MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Construction of Polyethylene Terephthalate Hydrolase Mimetic based on Self-assembled Peptide Functionalized with Graphene Oxide**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group
BEBT-21
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Scientific supervisor
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Kyiv 2025

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Educational and professional program Biotechnology

APPROVE
Head of Biotechnology, Leather and Fur Department, Professor,
Dr. Sc., Prof.
___________Olena MOKROUSOVA
«___»_______________2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Xu Anying

1. Thesis topic **Construction of Polyethylene Terephthalate Hydrolase Mimetic based on Self-assembled Peptide Functionalized with Graphene Oxide**
Scientific supervisor Dr.Sc., Prof. Olga Andreyeva

approved by the order of KNUTD “05” March 2025, № 50-уч

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments 05.03.2025

WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	28 May 2025	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	01 June 2025	Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	04 June 2025	

I am familiar with the task:

Student ____________ Xu ANYING

Scientific supervisor ____________ Olga ANDREYEVA

SUMMARY

Xu ANYING. Construction of Polyethylene Terephthalate Hydrolase Mimetic based on Self-assembled Peptide Functionalized with Graphene Oxide. – Manuscript.

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This study focused on the preparation of graphene oxide peptide fiber hybrid hydrogel and its application in the field of PET plastic degradation. The hybrid hydrogel was successfully prepared by carefully designing specific peptide sequence, promoting its self-assembly and compounding with graphene oxide. The influence of key factors such as solution type, reaction temperature, pH value and ionic strength on the self-assembled structure and the activity of the mimic enzyme was deeply explored. The hybrid hydrogel was characterized by fluorescence experiment, circular dichroism experiment, transmission electron microscope observation and other experimental techniques, and the activity of the mimic enzyme was detected by the experimental method using PET as the substrate. The results showed that the composite of graphene oxide and peptide fibers significantly enhanced the activity of simulated enzymes, and factors such as reaction time, pH value, and temperature had a significant impact on the activity of simulated enzymes. Under the optimized conditions, the hybrid hydrogel mimic enzyme performs well in PET degradation. This study provides a new and effective approach for PET plastic pollution control, which has important theoretical and practical significance for promoting the development of environmentally friendly materials and plastic waste disposal.

Key words: Graphene oxide, Peptide fibers, Hybrid hydrogel, PET degradation, Mimetic enzyme

TABLE OF CONTENTS

INTRODUCTION

Polyethylene Terephthalate (PET**)**, one of the world's most widely produced synthetic polyesters (with an annual output of 100 million tons, accounting for 18% of oil-based polymers), is extensively utilized in packaging and textile industries due to its excellent plasticity and durability. However, PET's chemical inertness results in a natural degradation cycle of hundreds of years, with the global average recycling rate of PET remaining below 30%. This pollution has become a significant threat to ecosystem sustainability. Traditional physical recycling methods (melt granulation, mechanical crushing) often degrade material performance, while chemical hydrolysis requires high-temperature and high-pressure conditions and generates toxic byproducts. Although bi enzymatic degradation is environmentally friendly, it still faces challenges such as enzyme activity instability under environmental fluctuations and high costs for large-scale production.

Recent advances in enzyme-mimicking technology, characterized by high design flexibility, superior catalytic efficiency, and robust environmental tolerance, have opened new avenues for PET degradation. Studies demonstrate that peptide-based mimetic enzymes can achieve efficient substrate recognition and catalytic conversion through rational design. In this study, we innovatively construct a hybrid hydrogel by combining graphene oxide (GO) with peptide nanofibers. The large surface area of GO provides active sites, while the self-assembly properties of peptides impart dynamic responsiveness to the system.

Preliminary experiments revealed that GO incorporation increases the structural disorder of peptide fibers by 32% (ID/IG value rises from 0.75 to 0.89), suggesting enhanced catalytic performance through interfacial synergistic effects.

Purpose of the study: PETase is a highly efficient hydrolase capable of degrading polyethylene terephthalate (PET) into monomers, offering a green solution for plastic pollution remediation. However, its practical application is limited by poor thermal stability, strict reaction conditions, and low reusability. In this study, we adopt PETase as a mimetic target and construct a composite mimetic enzyme by integrating rational peptide sequence design with functional graphene oxide (GO) to enhance PET degradation efficiency.

This research aims to overcome the limitations of existing PET degradation technologies through three innovative strategies:

（1）Designing aromatic amino acid-containing peptide sequences (Fmoc-FFGSDHS-CONH$_2$; Fmoc-FFGSW-CONH$_2$) via Fmoc solid-phase synthesis, leveraging their hydrophobic cores to form multiple non-covalent interactions with GO surface functional groups;

（2）Developing a 3D hybrid hydrogel system to elucidate GO-peptide interfacial mechanisms using TEM, FTIR, and Raman spectroscopy;

（3）Systematically investigating the effects of pH (2.5–10) and temperature (25–70°C) on PET degradation efficiency.

The outcomes will provide novel strategies for developing efficient and stable biomimetic catalysts, offering critical theoretical and practical insights for advancing plastic pollution remediation and circular economy development.

Chapter I

LITERATURE REVIEW

1.1 Polyethylene Terephthalate (PET)

Plastic is a type of high-molecular compound, primarily manufactured from petrochemical raw materials[1,2],characterized by its high molecular weight and plasticity. Driven by advancements in organic polymer technology[3], continuous efforts have been made to develop new plastic products and expand production scales. According to data from the United Nations Environment Programme (UNEP), global plastic production reached 535 million tons in 2024. Among these, polyethylene terephthalate (PET) stands out as one of the most representative synthetic polyesters in daily life. PET, composed of terephthalic acid (TPA) and ethylene glycol (EG) linked by ester bonds[4,5,6,] is widely used across industries due to its exceptional plasticity, durability, and strength. Globally, annual PET production has surpassed 100 million tons, accounting for 18% of all oil-based polymers and ranking third in global production. However, the recycling rate for PET remains below 30% in most countries, posing severe environmental challenges[7,8].

1.2 PET Degradation Methods

Polyethylene Terephthalate (PET), one of the most widely applied synthetic polyesters globally, is extensively utilized in packaging, textiles, and other fields. However, the environmental challenges posed by its massive consumption have spurred global researchers to actively explore effective recycling and degradation methods. Significant progress has been made in PET hydrolase research both domestically and internationally.

Traditional PET treatment methods mainly include physical recycling and chemical degradation[9,10]. Physical recycling techniques such as melt granulation and mechanical crushing enable partial material regeneration but often compromise PET's properties. These methods are limited to clean, homogeneous waste streams and struggle with complex real-world waste scenarios. Chemical degradation approaches like hydrolysis and alcoholysis require harsh conditions (e.g., high temperature and pressure), consume substantial energy, generate toxic byproducts, and yield low-purity products unsuitable for high-quality recycling[11]. As an emerging green technology, biological enzymatic degradation offers mild reaction conditions and environmental friendliness. However, it faces challenges such as enzyme sensitivity to temperature/pH fluctuations, low catalytic efficiency, difficulties in enzyme recovery/reuse, and high production costs of natural enzymes, which hinder large-scale industrial applications[2].

PET-degrading enzymes were first discovered in 2005[12], prompting research into integrating them with downstream green processes for sustainable PET recycling[13]. Current PET hydrolases are primarily isolated from microbial consortia[1], but enzymes with high activity under extreme conditions (e.g., high temperature, high salinity, variable pH) remain scarce. For example, researchers at Thailand's VISTEC Institute of Technology discovered the PET hydrolase MG8 from human saliva, which exhibits robust degradation activity across diverse temperature and salinity ranges, outperforming some naturally occurring engineered hydrolases. Collaborative efforts between China's Tianjin Institute of Industrial Biotechnology (TIB) and Nanjing University of Chinese Medicine led to the development of a fluorescence-based microplate screening method for PETase mutants. Through directed evolution, the DepoPETase mutant achieved complete depolymerization of various PET packaging materials under moderate temperatures, with its activity and stability validated in 3L fermenter experiments.

Advancements in catalytic performance enhancement and molecular engineering of PET hydrolases include:

Rational design based on IsPETase crystal structures: Studies using molecular dynamics simulations revealed that beneficial mutations strengthen loop interactions near the catalytic triad via hydrophobic forces, salt bridges, and hydrogen bonds, boosting enzymatic efficiency[14]. Collaborative research between TIB’s Structural Biology Platform and Germany’s University of Greifswald elucidated the structures of PES-H1/PES-H2 enzymes and their complexes with PET analogs, identifying key binding modes and hotspot residues. Engineered PES-H1 mutants showed significantly improved hydrolysis efficiency for low-crystallinity PET.

Application expansion: Site-specific enzyme modifications have enabled covalent bonding of PET hydrolases to plastic surfaces, broadening their functional applications [15]. Structural studies of carboxylesterases bound to (terephthalic acid) esters revealed their role in promoting PET depolymerization[16].

Beyond biological methods, chemical recycling remains a research hotspot. Neutral hydrolysis has been explored for heterogeneous PET waste streams to directly recover terephthalic acid (TPA)[12], but challenges persist, including reliance on high temperature/pressure and acid purification. Hybrid approaches combining glycolysis with hydrolysis and continuous-flow systems have been proposed to enhance efficiency and scalability.

1.3 Graphene Oxide-Peptide Fiber

Compared to natural enzymes, mimetic enzymes exhibit advantages such as design flexibility, superior catalytic efficiency, precise selectivity, and robust environmental tolerance, effectively overcoming the limitations of natural enzymes prone to deactivation in complex environments[17]. Peptide-based mimetic enzymes, derived from biological systems, offer excellent biocompatibility, structural versatility, and customizable features tailored to specific needs.

Graphene oxide (GO), a novel two-dimensional nanomaterial, possesses a unique microscopic structure with a large specific surface area and abundant oxygen-containing functional groups (e.g., hydroxyl, carboxyl, epoxy groups)[11,15]. These properties enable efficient composite formation with polymers through covalent bonds, hydrogen bonds, and other interactions, significantly enhancing mechanical strength, thermal stability, and catalytic activity. Incorporating GO into mimetic enzyme systems holds promise to leverage its interfacial effects and functional properties, thereby improving PET degradation performance.

1.4 Research Objectives

PETase is a highly efficient hydrolase capable of degrading polyethylene terephthalate (PET) into monomers, offering a green solution for plastic pollution remediation [18]. However, its practical application is limited by poor thermal stability, strict reaction conditions, and low reusability. In this study, we adopt PETase as a mimetic target and construct a composite mimetic enzyme by integrating rational peptide sequence design with functional graphene oxide (GO) to enhance PET degradation efficiency.

1.5 Research Content

This study focuses on fabricating a hybrid hydrogel by modifying peptides(Fmoc-FFGSDHS-CONH2；Fmoc-FFGSW-CONH2) with graphene oxide (GO), using transmission electron microscopy (TEM) to investigate its structural characteristics and evaluating its catalytic activity toward PET degradation.

1.6 Technical Roadmap

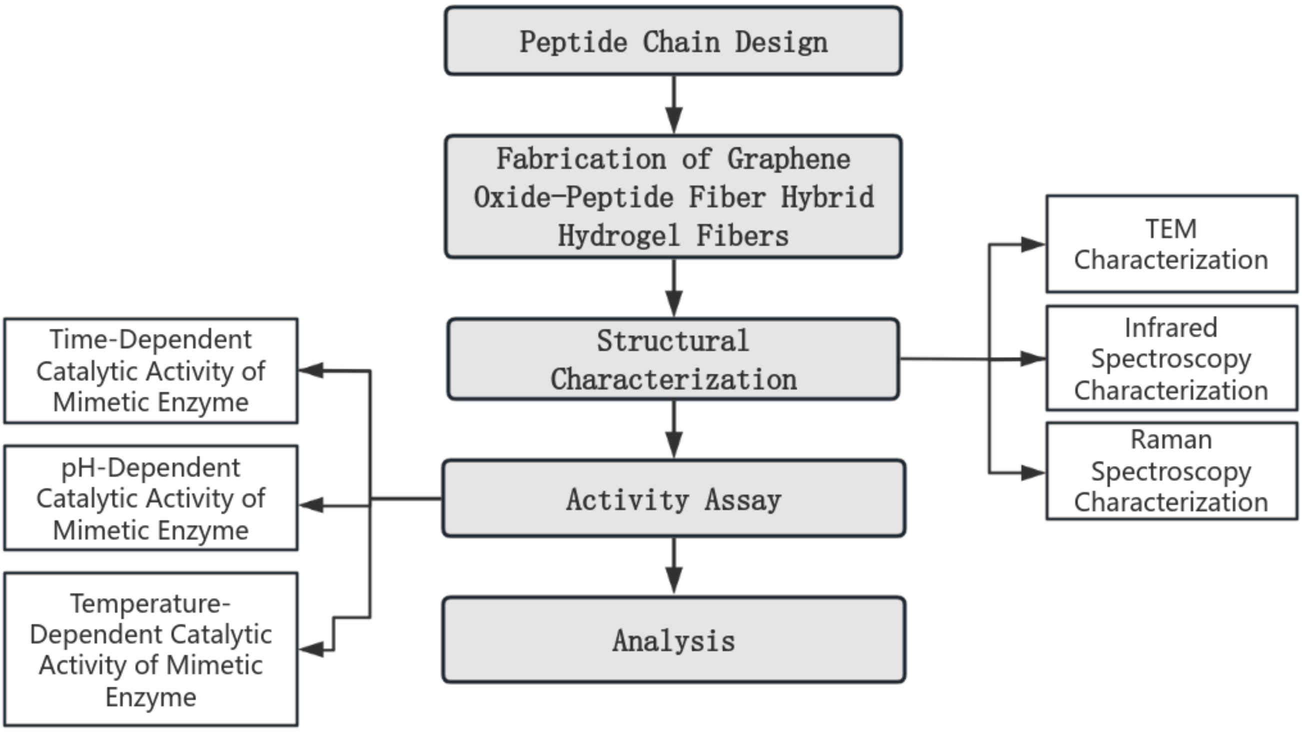


Figure 1.1 Experimental flowchart

Summary of the chapter I

1. PET and the traditional methods for degrading PET were introduced
2. Graphene oxide peptide fibers were introduced
3. The research purpose and content were clarified, and the research experimental route was determined

Chapter 2

OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Experimental Materials

2.1.1 Experimental Instruments

Transmission Electron Microscope (TEM), Raman Spectrometer, Analytical Balance, Pipette, Microporous Membrane Filter, pH Meter, Centrifuge, Thermostatic Incubator, Test Tube, Cuvette, Fluorometer, Drying Oven, Ultrasonic Cleaner, Filter Paper, TEM Copper Grid.

2.1.2 Experimental Reagents

Polypeptide， Graphene Oxide (GO)， Deionized Water， Polyethylene Terephthalate (PET) Granules,2% (mass/volume) Phosphotungstic Acid, Dimethyl Sulfoxide (DMSO),0.1% (w/w) Phosphoric Acid Aqueous Solution (pH 2.5), Acetonitrile.

2.2 Experimental Methods

2.2.1 Preparation of Graphene Oxide-Peptide Fiber Hybrid Hydrogel

(1) Preparation of Peptide Fibers

Lyophilized short peptide powder was dissolved in DMSO.A pH-adjusted buffer solution was added. Due to low solubility, a small amount of co-solvent (e.g., ethanol or propylene glycol) was introduced. The mixture was ultrasonicated for 90 seconds to enhance dissolution. The solution was diluted to 5 mM and incubated at 25°C under light-protected conditions for 24 hours to facilitate self-assembled fibrous matrices. Peptide nanofibrous materials (PNFs) were obtained.

(2) Preparation of Homogeneous Graphene Oxide-Peptide Nanofiber Composites

A portion of the PNFs obtained from the previous step was mixed with graphene oxide (GO) solution. The mixture was vortexed thoroughly to eliminate air bubbles, then incubated at 25°C under light-protected conditions for 12 hours. This process enabled uniform interfacial interaction between GO sheets and peptide nanofibers, yielding homogeneous graphene oxide-peptide nanofiber composites (GO-PNFs).

2.2.2 Structural Characterization of Graphene Oxide-Peptide Fiber Hybrid Hydrogel

(1) Transmission Electron Microscopy (TEM) Technology

7 μL of the diluted peptide self-assembly solution was dropped onto a TEM copper grid and allowed to stand at room temperature for 30 minutes. Excess solution was blotted with filter paper, followed by immediate application of 7 μL freshly prepared 2% phosphotungstic acid (PTA) for 1.5 minutes of staining. Excess PTA was removed, and the grid was washed three times with ultrapure water before air-drying in a desiccated petri dish. The TEM grid was loaded into the sample chamber, vacuum-pumped, and observed at 200 kV within an optimized field of view.

(2) Infrared Spectroscopy (IR)

A dry peptide powder was uniformly applied to the ATR crystal surface to ensure intimate contact. Spectra were acquired with a resolution of 4 cm^{-1} and a wavenumber range of 4000–500 cm^{-1}.Scans were collected and saved in a standardized format (e.g.,. jdx or .spc).

(3) Raman Spectroscopy

Dry peptide powder was directly spread on a glass slide. A laser was selected, starting at low power, and spectra were acquired in the wave number range of 800–2000 cm^{-1}.Spectra were saved and structural features were recorded.

2.2.3 Catalytic Activity Detection of Graphene Oxide-Peptide Fiber Hybrid Hydrogel

(1) Hybrid Hydrogel-Mediated Degradation of PET

PET particles were added to the prepared hybrid hydrogel solution and subjected to degradation reactions in different pH buffer solutions. After the reaction, the mixture was boiled at 100℃ for 5 minutes to inactivate the simulated enzymes and terminate the degradation. The mixture was centrifuged at 12,000 rpm for 10 minutes. Undegraded PET fragments settled at the bottom, and the supernatant was collected for further analysis.

(2) High-Performance Liquid Chromatography (HPLC) Analysis

Based on the comparison of the peak positions of HPLC with the standard products, it was determined that the main degradation products of PET were TPA and MHET. The standard curves of TPA and MHET were fabricated. Through the peak area of the degradation products, the number of products was calculated using the standard curves to evaluate the degradation activity of PET by the hybrid hydrogel. According to the pre-set chromatographic parameters (Table 2.1), the peak time of MHET was determined to be 5.185min, and the peak time of TPA was determined to be 4.591min. After detecting the peak area of the sample, substitute it into the standard curve equation to accurately calculate the contents of TPA and MHET.

Table 2.1 **Chromatographic Parameters detected by HPLC**

Parameters	Settings
Chromatographic column	Column: C18 reverse-phase（4.6×250mm,5μm，Agilent ZORBAX SB-C18）
Mobile phase	A 相：0.1% Phosphoric Acid Aqueous Solution (pH 2.5);B 相：Acetonitrile
Gradient program	0-5min: 5%B→5%B
	5-15min: 5%B→40%B
	15-20min: 40%B→5%B
Flow velocity	1.0mL/min
Column temperature	30℃
Detection wavelength	TPA:240nm; MHET:254nm
Injection volume	20μm

Summary of chapter II

1. It indicates the instruments and reagents required for the experiment
2. It indicates the accurate steps of the experiment
3. The structure of graphene oxide peptide fiber hydrogel was characterized
4. The catalytic activity of graphene oxide peptide fibers was detected

Chapter 3
EXPERIMENTAL PART

3.1 Structural Characterization of Graphene Oxide-Peptide Fiber Hybrid Hydrogel

3.1.1 Morphological Analysis

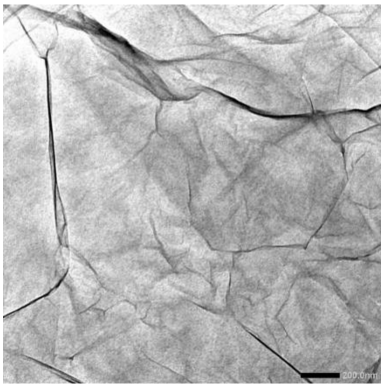


Figure 3.1 TEM image of graphene

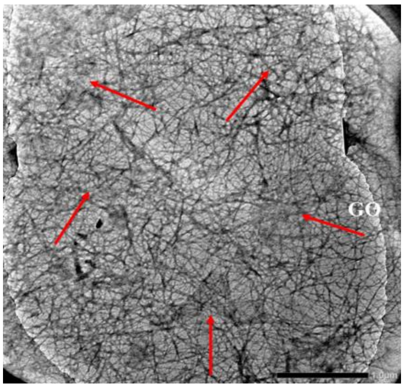
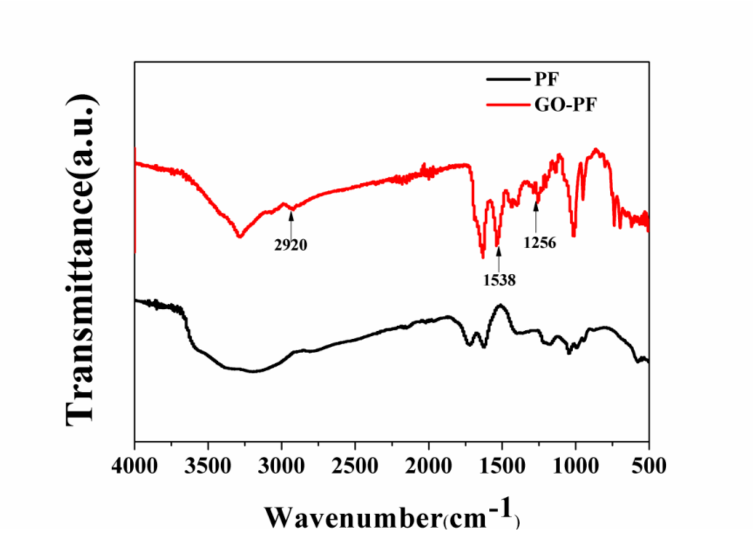


Figure 3.2 TEM image of graphene-peptide fiber composite

As clearly observed in the TEM characterization images (Figures 3.1 and 3.2), graphene exhibits a typical layered structure characterized by flat sheets with characteristic wrinkles, which are unique morphological features of graphene. In

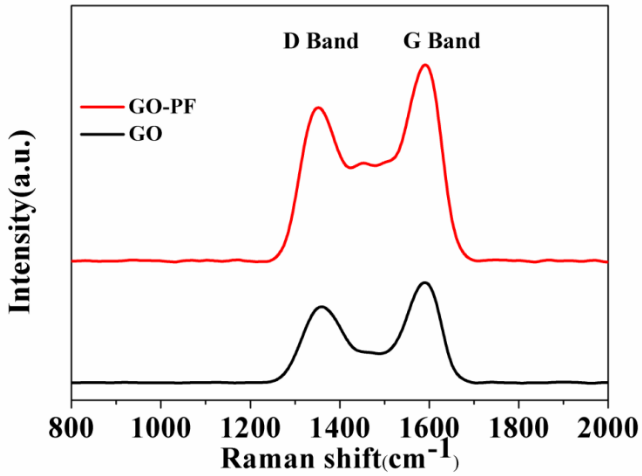


contrast, the graphene-peptide composite forms a more complex fibrous intertwined structure, where peptide fibers are uniformly distributed around the graphene sheets and interwoven with each other. This structural transformation directly confirms the successful composite formation between peptide fibers and graphene. Such a composite structure is likely to significantly influence the enzymatic-mimicking performance – for example, by increasing the number of active sites, altering the microenvironment of active sites, and thereby enhancing the catalytic activity of the enzyme-mimicking system.

3.1.2 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Figure 3.3 FTIR spectra of PF and GO-PF

The infrared spectrum of GO exhibits two broad and strong absorption peaks at 3400 cm^{-1} and 3230 cm^{-1}, attributed to the O-H stretching vibrations of carboxylic acid groups on the GO surface and intercalated water molecules, respectively. A peak at 1630 cm^{-1} corresponds to the C-C backbone stretching vibration of unoxidized graphite in the sp^2 hybridized structure. For the GO-peptide hydrogel, not only are the characteristic IR peaks of GO retained, but a new peak at 2892 cm^{-1} emerges, assigned to the alkyl C-H stretching vibrations in the peptide chains. Additionally, new peaks at 1538 cm^{-1} and 1256 cm^{-1} are



observed, corresponding to the N-H bending (amide II band) and C-N stretching vibrations of the peptide nanofibers, respectively. These results collectively confirm the successful decoration of GO nanosheets with peptide nanofibers.

3.1.3 Raman Spectroscopy Analysis

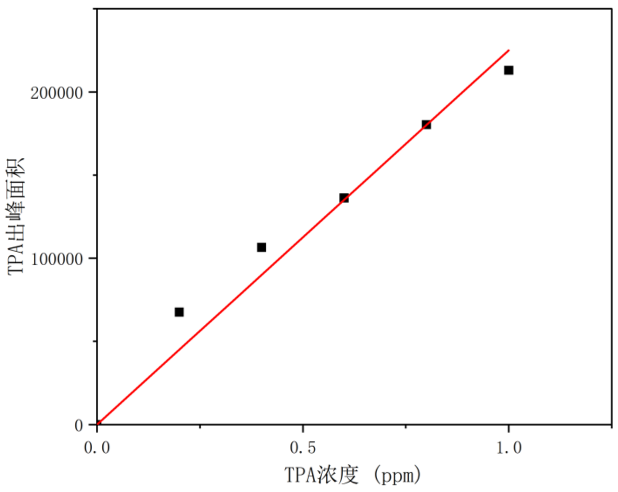
Figure 3.4 Raman spectra of GO and GO-PF

Raman spectra of graphene oxide-based composites (GO and GO-peptide nanofibers) exhibit two dominant peaks: the D band at 1359 cm^{-1} and the G band at 1585 cm^{-1}.

D band (disorder-induced band): Typically attributed to defects or disorder in the graphene structure, such as broken C=C sp^2 bonds in graphite layers, reflecting structural imperfections.

G band (graphitic band): Originates from the E_2g optical mode of sp^2-hybridized carbon atoms, representing the symmetry and order of the graphite lattice.

The degree of structural disorder in GO-based composites is often quantified by the I(D)/I(G) intensity ratio. A higher ratio indicates increased disorder and defects. For the samples GO and GO-peptide nanofibers, the calculated I(D)/I(G) values



are 0.75 and 0.89, respectively, demonstrating structural disruption and enhanced disorder in the graphene oxide within the composite system.

Comprehensive analysis combining Raman and FTIR spectroscopy reveals that the GO-peptide composite achieves structural optimization through oxygen-containing functional group introduction and defect engineering, providing a theoretical basis for designing functional composites. Future studies should integrate experimental data to deepen mechanistic insights and expand application validation.

3.2 Catalytic Activity Detection of Graphene Oxide-Peptide Fiber Hybrid Hydrogel

3.2.1 MHET Assay

Figure 3.5 Standard curve of the MHET product

The fitting equation derived from the regression analysis is $y_1=54202.36x_1$, with an R^2 value of 0.988, demonstrating a strong correlation between the fitted model and the experimental data.

TPA Assay

Figure 3.6 Standard curve of the TPA product

The fitting equation derived from the regression analysis is $y_2=225037.45x_2$, with an R^2 value of 0.990, demonstrating a strong correlation between the fitted model and the experimental data.

3.3 Degradation of PET by Graphene Oxide-Peptide Fiber Hybrid Hydrogel

3.3.1 Effect of Time on PET Degradation

Figure 3.7 shows that the product yield increases with reaction time in both the GO-peptide fiber and peptide fiber systems. However, the GO-peptide fiber system consistently exhibits higher product formation than the peptide fiber system within 12 hours. For example, at 12 hours, the product yield of the GO-peptide fiber system is 56.32 μM, compared to 44.39 μM for the peptide fiber system.

This result indicates that the integration of graphene oxide (GO) with peptide fibers significantly enhances the enzymatic-mimicking activity of the composite. The superior performance of the GO-peptide composite can be attributed to two key factors:

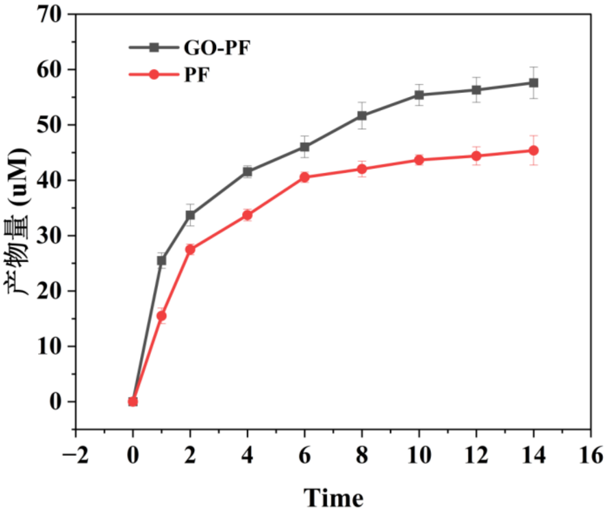


Figure 3.7 Effect of reaction time on simulated enzyme-catalyzed product yield

(1) Increased active sites: GO provides a high specific surface area, exposing more reactive sites for substrate binding and catalytic reactions.

(2) Structural stability: The interfacial interactions between GO and peptide fibers (e.g., π-π stacking or hydrogen bonding) stabilize the enzyme-mimicking structure, reducing inactivation during the reaction.

3.3.2 Effect of pH on PET Degradation

Figure 3.8 demonstrates that the enzymatic-mimicking activity varies significantly with pH. At pH 8.0, the GO-peptide fiber system exhibits a product yield of 45 μM, while the peptide fiber system yields 36.78 μM, indicating higher catalytic activity under these conditions.

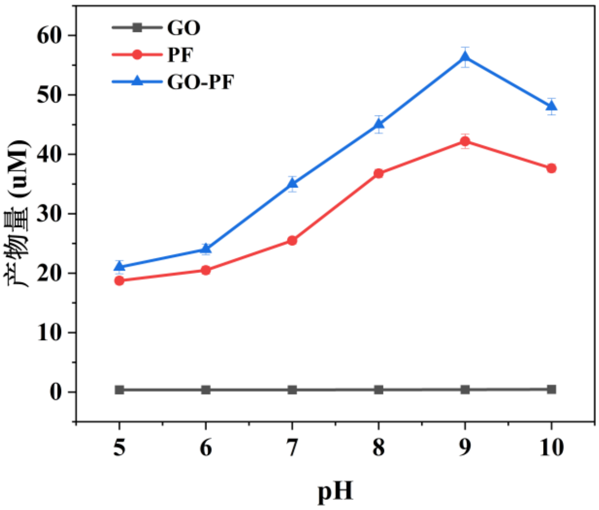


Figure 3.8 Effect of pH on simulated enzyme-catalyzed product yield

These results suggest that pH 8.0 is near the optimal reaction conditions for the enzyme-mimicking system. Under this environment, the active sites of the composite are more accessible for substrate binding, and the enzyme structure remains stable, facilitating efficient catalysis. Deviations from pH 8.0 lead to reduced activity, likely due to alterations in the enzyme's structural stability and microenvironment of active sites caused by changes in acidity/alkalinity, which weaken substrate-binding affinity and catalytic efficiency.

3.3.3 Effect of Temperature on PET Degradation

Figure 3.9 illustrates the effect of temperature on the enzymatic-mimicking activity of the GO-peptide fiber composite. At 50°C, the product yield reaches 56.32 μM, indicating that this temperature represents the optimal reaction condition for the simulated enzyme. When the temperature is below 50°C, reduced molecular motion decreases the frequency of collisions between the enzyme-mimicking system and substrates, leading to slower reaction rates and lower product yields. Conversely, temperatures exceeding 50°C may induce structural destabilization of the composite, reduce its catalytic activity and further decrease product formation.

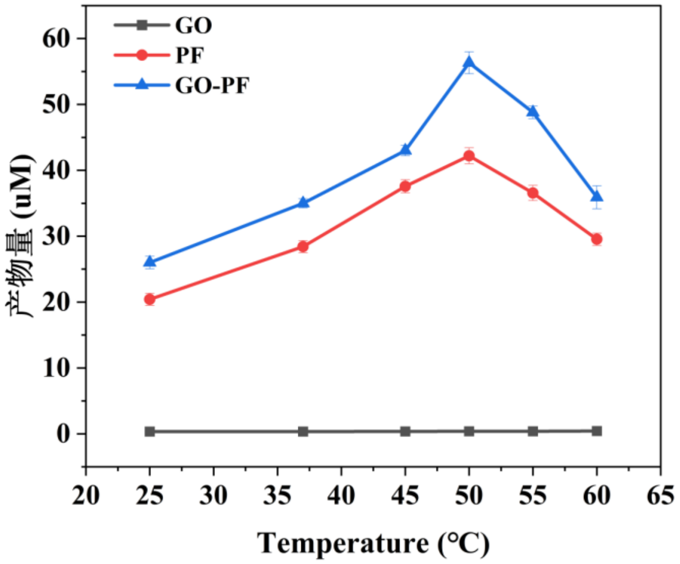


Figure 3.9 Effect of temperature on simulated enzyme-catalyzed product yield

Summary of chapter III

1. Structural characterization of the graphene oxide-peptide fiber hydrogel was conducted using transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and Raman spectroscopy.

2. The amount of product is calculated by fitting a standard curve using the peak area of the product, followed by quantitative analysis based on the established calibration model.

3. The catalytic activity of the hydrogel was systematically investigated by examining the effects of reaction time, temperature, and pH.

CONCLUSIONS

1. This study successfully developed a graphene oxide-peptide fiber hybrid hydrogel and systematically investigated its application in PET plastic degradation. By rationally designing peptide sequences and leveraging the superior properties of graphene oxide, a novel enzyme-mimicking system was constructed. Experimental results demonstrated significant enhancement in enzymatic-mimicking activity of the hybrid hydrogel, particularly highlighting its potential value in PET degradation.

2. In this research, the enzymatic-mimicking activity of the graphene oxide-peptide fiber composite was substantially improved compared to individual components. Structurally, graphene oxide provides an ultrahigh specific surface area, offering abundant active sites for reactions. This facilitates greater adsorption of PET substrates onto the simulated enzyme, enhancing product generation. Additionally, multiple non-covalent interactions—including electrostatic attraction, π-π stacking, and hydrogen bonding—between graphene oxide and peptide fibers stabilize the enzyme-mimicking structure, reducing inactivation during reactions and further promoting catalytic efficiency.

3. Structural characterization confirmed the successful formation of a hybrid system with nanofibrous architecture. Transmission electron microscopy (TEM) visually revealed the microstructure of the composite. Enzymatic activity assays indicated that graphene oxide integration significantly boosted catalytic performance. Reaction time, pH, and temperature critically influenced the enzyme-mimicking activity. Prolonged reaction time increased product yield, suggesting enhanced substrate conversion efficiency within optimal durations. The highest activity was observed at pH 8.0; deviations from this value reduced activity due to structural destabilization and altered microenvironments of active sites, weakening substrate binding. A temperature of 50°C was identified as optimal: lower temperatures slowed molecular motion and reduced collision frequency

between the enzyme and substrate, while higher temperatures disrupted the enzyme’s structure and active site configuration, leading to activity decline. These findings provide critical theoretical and practical insights for optimizing catalytic performance toward efficient PET degradation.

4. The simulated enzyme’s structure and active site functionality may degrade under suboptimal conditions, reducing activity. However, under optimized parameters, the hybrid hydrogel demonstrated exceptional PET degradation efficiency, offering a novel and effective strategy for addressing PET plastic pollution.

While significant progress has been made in the preparation of graphene oxide-peptide fiber hybrid hydrogels and their application in PET degradation, substantial improvements and refinements remain necessary. Although this study comprehensively elucidated the structural and functional properties of the hybrid hydrogel, its environmental tolerance and reusability under real-world conditions require deeper investigation. These two factors are critical criteria for evaluating the commercial viability of enzyme-mimicking systems. Therefore, future research must focus on evaluating the stability and reusability of the hydrogel under diverse environmental conditions (e.g., varying pH, temperature, and pollutant loads) to enhance its practical applicability.

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