and removal rates were achieved for AAP concentrations as high as 1 g/L, suggesting that at this concentration, the enzyme operates at its peak efficiency. In Fig. 5B, the linearization of the Lineweaver–Burk plot, or the double reciprocal representation of velocity and AAP concentration, yields a straight line with a maximum velocity (r_{max}) of 28.1 U/L and a Michaelis–Menten constant (K_m) of 0.1 g/L.

Effect of beads quantity

From Fig. 6A, it was observed that the removal of AAP increased from 84 to 97 % as the mass of immobilized enzyme beads increased from 0.3 to 0.8 g. This indicates a positive correlation between the quantity of enzyme beads and the

efficiency of paracetamol degradation. At lower masses (0.3 g), the reaction is less efficient due to the limited availability of enzyme molecules to bind with paracetamol. Increasing the mass of enzyme beads is likely to increase the surface area available for the reaction, allowing more paracetamol molecules to interact with the enzyme simultaneously, resulting in higher degradation of AAP.

Effect of contact time under optimal conditions

Fig. 6B illustrates the impact of contact time on the decomposition of paracetamol under optimal conditions. In environmental settings, optimizing contact time is crucial for maximizing the efficiency of paracetamol decomposition processes. The kinetics of degradation yield by ITP–Ca-AG under optimal conditions demonstrates a gradual increase in paracetamol decomposition over time. After 10 min, 26 % of the drug is decomposed, and the yield reaches 97 % after 1 h. The initial slower rate of decomposition followed by accelerated degradation suggests that the enzymatic reaction proceeds more efficiently over longer periods, leading to a higher overall yield of paracetamol degradation.

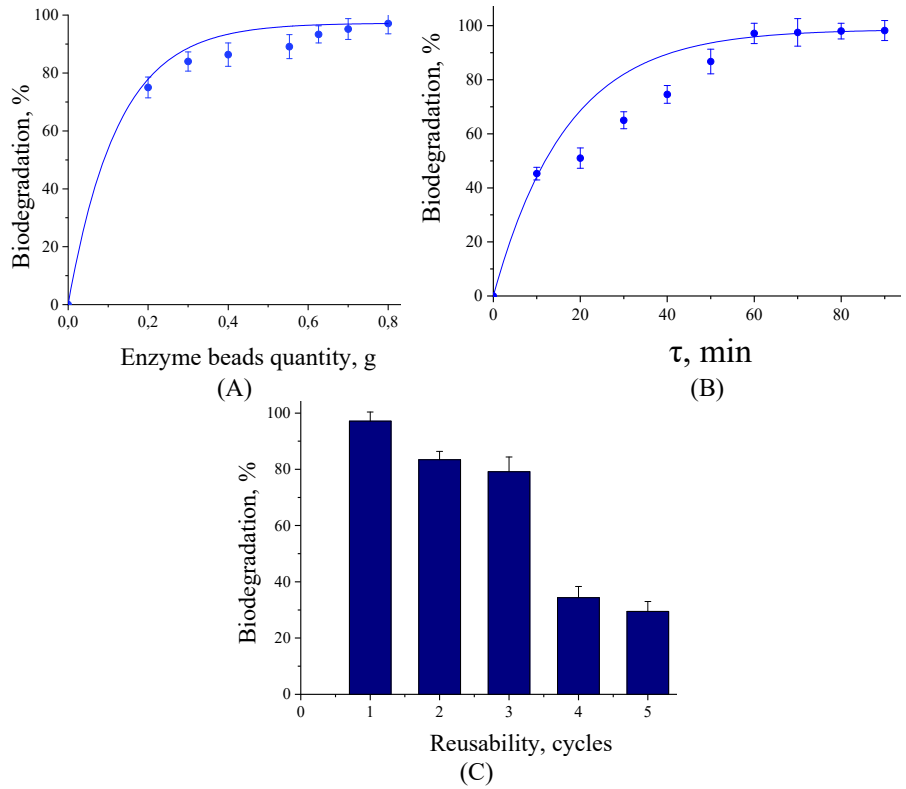


Fig. 6. AAP removal; A) effect of ITP–Ca-AG beads quantity, B) under optimal conditions and C) reuse cycles of immobilized enzyme.

Reuse of immobilized enzyme

When peroxidase enzyme is immobilized in calcium alginate, it is protected from denaturation and degradation, leading to a prolonged activity. The immobilized enzyme can be easily separated from the reaction mixture and reused, resulting in cost-effectiveness and reduced waste. In a study illustrated in Fig. 6C, it was observed that the activity of ITP–Ca-AG remained stable, with the degradation of paracetamol being maintained above 80 % up to the third batch, showing only a 20 % decrease compared to the first batch. Based on the results, it can be inferred that the immobilization process is effective, enabling the practical reuse of peroxidase for the degradation of pharmaceutical effluents. In the same study, paracetamol removal was achieved by combining the biocatalytic activity of horseradish peroxidase (HRP) with the adsorption capacity of a thermally cross-linked nanofibrous membrane, while the immobilized HRP showed a similar removal rate to the free enzyme (83.5 *vs.* 84.4 %).[27] Alternatively; in a separate work, paracetamol remained undegraded at pH 5 when using sol–gel encapsulated LiP- and HRP-based nanocomposites.[44] Whereas photocatalytic oxidation achieved 84.34 % degradation in over 1.5 h.[45] However, ITP–Ca-AG was able to degrade AAP to 99 % in less than 1 h. The study also included a comparison with previous research on the degradation of acetaminophen using various methods. It was noted that while photocatalytic oxidation, UV irradiation, and microbiological approaches can degrade AAP, they generally require a much longer time compared to enzymatic methods. The ITP–Ca-AG employed in the study demonstrated a significantly faster and more efficient degradation of AAP compared to the reported physical and microbiological methods. For instance, the yield of biological treatment of AAP by *Pseudomonas PrS10* strain isolated from pharmaceutical industrial effluents could reach 96.37 % within 7 days.[7] While photocatalytic oxidation gave 84.34 % degradation within 1.5 h,[45] whereas 97 % was obtained by ITP–Ca-AG after 60 min.

Despite its known catalytic properties, the application of turnip peroxidase (TP) in paracetamol degradation is less explored compared to HRP, presenting potential novelty due to its stability and efficiency in harsh conditions such as wastewater treatment. Turnip peroxidase (TP) has emerged as a promising biocatalyst for environmental remediation due to its oxidative capabilities and eco-friendly origin. Derived from a low-cost and readily available plant source, TP is an inexpensive and abundant enzymatic tool that can be readily exploited for the degradation of emerging pollutants, including pharmaceuticals, dyes and phenolic compounds. Its application in wastewater treatment offers a sustainable alternative to conventional chemical methods, particularly when immobilized or used in combination with hydrogen peroxide under mild conditions. The broad substrate specificity of TP, along with its potential for large-scale extraction from

agro-industrial waste, positions it as a viable candidate for green remediation technologies.

CONCLUSION

In conclusion, the breakdown of paracetamol utilizing partially purified turnip (*Brassica rapa*) peroxidase immobilized in calcium alginate beads under optimized conditions (1.3 U/mL enzyme loading, 1.5 % sodium alginate, and 0.05 M calcium chloride) with a maximum degradation yield of 97 % at pH 2.0 and 30 °C was achieved. The immobilized enzyme exhibited favorable kinetic parameters and maintained 80 % of its activity after three reuse cycles, indicating good stability and reusability. The findings suggest that the enzymatic mechanism was successful in breaking down pharmaceutical substances. While organic material was present at the end of the treatment, it was in a non-toxic and insoluble form. Future research could focus on scaling up the process, exploring degradation of other pharmaceutical contaminants and improving enzyme immobilization techniques to enhance stability and operational lifespan.

ИЗВОД

РАЗВОЈ ЕНЗИМА ИНКАПСУЛИРАНОГ У БИОПОЛИМЕРУ ЗА ЕФИКАСНУ РАЗГРАДЊУ АЦЕТАМИНОФЕНА

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У овој студији је испитивана ефикасност делимично пречишћене пероксидазе из репе (*Brassica rapa*) имобилизоване у калцијум алгинату, за разлагање парацетамола, познатог и као ацетаминофен (ААР), често коришћеног аналгетика и антипиретика који се набавља без рецепта. Инкапсулација пероксидазе је оптимизована у циљу минимизирања цурења ензима и задржавања максималне активности, подешавањем концентрације калцијум-алгината, полазне количине ензима и концентрације калцијум-хлорида. Оптимални услови инкапсулирања пероксидазе у калцијум-алгинатној матрици, са најповољнијим задржавањем и активношћу ензима су били: 1,3 U/mL почетна активност ензима, 1,5 % натријум-алгината и 0,05 M калцијум-хлорида. Фокус овог истраживања је био на испитивању ефикасности и могућности примене имобилизоване пероксидазе из репе у разлагању фармацеутских остатака, специфично циљајући парацетамол. Резултати су показали да је максимална разградња ААР на pH 2,0 и на температури од 30 °C, при концентрацији парацетамола и водоник пероксида од 1 g/L, односно 1,2 mol/L, дајући принос од 97 %. У студији су, такође, одређени кинетички параметри ензимске реакције, као што су максимална брзина и Микаелис–Ментенова (*Michaelis–Menten*) константа. Стабилизован ензим се може користити више пута, минимум три пута, задржавајући, у идеалним условима, 80 % своје способности да разгради ААР.

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