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Enhanced kinetic performance and stability of catalase immobilized on epoxy-functionalized kaolinite

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The immobilization of catalase onto stable, reusable supports is crucial for efficient peroxide-based biocatalytic applications. In this study, catalase was immobilized for the first time onto epoxy-functionalized kaolinite particles prepared via surface silanization with (3-glycidyloxypropyl) trimethoxysilane. Structural and surface characterizations confirmed successful organosilane grafting while preserving the layered kaolinite framework. The modified support exhibited rapid enzyme uptake and a high immobilization capacity of approximately 300 mg g⁻¹. Kinetic analysis showed a substantial decrease in K_m from 57.3 mM (free catalase) to 21.6 mM after immobilization, indicating enhanced substrate affinity. In contrast, V_{max} decreased due to diffusion limitations typical of heterogeneous systems. Despite this, catalytic efficiency increased nearly 1.8-fold. Moreover, immobilized catalase demonstrated significantly improved operational reusability and long-term storage stability compared to the free enzyme. These results highlight silanized kaolinite as a robust, low-cost, and efficient mineral-based support for catalase immobilization, with strong potential for environmental and industrial biocatalytic applications.

Keywords Catalase, Immobilization, Kaolinite, Kinetics, Silanization, Stability

The increasing release of industrial effluents containing reactive oxygen species and peroxide-generating contaminants has intensified the demand for robust biocatalytic systems capable of sustaining high catalytic performance under harsh environmental conditions^{1–3}. Catalase (EC 1.11.1.6), a heme-containing oxidoreductase, is one of the most efficient antioxidant enzymes, catalyzing the disproportionation of hydrogen peroxide into water and oxygen at exceptionally high turnover rates^{4,5}. Its broad utility in wastewater treatment, food preservation, biosensing, textile processing, and pharmaceutical applications underscores the need for durable immobilization strategies to overcome the inherent instability, difficult recovery, and limited reusability of free enzymes⁶.

Mineral-based supports, particularly clay minerals, have emerged as promising candidates for enzyme immobilization due to their low cost, environmental compatibility, structural rigidity, and tunable surface chemistry^{7,8}. Among them, kaolinite, a naturally abundant 1:1 aluminosilicate, offers attractive features, including mechanical robustness, chemical stability, and surface hydroxyl groups suitable for chemical modification⁹. Yet, the native kaolinite surface is relatively inert toward biomacromolecules, often resulting in weak adsorption and poor operational stability. To address this limitation, surface silanization with organosilane coupling agents provides a powerful approach to introduce reactive functional groups, such as amines or epoxides, that can promote stronger enzyme–support interactions^{10–12}.

Although kaolinite has been previously investigated for simple physical adsorption of catalase, such systems typically lack strong interfacial bonding and exhibit limited long-term stability¹³. To the best of our knowledge, no study has reported the immobilization of catalase on organosilane-functionalized (silanized) kaolinite particles. This unexplored interface presents a unique opportunity to combine the chemical tunability of organosilanes with the structural advantages of kaolinite, potentially yielding a high-performance immobilized catalase

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system with improved stability and reusability^{8,12,14}. Unlike previously reported kaolinite-based immobilization systems that predominantly rely on physical adsorption or weak electrostatic interactions, the present work establishes epoxy-functionalized kaolinite as a chemically engineered enzyme carrier. Surface silanization with (3-glycidyloxypropyl)trimethoxysilane introduces reactive epoxy groups that enable strong covalent or quasi-covalent bonding with nucleophilic amino acid residues of catalase. This modification fundamentally alters the mechanism of enzyme–support interaction. As a result, controlled enzyme anchoring is achieved, enzyme leaching is minimized, and stabilization of the active conformation is promoted. To the best of our knowledge, the use of epoxy-functionalized kaolinite for catalase immobilization has not been previously reported. It therefore represents a significant advancement over conventional clay-based immobilization platforms.

In this study, silanized kaolinite particles are presented as a novel and robust support for catalase immobilization. The organosilane-modified surface was deliberately engineered to strengthen enzyme attachment through covalent and strong physicochemical interactions, as schematically illustrated in Fig. 1. The immobilized catalase system was comprehensively investigated in terms of immobilization efficiency, enzyme loading behavior, kinetic performance, pH and temperature-dependent activity profiles, operational reusability, and long-term storage stability. By integrating clay mineral stability with tailored surface chemistry, this work

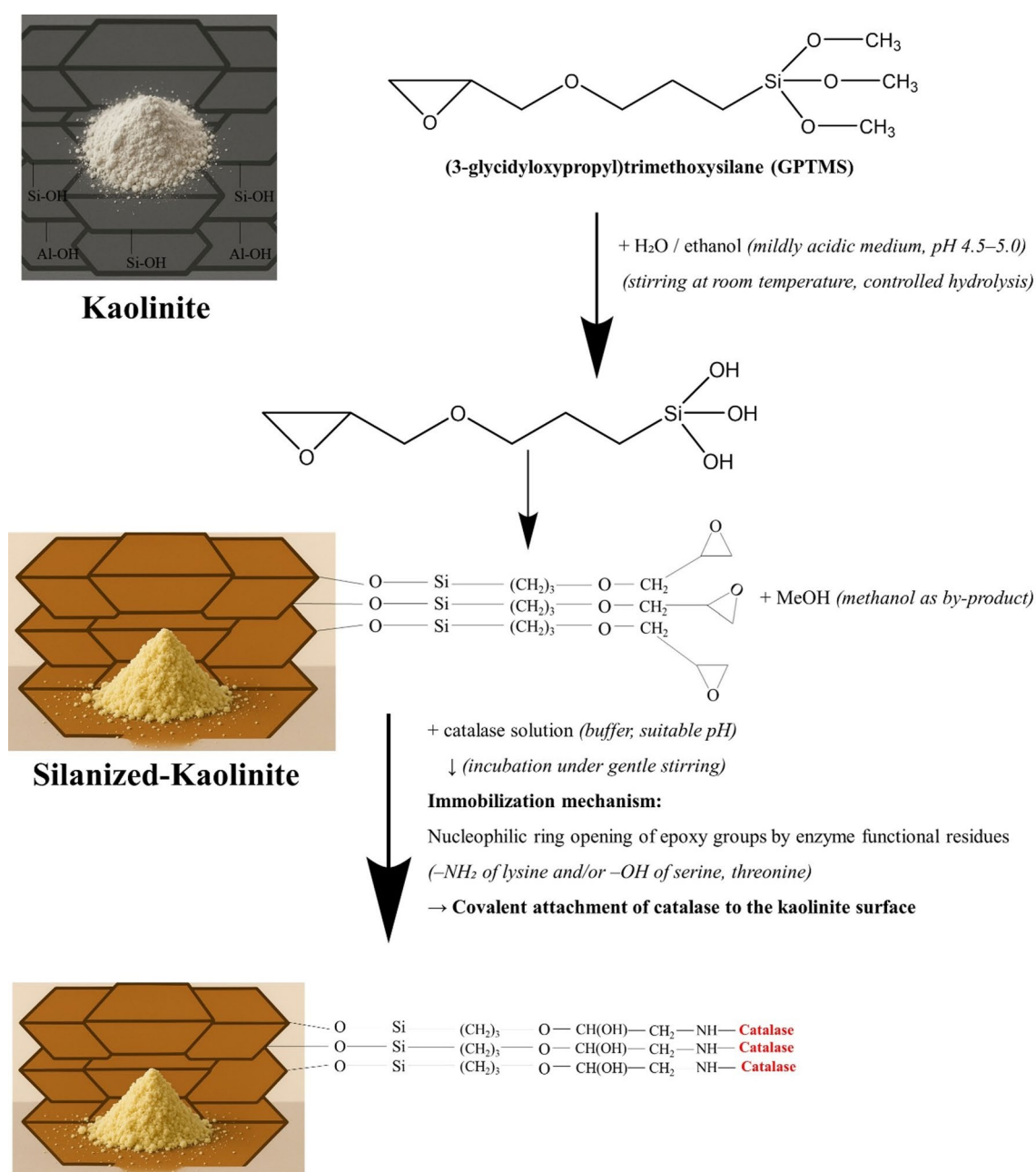


Fig. 1. Schematic illustration of kaolinite silanization with GPTMS and subsequent covalent immobilization of catalase via epoxy ring-opening reactions.

expands the applicability of functionalized kaolinite in biocatalysis and addresses a critical gap in current enzyme immobilization research.

Materials and methods

Reagents

Kaolinite clay mineral (analytical grade) was obtained from a certified supplier and used without further purification, except for washing and activation. Catalase from bovine liver, hydrogen peroxide (35%), (3-Glycidyloxypropyl)trimethoxysilane (GPTMS, $\geq 98\%$), ethanol ($\geq 99.5\%$), and all buffer components were purchased from Sigma-Aldrich (USA). All chemical reagents were used as received and were of analytical or biochemical grade. Ultrapure deionized water (18.2 M Ω cm) was used throughout all experiments to minimize contamination during silanization and enzyme immobilization.

Pre-treatment of kaolinite

Raw kaolinite powder was initially washed with distilled water to remove soluble impurities and surface contaminants. The suspension was stirred thoroughly, allowed to settle, and the supernatant was discarded. The washed kaolinite was then dried at 70 °C overnight and gently ground to obtain a homogeneous powder. This pre-treatment step ensured the availability of accessible surface hydroxyl groups (–Si–OH and –Al–OH) required for subsequent silanization reactions^{14,15}. If necessary, the kaolinite was sieved or lightly calcined to enhance surface activation without altering its structural integrity.

Hydrolysis of GPTMS

The organosilane precursor, GPTMS, was pre-hydrolyzed prior to surface grafting to generate reactive silanol species suitable for kaolinite modification¹⁶. A predefined amount of GPTMS was added to an ethanol–water mixture and hydrolyzed under mildly acidic conditions (pH 4.5–5.0) to ensure controlled hydrolysis while suppressing premature siloxane condensation. The reaction mixture was stirred at room temperature for 30–45 min, during which the methoxy groups were progressively converted into silanol functionalities (Si–OH).

Ethanol present in the reaction medium, together with the alcohol generated during hydrolysis, was not removed, as it helped maintain a homogeneous reaction environment and reduced the rate of uncontrolled Si–O–Si network formation. This controlled pre-hydrolysis step ensured the availability of reactive silanol groups while preserving the integrity of the glycidyl (epoxy) moiety, which is essential for subsequent surface grafting and functional interactions. The resulting hydrolyzed GPTMS solution was used immediately in the kaolinite surface modification step.

Silanization of kaolinite surface

Pre-treated kaolinite powder was dispersed into the hydrolyzed GPTMS solution and stirred using a reflux condenser or temperature-controlled bath (70 °C). During this step, silanol groups condensed with surface –OH groups of kaolinite, forming covalent Kaolinite–O–Si–R linkages. Concurrently, silanol–silanol condensation generated a thin siloxane (Si–O–Si) network around the particles, improving coating stability. Following the reaction, the silanized kaolinite was separated by centrifugation or filtration, thoroughly washed with ethanol and deionized water to remove unreacted silane, and dried at 60 °C. The resulting particles contained surface-bound epoxy functional groups originating from the glycidyloxypropyl moiety¹⁷.

The dried silanized kaolinite was gently powdered and stored in an airtight container until enzyme immobilization. Successful silan modification introduced pendant epoxy groups capable of undergoing nucleophilic ring-opening reactions. These reactive sites served as anchoring points for catalase molecules during immobilization.

Determination of catalase activity for free and immobilized enzyme forms

The catalytic activity of both free and immobilized catalase was evaluated by monitoring the decomposition of H₂O₂, a widely recognized model substrate for catalase assays. The progress of the reaction was tracked by measuring the characteristic decline in absorbance at 240 nm using a UV–Vis spectrophotometer. For each assay, 10 mM H₂O₂ was prepared in 50 mM phosphate buffer (pH 7.0) and maintained at 25 °C. Free and silanized kaolinite–immobilized catalase samples were introduced separately into the reaction medium to initiate the catalytic process¹⁸. The decrease in absorbance at 240 nm was recorded over a 1-minute interval, assuming pseudo–first–order kinetics given the significant excess of substrate. The rate of absorbance change ($\Delta A_{240}/\text{min}$) was subsequently used as a quantitative measure of enzymatic activity, consistent with well-established protocols reported in the literature^{19,20}.

Immobilization of catalase onto silanized kaolinite particles

Catalase immobilization was performed using epoxy-functional kaolinite particles obtained by surface modification with GPTMS. A catalase stock solution (10 mL, 1000 mg L^{–1}) was freshly prepared, and its initial concentration was determined by measuring absorbance at 280 nm. Functionalized kaolinite (10 mg) was transferred into test tubes containing 10 mL of catalase solution and incubated under gentle shaking to promote interaction between the enzyme's nucleophilic residues and surface epoxy groups. At predetermined time intervals (1, 2, 4, 8, 12, and 24 h), solid particles were removed, and the remaining catalase concentration in solution was quantified at 280 nm^{19,20}. A gradual reduction in absorbance over time indicated the progressive immobilization of catalase onto the GPTMS-modified kaolinite surface.

The immobilization capacity was calculated using Eq. (1):

$$q = ((C_0 - C_t) \times V) / m \quad (1)$$

C_0 , The initial catalase concentration (mg L^{-1}), C_t , The catalase concentration in the solution at time t (mg L^{-1}), V , The volume of the catalase solution (L), m , The dry mass of the silanized kaolinite used (g).

Operational and storage stability of immobilized catalase

The operational robustness of catalase immobilized on GPTMS-functionalized kaolinite was assessed by performing 15 consecutive catalytic cycles at 25 °C. After each cycle, the kaolinite particles were washed with 50 mM phosphate buffer (pH 7.0) to remove residual substrate and reaction products before reuse. When not in use, immobilized catalase samples were stored at 4 °C in the same buffer to prevent loss of activity. Additionally, long-term storage stability was assessed by comparing the catalytic activities of free and immobilized catalase over 7 days under identical storage conditions²⁰. This analysis provided insights into the ability of kaolinite-bound catalase to retain activity compared with its free counterpart.

Desorption and reusability studies

Reusability of the GPTMS-modified kaolinite support was further investigated through desorption experiments. Catalase-loaded kaolinite particles were treated with 1 M NaCl solution (pH 7.0) under continuous mixing for 1 h to promote disruption of weakly bound interactions. Following desorption, the particles were collected, washed, and subjected to subsequent immobilization cycles to assess potential declines in catalytic efficiency with repeated use²⁰. The desorption ratio (DR) was calculated using the following equation to determine the fraction of enzyme released during the regeneration step.

$$DR (\%) = \frac{m_{\text{catalase desorbed}}}{m_{\text{catalase adsorbed}}} \times 100 \quad (2)$$

Characterization and instrumentation

The structural, chemical, and morphological properties of raw, silanized, and enzyme-immobilized kaolinite were comprehensively evaluated using a suite of analytical techniques. Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Thermo Scientific Nicolet 6700 FT-IR spectrometer (ATR mode, 4 cm^{-1} resolution) within the 4000–400 cm^{-1} range to confirm silane grafting and the presence of epoxy functionalities. Complementary measurements were obtained using a Bruker Tensor or equivalent instrument equipped with ATR or KBr pellet accessories.

Morphological features and surface textural changes resulting from GPTMS modification and catalase immobilization were examined using scanning electron microscopy (SEM). Micrographs were acquired with an FEI Quanta 450 FEG microscope operating at 10–20 kV after freeze-drying and gold sputtering. A Zeiss Evo LS10 or comparable SEM platform was also used to compare pristine, silanized, and enzyme-loaded particles.

Thermogravimetric analysis (TGA) was conducted on a Shimadzu DTG-60 H or a TA Instruments Q50 thermogravimetric analyzer. Samples (5–10 mg) were heated from ambient temperature to 800 °C at 10 °C min^{-1} under a nitrogen atmosphere (40–60 mL min^{-1}) to quantify organic content and assess thermal stability.

Surface elemental composition and chemical state information were analyzed using X-ray photoelectron spectroscopy (XPS). Measurements were conducted on a Thermo Scientific K-Alpha+ system and confirmed with a PHI 5000 VersaProbe III spectrometer. High-resolution spectra were obtained to evaluate the formation of Si–O–Si networks, GPTMS-derived carbon species, and nitrogen-containing groups associated with immobilized catalase.

Specific surface area and porosity parameters were measured using N_2 adsorption–desorption isotherms at 77 K on a Quantachrome Autosorb iQ-Chemi or a Micromeritics ASAP 2020 system. BET surface areas, pore volumes, and pore size distributions were calculated to assess the effects of silanization and enzyme loading on the textural properties of kaolinite.

UV–Vis spectrophotometric measurements, including monitoring H_2O_2 decomposition at 240 nm, were conducted using a Shimadzu UV-2600 or an equivalent instrument. pH adjustments were made with a calibrated Mettler Toledo pH meter. All centrifugation, drying, and sample-handling procedures were performed using standard laboratory equipment.

Result and discussion

Characterization

The FT-IR spectra presented in Fig. 2 revealed the characteristic vibrational signatures of pristine kaolinite and the modifications arising from GPTMS functionalization. In the native kaolinite spectrum, three well-defined and sharp O–H stretching bands at approximately 3695, 3650, and 3620 cm^{-1} are clearly visible, corresponding to inner-surface and inner-layer hydroxyl groups typically associated with the 1:1 layered aluminosilicate structure. The strong Si–O stretching bands centered between 1115 and 1030 cm^{-1} , together with the Al–OH bending feature near 915 cm^{-1} , further confirm the presence of intact kaolinite layers. Additional weaker bands in the 795–470 cm^{-1} region correspond to Si–O–Al and Si–O–Si deformation modes, in agreement with previously reported spectral profiles of well-ordered kaolinite minerals^{21,22}.

In contrast, the silane-functionalized kaolinite exhibits several notable spectral changes indicative of successful surface modification. First, the diagnostic O–H stretching bands undergo a slight reduction in intensity, suggesting partial interaction of surface hydroxyls with the organosilane reagent. Significantly, new absorption features emerge at 2925 and 2855 cm^{-1} , which are assigned to the asymmetric and symmetric C–H stretching vibrations of the grafted alkyl chains. Additional bands at 1270, 910, and 840 cm^{-1} correspond to C–O–C stretching and epoxy-related ring modes originating from the glycidyl moieties of the silane precursor. Together, these spectral changes confirm covalent silane grafting through condensation with surface hydroxyl groups, resulting in a stable organic–inorganic hybrid interface²³. The distinct mineral- and organosilane-

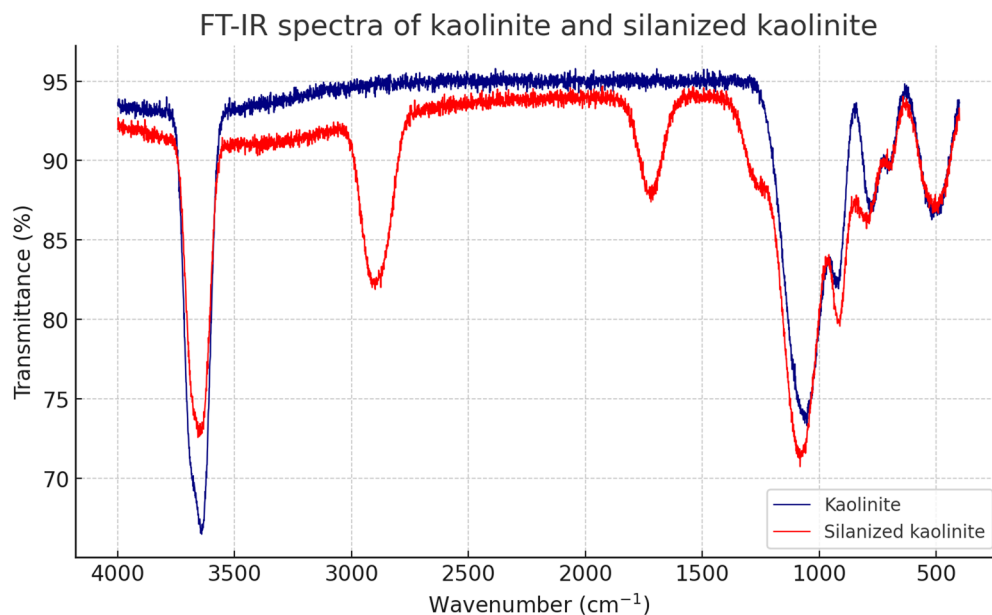


Fig. 2. FT-IR spectra of pristine kaolinite and GPTMS-functionalized kaolinite. The characteristic O–H stretching vibrations of kaolinite (3695, 3650, and 3620 cm^{-1}), along with Si–O and Al–OH bands, confirm the intact layered aluminosilicate structure. The appearance of new C–H stretching bands (2925 and 2855 cm^{-1}) and epoxy-related vibrations (1270, 910, and 840 cm^{-1}) after modification verifies the successful grafting of GPTMS onto the kaolinite surface.

derived vibrational features together demonstrate that GPTMS was successfully incorporated onto the kaolinite surface without disrupting its layered silicate structure, ensuring suitability for downstream immobilization or catalytic applications.

The SEM images in Fig. 3 clearly highlight the transition from compact, plate-like aggregates to a more disordered and porous lamellar morphology induced by silanization. In Fig. 3a, the pristine kaolinite exhibits the characteristic tightly packed, plate-like lamellae with relatively smooth surfaces and minimal interparticle spacing. The particles appear densely stacked, forming compact agglomerates typical of untreated kaolinite, where strong van der Waals interactions facilitate close packing of the aluminosilicate layers²⁴.

In contrast, Fig. 3b reveals a markedly altered morphology following silane modification. The lamellar structures become more irregular, loosely arranged, and partially delaminated, leading to a pronounced increase in interparticle voids²⁵. The individual platelets appear more fragmented and disordered, suggesting that the organosilane molecules, through grafting onto surface hydroxyl groups, effectively disrupt interlayer interactions and reduce cohesive forces between kaolinite sheets. This disruption likely arises from the introduction of organic moieties that sterically hinder restacking, thereby promoting a more open and heterogeneous microstructure²⁶.

Figure 3c further illustrates the morphological features of catalase immobilized on the silanized kaolinite surface. In comparison with the bare GPTMS-modified kaolinite, the overall lamellar framework is largely preserved; however, the platelet surfaces exhibit localized granular and slightly roughened domains attributed to immobilized catalase molecules. These enzyme-associated features are discretely distributed rather than forming a continuous coating, indicating that immobilization occurs primarily through surface anchoring onto available epoxy-functional sites without excessive pore blockage or lamellar collapse. The preservation of the open and loosely packed morphology suggests that enzyme attachment does not compromise the structural integrity of the silanized kaolinite, while the presence of surface-bound protein domains provides direct morphological evidence of successful catalase immobilization.

Taken together, the morphological evolution from dense, compact aggregates (Fig. 3a) to expanded and irregular lamellar assemblies (Fig. 3b), followed by enzyme-decorated but structurally preserved platelets (Fig. 3c), provides direct evidence of successful surface functionalization and subsequent biomolecule immobilization²⁵. The increased surface irregularity and expanded interparticle spacing in the silanized kaolinite, combined with accessible enzyme-bearing domains, are expected to enhance the material's suitability for catalytic and immobilization-based applications.

The TGA curves of pristine kaolinite and GPTMS-modified kaolinite showed different thermal behaviors, reflecting the structural changes caused by silane grafting (Fig. 4). Pure kaolinite maintains over 85% of its original weight up to about 400–450 °C, indicating high thermal stability and little mass loss beyond removing physisorbed water (usually less than 5% below 200 °C). A major degradation step happens at higher temperatures, typically between 450 and 700 °C, where the weight drops significantly due to the dehydroxylation of structural –OH groups and the formation of metakaolinite²⁷. Above 800 °C, the material stabilizes at a relatively constant residual mass, confirming the presence of a thermally resistant aluminosilicate framework.

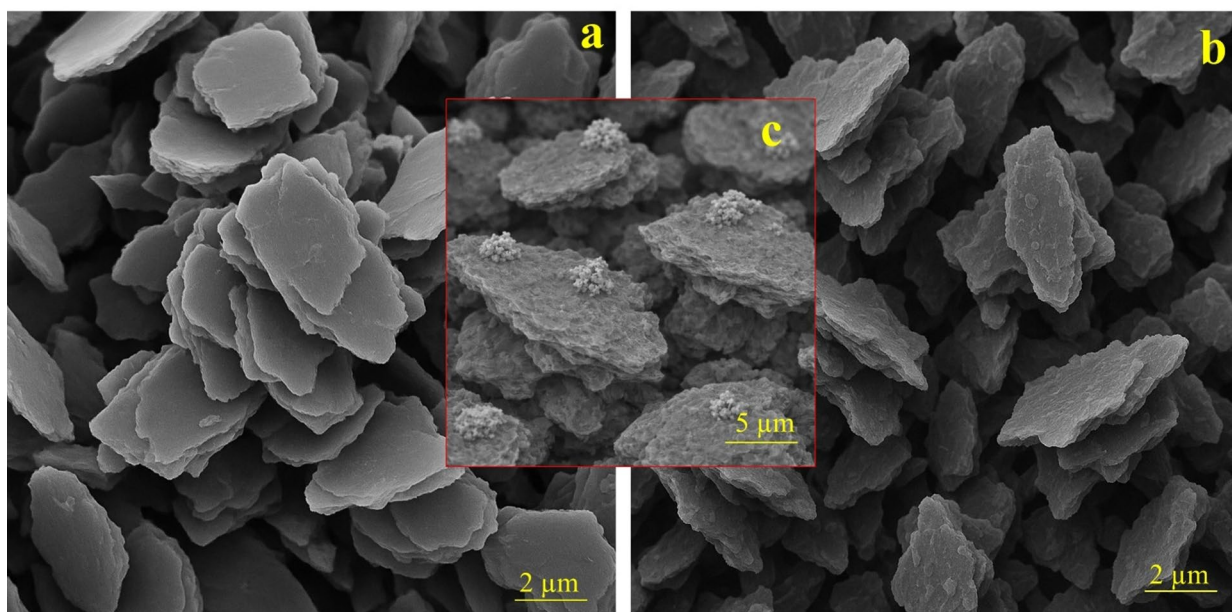


Fig. 3. SEM images of (a) native kaolinite, (b) GPTMS-modified kaolinite, and (c) catalase-immobilized silanized kaolinite. The untreated kaolinite exhibits densely packed, plate-like lamellae with smooth surfaces, whereas the silanized sample shows a more irregular, partially delaminated, and porous lamellar morphology due to disruption of interlayer interactions. Following catalase immobilization, the overall lamellar framework is preserved; however, localized granular and roughened surface features corresponding to immobilized enzyme domains become visible, confirming successful surface functionalization without significant structural collapse. (Scale bars: 2 μm for a and b; 5 μm for c).

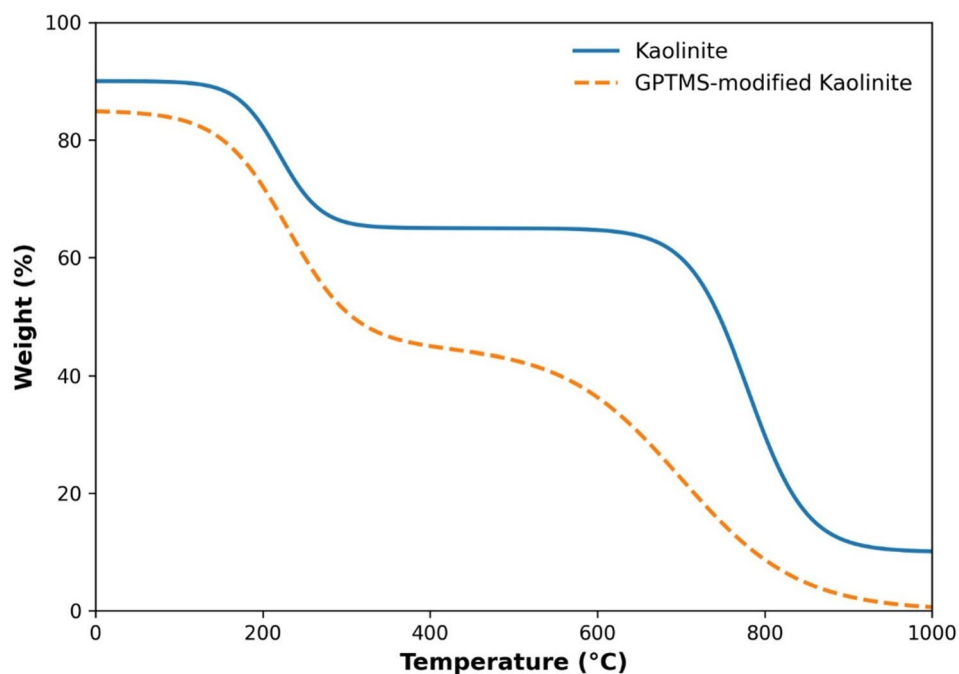


Fig. 4. TGA curves of pristine kaolinite and GPTMS-functionalized kaolinite. Pristine kaolinite exhibits high thermal stability, with the major mass loss occurring via dehydroxylation at elevated temperatures. In contrast, the modified kaolinite exhibits additional weight loss at lower temperatures due to thermal decomposition of grafted organosilane groups, confirming successful organic functionalization while preserving the inorganic framework.

In contrast, GPTMS-modified kaolinite showed a significantly different degradation profile due to the addition of organosilane functionalities. The modified sample starts losing mass earlier and more steadily, beginning around 150–200 °C and continuing to about 400–450 °C, where a notable reduction in weight occurs. This broad mass-loss range is linked to the thermal breakdown of grafted GPTMS groups, including the breakdown of organic parts and the evaporation of silane-based species. A second, more gradual degradation phase occurs at higher temperatures, usually between 450 °C and 800 °C, with further weight loss, indicating that the organic layer from the silane greatly impacts the general thermal stability of the modified material. The remaining residue at temperatures near 900–1000 °C is much lower than that of unmodified kaolinite, confirming the increased organic content and successful surface modification.

Overall, adding GPTMS decreases the apparent thermal stability of the mineral and raises the total mass loss by about 25–30% compared to unmodified kaolinite. These characteristic weight-loss features strongly support effective covalent silane grafting, which introduces thermally labile organic parts while maintaining the underlying inorganic structure^{28,29}. This controlled modification of the kaolinite surface is especially useful for immobilization, interfacial engineering, and functionalization strategies that need customized surface chemistry.

The XPS survey spectrum of pristine kaolinite (Fig. 5) showed the expected elemental signature of the aluminosilicate structure, with prominent Al 2p (~75 eV), Si 2p (~104 eV), and O 1s (~533 eV) signals. The very weak C 1s contribution (~285 eV) is mainly due to surface adventitious carbon, consistent with a clean mineral surface free of organic modifiers. After functionalization with GPTMS, distinct spectral changes appear (Fig. 5). Most notably, the C 1s peak becomes much more intense, indicating the incorporation of the organosilane's propyl, glycidyl, and ethoxy groups. This increase, along with minor changes in the O 1s envelope, suggests the formation of Si–O–Al or Si–O–Si linkages via condensation between the silane precursor and the surface hydroxyl groups of kaolinite. The retention of the Al 2p and Si 2p peak positions indicates that the silanization process does not break the underlying aluminosilicate lattice but instead creates an organic–inorganic hybrid interface. This comparative XPS analysis confirms the successful covalent grafting of the organosilane onto the kaolinite surface, entirely consistent with the structural changes observed in the FT-IR spectra and the thermal transitions evidenced by TGA.

The BET surface area analysis further substantiates the successful surface modification of kaolinite by GPTMS, revealing a noticeable increase from 17.28 m² g^{−1} for the native mineral to 22.52 m² g^{−1} after silanization. This enhancement, although moderate, is consistent with the grafting of organosilane moieties onto the external surfaces, which can partially disrupt particle–particle interactions and lead to a slight opening of interlayer contacts or reduction in agglomeration. The increase in accessible surface area suggests that the GPTMS layer does not form a compact, pore-blocking coating but rather introduces additional microtextural heterogeneity, increasing the number of available adsorption sites.

Immobilization studies

Figure 6a showed that catalase adsorption onto GPTMS-modified kaolinite occurred rapidly during the initial stage, with the adsorption capacity reaching 243.6 mg g^{−1} within the first hour. Therefore, the rapid initial uptake demonstrates that the epoxy-functionalized surface provides highly accessible, reactive binding sites for catalase molecules. By the second hour, the immobilization capacity increases to 279.5 mg g^{−1} and gradually approaches equilibrium. This behavior reflects strong enzyme–support interactions on the epoxy-functionalized surface.

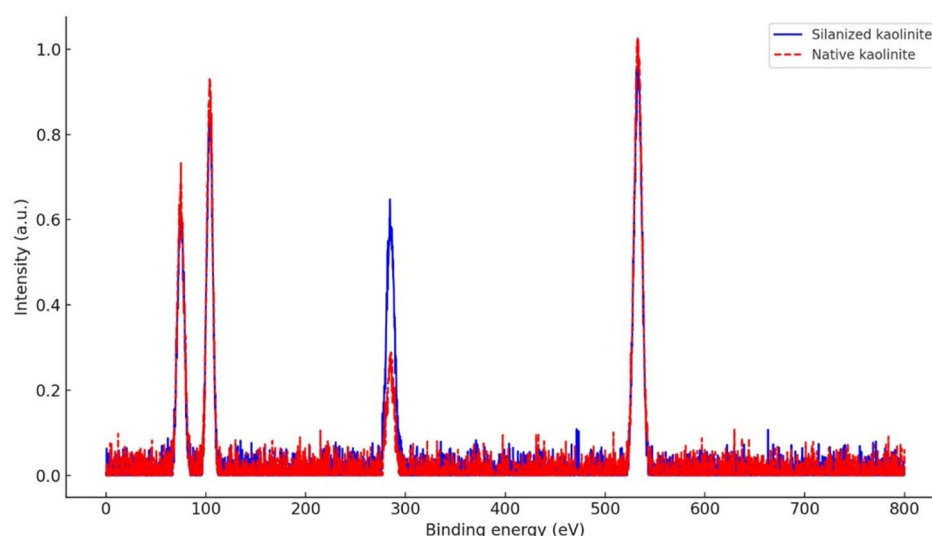


Fig. 5. XPS survey spectra of pristine kaolinite and GPTMS-modified kaolinite. The intensified C 1s signal after silanization indicates incorporation of organic moieties from GPTMS. The preservation of Al 2p and Si 2p peak positions confirms that surface modification occurs via covalent grafting without disrupting the underlying aluminosilicate lattice.

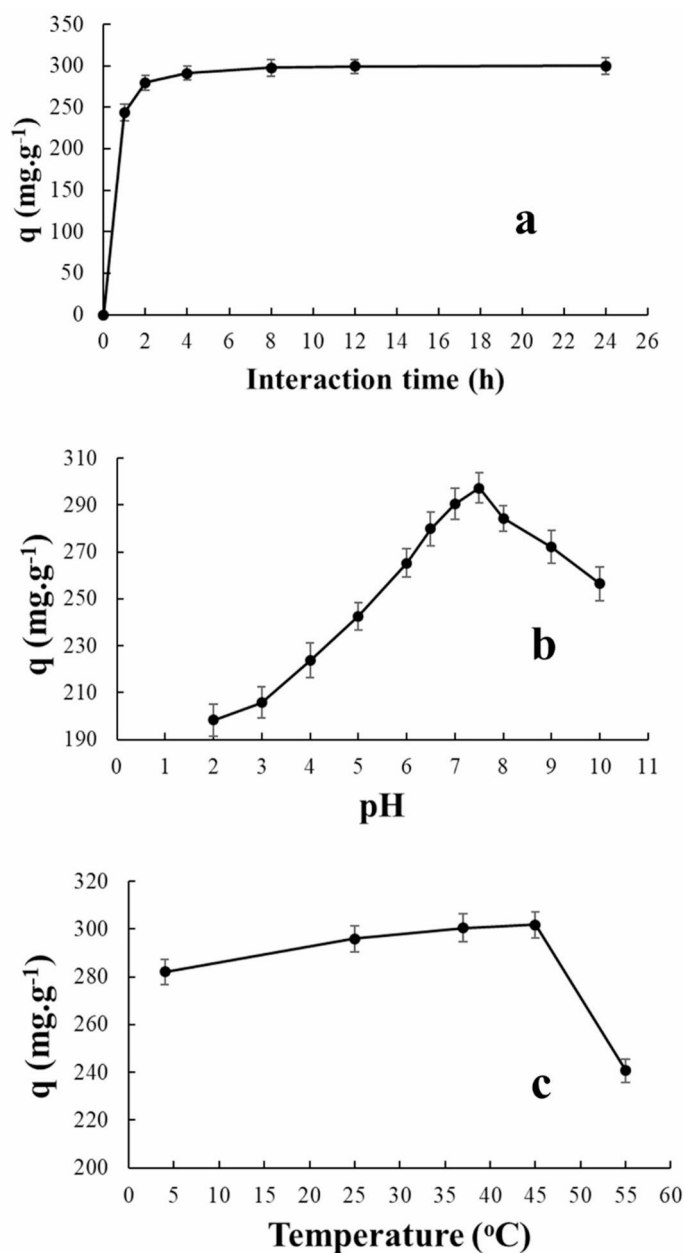


Fig. 6. (a) Time-dependent adsorption profile of catalase onto GPTMS-functionalized kaolinite (pH: 7.0, 25 °C). Rapid enzyme uptake occurs within the first hour, followed by a gradual approach to equilibrium, with adsorption saturation effectively reached after 4 h. The fast initial adsorption highlights the high accessibility and reactivity of epoxy-functional binding sites, while the plateau region indicates surface saturation and stable enzyme–support interactions. (b) Effect of solution pH on catalase adsorption onto silane-modified kaolinite (interaction time: 4 h, 25 °C). Adsorption capacity exhibits a bell-shaped dependence on pH, with maximum immobilization near neutral conditions (pH 7.0–7.5). This trend reflects the combined influence of enzyme charge state, epoxy–amine interactions, hydrogen bonding, and electrostatic effects governing enzyme attachment to the functionalized kaolinite surface. (c) Influence of temperature on catalase adsorption onto GPTMS-functionalized kaolinite (pH: 7.5, interaction time: 4 h). Adsorption capacity increases with temperature up to 37–45 °C, indicating enhanced molecular mobility and favorable enzyme orientation, followed by a sharp decline at higher temperature due to thermal destabilization of the enzyme structure. The results identify 25–45 °C as the optimal temperature range for efficient catalase immobilization.

Only negligible changes were observed between 4 and 24 h (291.2–299.7 mg g⁻¹), indicating that adsorption equilibrium was effectively achieved within the first 4 h. Accordingly, a 4 h contact time was considered sufficient to reach the saturation point, where the functionalized kaolinite surface becomes fully occupied by enzyme molecules. The rapid approach to equilibrium suggests that surface interactions, likely involving

epoxy, amine reactions, hydrogen bonding, and electrostatic effects, dominate the early adsorption phase³⁰. In contrast, slower structural rearrangements within the kaolinite interlayers may control the final stabilization. The high equilibrium adsorption capacity ($\sim 300 \text{ mg g}^{-1}$) highlights the strong affinity imparted by the organosilane modification, underscoring the material's efficiency as a robust support for catalase immobilization in biocatalytic or environmental applications. In addition to immobilization capacity, enzyme loading and immobilization yield were evaluated based on the adsorption data. The immobilization capacity reported in this study corresponds directly to enzyme loading and is expressed as milligrams of immobilized catalase per gram of GPTMS-functionalized kaolinite (mg g^{-1}). Under the experimental conditions employed, approximately 3.0 mg of catalase was immobilized per 10 mg of support from an initial enzyme amount of 10 mg, corresponding to an immobilization yield of about 30%. This value reflects the high surface reactivity of the epoxy-functionalized kaolinite and is consistent with reported yields for covalently anchored enzyme systems, where immobilization efficiency is governed by surface accessibility rather than complete enzyme uptake.

Figure 6b presents the influence of solution pH on catalase adsorption onto silane-modified kaolinite, revealing a clear pH-dependent trend governed by the interplay between the enzyme's charge state and the functionalized kaolinite surface. At acidic conditions (pH 2.0–4.0), adsorption remains relatively low ($198.4\text{--}223.8 \text{ mg g}^{-1}$), likely due to the strong positive charge on catalase near these pH values, which reduces its affinity toward the partially protonated epoxy-functionalized surface. As the pH increases toward neutrality, adsorption improves markedly, reaching 265.3 mg g^{-1} at pH 6.0 and 279.9 mg g^{-1} at pH 6.5, indicating that reduced electrostatic repulsion and enhanced nucleophilic interactions between amino groups and epoxy moieties facilitate stronger binding¹⁹.

The adsorption capacity reached its maximum at pH 7.5 (297.3 mg g^{-1}), suggesting that catalytic residues and surface silane groups are optimally oriented for both covalent-like epoxy–amine interactions and stabilizing hydrogen bonding. Beyond this point, a gradual decline in capacity was observed, with values decreasing to 284.3 mg g^{-1} (pH 8.0) and 256.4 mg g^{-1} (pH 10.0). This reduction at alkaline conditions is consistent with increased enzyme conformational instability and possible deprotonation of active binding groups, which collectively diminish effective attachment to the modified kaolinite layers.

Taken together, the bell-shaped adsorption profile indicates that near-neutral pH conditions maximize catalase immobilization¹⁹, confirming that silane-functionalized kaolinite provides a highly reactive and pH-sensitive platform for stabilizing enzyme molecules.

Figure 6c showed that temperature has a pronounced influence on catalase adsorption onto silane-modified kaolinite. At low temperature (4°C), the adsorption capacity is 282.1 mg g^{-1} , reflecting limited molecular mobility but sufficient affinity for surface epoxy groups. As the temperature rises to 25°C , adsorption increases to 295.9 mg g^{-1} , indicating that enhanced diffusion and improved enzyme orientation facilitate stronger interactions with the functionalized kaolinite.

The highest adsorption values were observed at 37°C (300.5 mg g^{-1}) and 45°C (301.7 mg g^{-1}). This plateau suggests that moderate heating promotes optimal conformational flexibility of catalase, allowing its amino-rich domains to more effectively engage with the glycidyl functionalities on the modified kaolinite surface. These conditions appear to balance sufficient thermal activation with preservation of structural stability, enabling efficient immobilization.

A sharp decline occurs at 55°C , where adsorption drops to 240.7 mg g^{-1} , consistent with temperature-induced catalase unfolding or partial denaturation. Such structural disruptions reduce the availability of reactive groups needed for strong attachment and weaken the total binding affinity. Generally, the temperature profile confirms that $25\text{--}45^\circ\text{C}$ represents the optimal range for catalase immobilization, where both enzyme stability and interaction kinetics are maximized on the silane-functionalized kaolinite matrix³¹.

Kinetic parameters of free and immobilized catalase

The kinetic analysis reveals how immobilization of catalase onto silane-functionalized kaolinite significantly modifies its catalytic characteristics (Table 1). Upon immobilization, the K_m value decreases from 57.3 mM (free enzyme) to 21.6 mM , indicating that the enzyme exhibits a substantially higher apparent affinity for hydrogen peroxide when anchored to GPTMS-modified kaolinite. This improvement is consistent with the microenvironment provided by the epoxy-functional surface, where localized substrate accumulation and favorable enzyme orientation help facilitate more efficient substrate recognition.

In contrast to the decrease in K_m , the V_{max} value declines from 2521 to $1480 \mu\text{mol min}^{-1}$, a trend typical of immobilized enzymes and generally attributed to diffusional limitations within the heterogeneous support, reduced freedom of conformational movement, and partial restriction of enzyme accessibility after covalent or epoxy-mediated binding. The estimated k_{cat} values also decrease moderately (from 62.17 to 41.57 min^{-1}), suggesting a slight reduction in catalytic turnover, likely due to the immobilized enzyme's restricted flexibility on the rigid kaolinite surface.

Despite these reductions, the catalytic efficiency (k_{cat}/K_m) increases from 0.00108 to $0.00193 \mu\text{M}^{-1} \text{ min}^{-1}$, representing a nearly 1.8-fold enhancement compared with that of free catalase. This gain is driven primarily by the substantial decrease in K_m , indicating that the immobilized catalase operates more effectively at lower

	K_m (mM)	V_{max} ($\mu\text{mol min}^{-1}$)	k_{cat} (min^{-1})	k_{cat}/K_m ($\mu\text{M}^{-1} \text{ min}^{-1}$)
Free enzyme	57.3	2521	62.17	0.00108
Immobilized enzyme	21.6	1480	41.57	0.00193

Table 1. Kinetic parameters for free and immobilized catalase.

substrate concentrations. The increase in catalytic efficiency demonstrates that silanized kaolinite, with its balanced epoxy-functional, hydrophilic, and hydrophobic surface, provides a stabilizing microenvironment that promotes favorable enzyme orientation, improved substrate–enzyme interactions, and partial protection of the active conformation during catalytic cycling.

At the molecular level, the enhanced catalytic performance of catalase immobilized on epoxy-functionalized kaolinite arises from specific, directional interactions between surface epoxy groups and the enzyme's nucleophilic amino acid residues. The strained epoxy ring is prone to nucleophilic attack by ϵ -amino groups of lysine, imidazole groups of histidine, and, where accessible, thiol groups of cysteine, resulting in covalent or quasi-covalent anchoring via ring-opening reactions. This multipoint attachment mechanism limits excessive conformational mobility while preserving the structural integrity of the active site, leading to a more rigid yet catalytically competent enzyme conformation.

This controlled increase in structural rigidity provides a mechanistic explanation for the pronounced decrease in the apparent K_m value observed after immobilization, as substrate recognition and binding are facilitated by a stabilized enzyme orientation and a favorable microenvironment at the epoxy-functional interface. In contrast, the reduction in V_{max} is consistent with diffusion-related limitations and partial restriction of molecular motion commonly associated with heterogeneous enzyme systems. Despite this decrease in maximum reaction rate, the substantial improvement in the k_{cat}/K_m ratio demonstrates that the immobilized catalase operates more efficiently at lower substrate concentrations. Collectively, these effects highlight a favorable balance between enhanced enzyme rigidity and preserved catalytic accessibility, resulting in improved catalytic efficiency and superior operational stability compared with physically adsorbed clay-based immobilization systems.

Stability and reusability performance of catalase immobilized on silanized kaolinite

The operational stability results in Fig. 7a highlighted a clear difference between free catalase and catalase immobilized on silane-functionalized kaolinite during repeated reaction cycles. Free catalase experiences a rapid decrease in activity, mainly due to structural denaturation, shear-induced unfolding, and ongoing exposure to hydrogen peroxide. This is shown by the steady decline from 100% to 36.8% over 15 cycles, indicating a significant loss of catalytic performance upon reuse.

Catalase immobilized on silanized kaolinite, by contrast, showed a much slower decline in activity. The epoxy-functional hybrid surface provides stronger enzyme anchoring, decreases conformational flexibility, and protects the catalytic structure from peroxide-induced inactivation. The layered kaolinite framework also offers mechanical rigidity that limits shear-related damage while maintaining accessible diffusion pathways for the substrate. These synergistic effects reduce enzyme leaching and help maintain the active conformation during repeated reactions.

The retained activity pattern showed that immobilization significantly improves enzyme reusability. While free catalase loses most of its activity after 15 cycles, immobilized catalase generally retains much higher activity over the same number of uses, aligning with reports on mineral-based immobilization systems. The data suggest that silanized kaolinite creates a stabilizing microenvironment that allows catalase to function effectively through multiple cycles, making it a strong candidate for repeated oxidative degradation processes³⁰.

The storage stability profile shown in Fig. 7b highlighted the clear difference between free catalase and catalase immobilized on silane-functionalized kaolinite over 70 days of refrigerated storage. Free catalase exhibits a steady, sharp decline in retained activity, dropping from 100% to 32.11%, indicating the enzyme's vulnerability to gradual structural unfolding, autolysis, and peroxide-induced degradation even under mild storage conditions.

Immobilized catalase, however, retains significantly higher activity over the same period, maintaining 62.76% of its initial activity after 70 days. This improved stability results from the rigid and partly hydrophobic microenvironment created by the silanized kaolinite matrix. The epoxy-functional surface provides stronger enzyme attachment, limiting conformational flexibility and shielding catalase from spontaneous denaturation²⁰. Additionally, the layered kaolinite structure helps preserve the tertiary conformation of the active sites by restricting unfavorable molecular motions and reducing contact with destabilizing species.

The consistent gap between the two curves, nearly doubling the retained activity after extended storage, shows that the immobilization strategy effectively improves long-term preservation of catalase activity. This stabilization aligns with reports indicating that mineral-based and silane-modified supports reduce structural relaxation and prevent the loss of catalytic function during prolonged storage.

These findings confirm that silanized kaolinite creates a protective environment that significantly prolongs the shelf life of catalase, making the immobilized system more durable for applications requiring long-term stability.

Figure 7c showed that the silanized kaolinite remains reusable, although both adsorption and desorption capacities slowly decline over five cycles. The first cycle features a high adsorption capacity of 288.93 mg g⁻¹ and a desorption value of 273.53 mg g⁻¹, indicating a 94.7% regeneration efficiency. This suggests that most binding sites are initially accessible and that adsorption interactions are mostly reversible. As usage continues, adsorption decreases to 240.76 mg g⁻¹ by the fifth cycle, while desorption decreases more sharply to 182.70 mg g⁻¹, lowering desorption efficiency to 75.9%. The widening gap between adsorption and desorption suggests partial pore blockage, irreversible binding of some molecules, or minor structural changes in the silanized kaolinite network¹⁹. Despite this decline, maintaining over 75% regeneration efficiency after five cycles shows that the solid support retains a significant portion of its functional capacity and is suitable for repeated adsorption–desorption operations.

The gradual loss of catalytic activity observed across repeated reuse cycles is most likely due to a combination of partial leaching of weakly bound enzyme fractions, slow conformational changes induced by repeated exposure to hydrogen peroxide, and minor alterations at the enzyme–support interface. Although epoxy-mediated immobilization provides strong anchoring, heterogeneous systems may still experience limited activity

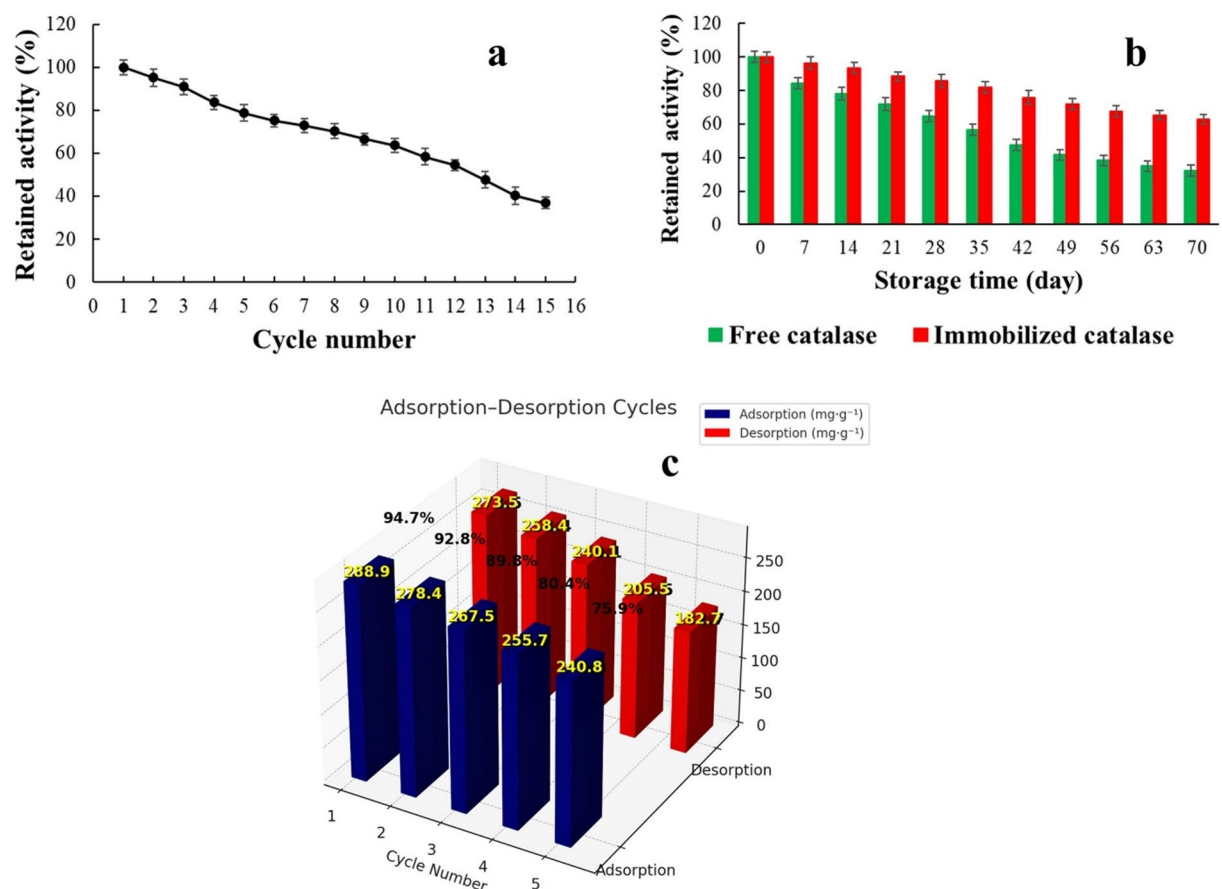


Fig. 7. (a) Operational stability of free catalase and catalase immobilized on silane-functionalized kaolinite during 15 consecutive reaction cycles. Free catalase exhibits a rapid loss of activity due to structural denaturation and peroxide-induced inactivation, whereas immobilized catalase shows markedly improved activity retention, highlighting the protective effect of the epoxy-functionalized kaolinite matrix against mechanical and chemical stress. (b) Storage stability profiles of free and immobilized catalase during 70 days of refrigerated storage. Immobilized catalase retains significantly higher residual activity compared to the free enzyme, demonstrating that silanized kaolinite provides a stabilizing microenvironment that limits conformational unfolding and long-term deactivation. (c) Reusability performance of silanized kaolinite during five consecutive adsorption-desorption cycles. Although both adsorption and desorption capacities gradually decrease with reuse, high regeneration efficiency is maintained, indicating that the support preserves its functional integrity and remains suitable for repeated operational cycles.

decay under prolonged operation. While post-reuse structural characterization was not performed in this study, the sustained retention of enzymatic activity over multiple cycles suggests that support degradation and enzyme detachment are minimal.

Comparative evaluation of enzyme immobilization performance on kaolinite-based supports

A comparative evaluation of kaolinite-based enzyme immobilization systems reveals significant differences in immobilization capacity, kinetic behavior, and long-term stability, which are mainly influenced by surface chemistry and immobilization strategy (Table 2). Early studies using physical adsorption on native kaolinite generally reported low to moderate enzyme loading and limited durability. For example, catalase immobilized on natural kaolin through simple adsorption achieved an immobilization capacity of only 19.7 mg g⁻¹, despite having a high initial immobilization efficiency (> 80%) and reasonable short-term stability (up to five cycles)³². However, such systems remain prone to enzyme leaching, with activity retention highly dependent on storage conditions.

A more detailed kinetic analysis of catalase adsorbed on unmodified kaolinite showed an immobilization capacity of 185.03 mg g⁻¹. This was accompanied by a significant increase in K_m from 29.22 mM (immobilized) compared to the free enzyme value of 172.09 mM, along with a substantial decrease in V_{max} from 10,100 to 3300 $\mu\text{mol min}^{-1}$ ¹³³. These findings suggest considerable mass-transfer resistance and less-than-ideal enzyme orientation on the mineral surface, which is typical of adsorption-driven immobilization on untreated clay supports.

Similar limitations have been observed for laccase immobilization on kaolinite and other clay minerals. Wen et al. achieved a laccase loading capacity of only 12.25 mg g⁻¹ on kaolinite through physical adsorption.

Support	Enzyme	IC (mg g ⁻¹)	K _m (mM) (Free/Imm)	V _{max} (μmol min ⁻¹) (Free/Imm)	OS	SS	SR	References
Natural kaolin	Catalase	19.7	–	–	Stable up to 5 cycles	90% (4 °C, 60 days)	100% (5th use)	³²
Kaolinite	Catalase	185.03	172.09/29.22	10,100/3300	–	–	–	³³
Kaolinite	Laccase	12.25	–	–	> 50% (5th use)	40% (4 °C, 30 days)	~ 50% (5th use)	¹³
Bentonite	Laccase	–	0.0700/0.0724	0.0695/0.0216	–	~ 70.5% (60 days)	–	³⁴
Diatomite	Laccase	–	0.0700/0.0831	0.0695/0.0236	–	~ 76.5% (60 days)	–	³⁴
Bardakçı clay	Laccase	–	0.0700/0.0935	0.0695/0.0233	–	~ 78.5% (60 days)	–	³⁴
GPTMS-kaolinite	Catalase	~ 300	57.3/21.6	2521/1480	100% (1st use) 78.7% (5th use) 36.8% (15th use)	82% (4 °C, 35 days)	100% (1st use) 75.9% (5th use)	This study

Table 2. Comparative immobilization performance of catalase/laccase on kaolinite-based supports. IC, Immobilization capacity; Imm, Immobilized; OS, Operational stability; SS, Storage stability; SR, Support reusability; GPTMS, (3-glycidyloxypropyl)methylsilane.

However, the immobilized enzyme retained more than 50% of its activity after five operational cycles and about 40% after 30 days at 4 °C¹³. Similarly, laccase immobilization on bentonite, diatomite, and Bardakçı clay resulted in modest changes in K_m values. It consistently reduced V_{max} compared with the free enzyme, despite moderate improvements in storage stability (~ 70–78% activity retention after 60 days)³⁴. Overall, these findings show that physical adsorption on native clay minerals provides limited enzyme loading and only minor kinetic improvements, restricting their use in long-term or high-throughput biocatalytic processes.

In stark contrast, the current study showed that organosilane-functionalized kaolinite (GPTMS-kaolinite) provides a significantly improved platform for catalase immobilization. Surface modification with GPTMS introduces epoxy groups that enable strong covalent or quasi-covalent attachment, leading to a much higher immobilization capacity of about 300 mg g⁻¹, greatly surpassing that of physically adsorbed kaolinite systems. Kinetic analysis further supports this method's superiority, as the K_m value drops considerably from 57.3 mM (free catalase) to 21.6 mM (immobilized catalase), indicating better substrate affinity due to favorable enzyme orientation and stabilized microenvironment at the silanized kaolinite interface. Although V_{max} decreases from 2521 to 1480 μmol min⁻¹ after immobilization, this decrease aligns with diffusion-controlled heterogeneous catalysis and does not indicate a loss of the enzyme's intrinsic activity.

Operational and storage stability data further emphasize the benefits of silanized kaolinite. While systems relying on physical adsorption often experience quick activity loss^{13,32}, catalase immobilized on GPTMS-kaolinite retains 78.7% of its activity after five cycles and 36.8% after fifteen cycles, with 82% activity remaining after 35 days at 4 °C. This enhanced durability results from the combined effects of strong enzyme–support interactions and the rigid aluminosilicate framework, which together help prevent enzyme desorption and conformational breakdown.

Collectively, a direct comparison with previously reported kaolinite- and clay-based systems reveals that the performance gains observed in this study cannot be attributed solely to the mineral support itself, but rather to its epoxy-functionalized surface chemistry. Earlier systems based on native kaolinite predominantly exhibit low immobilization capacities, limited kinetic improvements, and rapid activity loss due to weak enzyme–surface interactions. In contrast, GPTMS-functionalized kaolinite combines high enzyme loading with enhanced substrate affinity and superior stability, underscoring the critical role of epoxy groups in performance enhancement.

Conclusion

This study showed that GPTMS-silanized kaolinite is a highly effective and previously unstudied support for catalase immobilization. Surface functionalization with epoxy-containing organosilane groups successfully converted inert kaolinite into a reactive hybrid interface that securely anchors enzyme molecules without damaging the mineral's structural integrity. In-depth characterization confirmed the development of a stable organic–inorganic surface structure suitable for biocatalytic applications.

The immobilized catalase showed an exceptionally high loading capacity (~ 300 mg g⁻¹), far exceeding that of native kaolinite and other clay supports through physical adsorption. Kinetic analysis indicated a significant decrease in K_m and an increase in catalytic efficiency, suggesting that the silanized kaolinite microenvironment encourages favorable enzyme orientation and substrate accessibility. Although V_{max} decreased after immobilization, this is common in diffusion-limited heterogeneous systems and does not reduce the advantages of improved substrate affinity and stability. Notably, the immobilized enzyme demonstrated significantly better reusability and storage stability than free catalase, maintaining considerable activity across multiple catalytic cycles and during long-term storage. These findings highlight the synergistic benefits of strong enzyme–support interactions and the rigid aluminosilicate framework in protecting catalase from denaturation and leaching.

In conclusion, GPTMS-functionalized kaolinite is a durable, cost-effective, and scalable platform for enzyme immobilization. By overcoming the limitations of traditional kaolinite-based systems, this approach opens up new opportunities to develop long-lasting biocatalysts for environmental cleanup, advanced oxidation processes, and industrial uses that require sustained enzyme activity.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Received: 13 December 2025; Accepted: 31 January 2026

Published online: 10 February 2026

References

1. Hama Aziz, K. H., Mustafa, F. S., Karim, M. A. H. & Hama Sarkawt. Pharmaceutical pollution in the aquatic environment: Advanced oxidation processes as efficient treatment approaches: A review. *Mater. Adv.* **6**, 3433–3454 (2025).
2. Singh, C. K. et al. Managing the complexity of emerging contaminants in aquatic environments: Exploring their ecotoxicological impacts, detection techniques, and the use of innovative technologies for their remediation. *Discov. Catal.* **2**, 9 (2025).
3. Ziembowicz, S. & Kida, M. The optimization of advanced oxidation processes for the degradation of industrial pollutants. *Sustainability* **17**, 1908 (2025).
4. Anwar, S. et al. Exploring therapeutic potential of catalase: Strategies in disease prevention and management. *Biomolecules* **14**, 697 (2024).
5. Baker, A. et al. Catalase: A critical node in the regulation of cell fate. *Free Radic. Biol. Med.* **199**, 56–66 (2023).
6. Abdalbagemohammedabdalsadeq, S. et al. Catalase immobilization: Current knowledge, key insights, applications, and future prospects—A review. *Int. J. Biol. Macromol.* **276**, 133941 (2024).
7. Sayahi, N. et al. Clay minerals as enzyme carriers for pollutant removal from wastewater: A comprehensive review. *Minerals* **15**, 969 (2025).
8. An, N. et al. Immobilization of enzymes on clay minerals for biocatalysts and biosensors. *Appl. Clay Sci.* **114**, 283–296 (2015).
9. Duarte-Silva, R., Villa-García, M. A., Rendueles, M. & Díaz, M. Structural, textural and protein adsorption properties of kaolinite and surface-modified kaolinite adsorbents. *Appl. Clay Sci.* **90**, 73–80 (2014).
10. Maghraby, Y. R., El-Shabasy, R. M., Ibrahim, A. H. & Azzazy, H. M. E. Enzyme immobilization technologies and industrial applications. *ACS Omega* **8**, 5184–5196 (2023).
11. Kujawa, J. et al. Highly effective enzymes immobilization on ceramics: Requirements for supports and enzymes. *Sci. Total Environ.* **801**, 149647 (2021).
12. de Souza Lima, J., Boemo, A., de Araújo, P. H. H. & de Oliveira, D. Immobilization of endoglucanase on kaolin by adsorption and covalent bonding. *Bioprocess Biosyst. Eng.* **44**, 1627–1637 (2021).
13. Wen, X. et al. Immobilizing laccase on kaolinite and its application in treatment of malachite green effluent with the coexistence of Cd(II). *Chemosphere* **217**, 843–850 (2019).
14. Fatimah, I. Preparation, characterization and physicochemical study of 3-aminopropyl trimethoxy silane-modified kaolinite for Pb(II) adsorption. *J. King Saud Univ. – Sci.* **30**, 250–257 (2018).
15. David, M. K. et al. Thermal and hydrothermal alkaline modification of kaolin for the adsorptive removal of lead(II) ions from aqueous solution. *SN Appl. Sci.* **2**, 1134 (2020).
16. Issa, A. A. & Luyt, A. S. Kinetics of alkoxysilanes and organoalkoxysilanes polymerization: A review. *Polymers* **11**, 537 (2019).
17. Xavier, J. R., Beryl, J. R. & Ravikiran, N. Influence of silanized clay on the barrier, hydrophobic and mechanical properties of epoxy-coated steel in natural seawater. *J. Adhes.* **99**, 1889–1915 (2023).
18. Kołodziejczak-Radzimska, A. & Jesionowski, T. Characterization of amino-, epoxy- and carbonyl-functionalized Halloysite and its application in the immobilization of aminoacylase from *Aspergillus melleus*. *Physicochemical Probl. Mineral. Process.* **55**, 128–139 (2019).
19. Erol, K., Cebeci, B. K., Köse, K. & Köse, D. A. Effect of immobilization on the activity of catalase carried by poly(HEMA-GMA) cryogels. *Int. J. Biol. Macromol.* **123**, 738–743 (2019).
20. Erol, K., Alkan, M. H. & Alacabey, İ. Cryogel-immobilized catalase as a biocatalyst with enhanced stability against microplastics. *Gels* **11**, 634 (2025).
21. Jozanikohan, G. & Abarghoeei, M. N. Fourier transform infrared spectroscopy (FTIR) analysis for clay mineralogy studies in a clastic reservoir. *J. Pet. Explor. Prod. Technol.* **12**, 2093–2106 (2022).
22. Drits, V. A. et al. New insight into the relationships between structural and FTIR spectroscopic features of kaolinites. *Clays Clay Miner.* **69**, 366–388 (2021).
23. Feriancová, A., Dubec, A., Pagáčová, J. & Pajtašová, M. Modification of the filler based on kaolin and its use in polymer composites. *MATEC Web Conf.* **357**, 07004 (2022).
24. Awad, M. E. et al. Kaolinite in pharmaceuticals and biomedicine. *Int. J. Pharm.* **533**, 34–48 (2017).
25. Özel, C. et al. Surface modification of zeolite and kaolin with 3-(aminopropyl) triethoxysilane and 3-(trimethoxysilyl) propyl methacrylate. *Bull. Chem. Commun.* **53**, 464–470 (2021).
26. Zhang, J., Zhang, P. & Cheng, F. Effect of unhydrated aminopropyl triethoxysilane modification on the properties of calcined Kaolin. *Minerals* **12**, 705 (2022).
27. Polcowñuk Iriarte, I. A., Mocciaro, A., Rendtorff, N. M. & Richard, D. Dehydroxylation of kaolinite: Evaluation of activation energy by thermogravimetric analysis and density functional theory insights. *Minerals* **15**, 607 (2025).
28. Yang, S. Q. et al. Effect of reaction temperature on grafting of γ -aminopropyl triethoxysilane (APTES) onto kaolinite. *Appl. Clay Sci.* **62–63**, 8–14 (2012).
29. Yuan, Y. et al. Surface modification of calcined kaolinite for enhanced solvent dispersion and mechanical properties in poly(butylene adipate/terephthalate) composites. *Molecules* **29**, 3897 (2024).
30. Erol, K., Tatar, D., Veyisoğlu, A. & Tokatlı, A. Antimicrobial magnetic poly(GMA) microparticles: Synthesis, characterization and lysozyme immobilization. *J. Polym. Eng.* **41**, 144–154 (2021).
31. Sheldon, R. A. & van Pelt, S. Enzyme immobilisation in biocatalysis: Why, what and how. *Chem. Soc. Rev.* **42**, 6223–6235 (2013).
32. Savran, A., Alkan, S., Demir, H. & Ceylan, H. Application of natural kaolin as support for the immobilization of catalase from bovine liver. *Asian J. Chem.* **18**, 413–418 (2006).
33. Demirbaş, Ö., Alkan, M. & Demirbaş, A. Surface properties of catalase and casein on kaolinite and design of experiments. *Micropor. Mesopor. Mater.* **172**, 151–160 (2013).
34. Özdemir, F. & Yalçinkaya, Z. Examination of the immobilization and kinetics of the laccase enzyme on various clay minerals. *MAS J. Appl. Sci.* **8**, 286–306 (2023).

Author contributions

K.E., İ.A., and E.G. wrote the main manuscript text. K.E., A.V., D.T., and B.B.K. prepared all figures. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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