



Bioremediation of PET-Contaminated Wastewater using Shrimp Waste Activated Sludge: A Combined in Silico–Experimental Study

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ABSTRACT

Polyethylene terephthalate (PET) plastic pollution is a long-standing environmental problem because it is highly resistant to natural degradation. This study investigates a poorly studied low-cost biocatalytic approach for the degradation of PET-derived wastewater using esterase-producing bacteria isolated from activated sludge of shrimp waste. Theoretical binding affinity and degradation performance were evaluated by the dual-approach strategy of molecular docking simulation (MOE software) and laboratory-scale degradation experiments. The docking analysis revealed that PET had a strong and stable binding interaction with the esterase enzyme, suggesting its catalytic ability. Shrimp waste activated sludge treatment experimentally resulted in reduction of Total Suspended Solids (TSS), Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) up to 95% within 10 hours. The degradation product of diethylene glycol (DEG) was confirmed by gas chromatography. These studies demonstrate a promising pathway towards an eco-friendly PET wastewater treatment based on waste valorization.

Keyword: PET Degradation, Shrimp Waste, Molecular Docking Simulation, Esterase Enzyme

ABSTRAK

Pencemaran plastik *polyethylene terephthalate* (PET) merupakan salah satu permasalahan lingkungan yang telah berlangsung lama dikarenakan material ini memiliki ketahanan yang sangat tinggi terhadap degradasi alami. Penelitian ini mengkaji pendekatan biokatalitik berbiaya rendah yang masih belum banyak diteliti untuk mendegradasi limbah cair yang berasal dari PET dengan memanfaatkan bakteri dari lumpur aktif limbah udang. Afinitas pengikatan secara teoritis dan kinerja degradasi dievaluasi menggunakan strategi dua pendekatan, yaitu simulasi molecular docking (software MOE) serta eksperimen degradasi pada skala laboratorium. Hasil analisis *docking* menunjukkan bahwa PET memiliki interaksi pengikatan yang kuat dan stabil dengan enzim esterase, yang mengindikasikan potensi katalitik enzim tersebut dalam mendegradasi PET. Secara eksperimental, pengolahan menggunakan lumpur aktif limbah udang mampu menurunkan *Total Suspended Solids* (TSS), *Chemical Oxygen Demand* (COD), dan *Biological Oxygen Demand* (BOD) hingga 95% dalam waktu 10 jam. Produk degradasi berupa dietilen glikol (DEG) berhasil dikonfirmasi melalui analisis kromatografi gas. Hasil penelitian ini menunjukkan bahwa pemanfaatan lumpur aktif limbah udang sebagai sumber biokatalis merupakan pendekatan yang menjanjikan untuk pengolahan limbah cair PET yang ramah lingkungan.

Kata Kunci: Degradasi PET, Limbah Udang, Simulasi Penambatan Molekul, Enzim Esterase



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1. Introduction

Polyethylene terephthalate is a thermoplastic polymer that has become one of the most widely used plastic materials in the world. PET has the chemical formula $(C_{10}H_8O_4)_n$ and is formed through a polycondensation reaction between ethylene glycol (EG) and terephthalic acid (TPA) (1). This material possesses superior properties such as high mechanical strength, good transparency, impact resistance, and gas impermeability, making it extensively used for beverage bottles, food packaging, textile fibers, and various other industrial applications (2).

The resistance of PET to natural degradation is one of the most significant environmental challenges. PET has extremely slow degradation rates in natural environments. The chemical structure of PET containing high aromatic components renders it highly resistant to microbial attack and extremely persistent in the environment (3). This degradation resistance is caused by several factors, such as the stability of ester bonds in the polymer chain, high crystalline structure, surface hydrophobicity, and high molecular weight. Exposure to PET can cause harmful effects on both humans and the environment. Consumed microplastics can cause chronic inflammation, cardiovascular diseases, diabetes, neurodegenerative diseases, cancer, and even stroke (4,5).

Plastic PET pollution in waters has reached alarming levels globally. Approximately 14 million tons of plastic, including PET, enter the oceans annually. By 2025, it is estimated that 75-199 million tons of plastic waste are in our oceans, with an additional 33 billion pounds of plastic entering the marine environment each year (6). Approximately 269,000 tons of plastic float on the ocean surface, while 4 billion microfibers per km² exist below the surface (7).

In the past decade, the enzymatic degradation of PET has been the focus of intensive research as an environmentally friendly alternative to address plastic pollution. A significant breakthrough occurred in 2016 with the discovery of the bacterium *Ideonella sakaiensis*, which can use PET as a primary carbon source (8). Besides *I. sakaiensis*, various other microorganisms, including *Gordonia* sp., *Bacillus* sp., *Rhodococcus pyridinivorans*, and *Thermobifida fusca*, have been reported to be capable of degrading PET [9], [10].

Shrimp waste is a nutrient rich microbial niche that contains proteins, lipids, minerals and chitin and has been found to contain bacteria with extracellular hydrolytic activities, including lipolytic and proteolytic activities (11). These properties make shrimp waste a suitable and low-cost source for screening hydrolytic potential bacteria. In this work, the discovery of coliforms and *Escherichia coli* in shrimp waste was regarded an initial microbiological observation as *E. coli* is known to have esterase activity, but the mere presence of *E. coli* did not confirm PET-specific esterase activity. Thus, a combination of the experimental and molecular-docking technique was used to study the possible role of esterase-producing microbes involved in PET transformation.

2. Materials and Methods

2.1. Materials

The materials used in this study included pre-degradation and post-degradation PET wastewater samples, activated sludge, and COD Cell reagent for chemical oxygen demand analysis. For the biochemical oxygen demand (BOD) dilution water, the following analytical-grade reagents were used: potassium dihydrogen phosphate (KH₂PO₄), disodium hydrogen phosphate (Na₂HPO₄), ammonium chloride (NH₄Cl), magnesium sulfate (MgSO₄), calcium chloride (CaCl₂), iron(III) chloride (FeCl₃), and 0.025 N sodium sulfite (Na₂SO₃) as the dechlorinating agent. Microbiological analysis was performed using differential culture media, namely Chromocult® Coliform Agar or m-ColiBlue24®, together with 0.45 µm pore-size, 47 mm diameter membrane filters for sample filtration. Additional materials included 70% ethanol for aseptic and sterilization purposes, and distilled water used throughout sample preparation and reagent dilution.

2.2. Methods

2.2.1. *E. coli* and Identification

E. coli and coliforms were identified using membrane filtration (0.45 µm) with Chromocult® or m-ColiBlue24® media. The media and apparatus were sterilized in an autoclave at 121°C for 15 min. The shrimp sample was filtered through a filter membrane, placed on the surface of the media, and incubated at 37°C for 24 h. For *E. coli* identification, additional incubation was performed at 44,5°C for 2 h. Identification was based on changes in colony color.

2.2.2. Molecular Docking using Molecular Operating Environment (MOE) Software

The esterase enzyme structure was obtained from rcsb.org with ID 3BF7 (*E. coli*). Esterase enzyme structures were removed, leaving only the first sequence and water molecule. Furthermore, the preparation was conducted using MOE software (version 2014.0901). Initially, protonation was conducted using the LigX method with a strength setting of 100,000 and energy minimization by adjusting the gradient to 0.05 kcal/mol. Å², and the result was saved in PDB format. The active enzyme site was determined by conducting a site finder analysis and selecting the appropriate site, as described in the reference. The PET structure was obtained from Pubchem (<http://pubchem.ncbi.nlm.nih.gov>) with ID 8721140. Before conducting molecular docking, 2 nearly

identical stages were performed, including washing, partial charge assignment, and energy minimization. The force field was changed to the AMBER10 force field, and the structures were saved in the.mdb format, while the solvation of the protein and ligand structures was set to the gas phase. Docking was performed using a retention ratio of 100:3. The best conformations from the docking test were selected based on the lowest Gibbs free energy of binding ($\Delta G_{\text{binding}}$) and simulations with root-mean-square-deviation (RMSD) values $< 2 \text{ \AA}$, which were further processed using a flexible docking method (12–15).

2.2.3 PET Degradation

This study used a prototype laboratory-scale wastewater treatment plant with a total effective volume of 20 L. PET industrial wastewater obtained from PT Polyprex Indonesia was degraded using the activated sludge method inoculated with shrimp waste microorganisms which collected food industry factory around Cikande, Banten. The volume of the aeration tank was designed to be around 12-16 liters of the total system volume, with a length of 40 cm, a width of 25 cm, and a height of 20 cm. Figure 1 shows the prototype wastewater treatment with activated sludge.



Figure 1. Waste water treatment installation prototype with shrimp waste

The wastewater entered the aeration tank at a controlled flow rate of 90 mL/min. The degradation process was conducted for up to 10 h, and the BOD, COD, and TSS parameters of the wastewater were measured before and after the degradation process.

2.2.4. Determination of Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD)

The chemical oxygen demand was determined with these procedures. PET wastewater was collected before and after the degradation process. A 2-mL sample was placed into a prepared HACH vial reagent and gently shaken until homogeneous. The sample was heated using a HACH DRB 200 COD heater at 150°C for 120 min. The sample was cooled to room temperature after reaching the desired temperature. The COD parameters were measured using a HACH DR 900 spectrophotometer, and the values were recorded on the instrument display.

While biochemical oxygen demand was measured with 1000 mL of distilled water was oxygenated for 30 min, then remeasured and transferred to a 1000 mL beaker. Phosphate buffer (pH 7.2), iron (III) chloride (0.0001 M), calcium chloride (0.2 M), and magnesium sulfate (0.1 M) were added to 1 mL of each and stirred until homogeneous. The pH was measured and adjusted to 7 by adding NaOH dropwise if necessary. The volume of the solution was reduced to 900 mL, and 100 mL of the sample was added and stirred until homogeneous.

Two BOD bottles were filled to the brim with the solution. The first bottle was incubated for 5 days, and the second bottle was immediately analyzed. For the initial analysis (DO_0), 2 M manganese sulfate (1 mL) and 1 M alkali-iodide-azide (1 mL) were added, shaken until homogeneous, and then allowed to settle. After settling, 2 mL of sulfuric acid was added, and the solution was shaken until dissolved and allowed to settle again until the sediment was completely dissolved.

A 100 mL solution was transferred to a 250 mL Erlenmeyer flask and titrated with 0.025 N sodium thiosulfate using 3 drops of starch indicator until the color changed from dark brown to clear. The BOD levels are calculated using the following Equation 1.

$$BOD = (DO_0 - DO_s) \times \text{dilution factor} \quad (1)$$

Where, DO_0 is initial dissolve oxygen and DO_s is final dissolve oxygen

2.2.5. Determination of the Total Suspended Solid (TSS)

The empty filter paper was dried in an oven at 105°C for 1 h, placed in a desiccator for 10 min, and then weighed. The filter paper was placed in a filter funnel, and a 20 mL sample was filtered, followed by washing with 20 mL of distilled water. The filter paper was dried again at 105°C for 1 h, cooled in a desiccator for 10 min, and weighed. The TSS content was calculated using the following Equation 2.

$$TSS \left(\frac{mg}{L} \right) = \left[\left(\frac{A-B}{V} \right) \right] \times 1000 \quad (2)$$

Where, A is weight of residue-containing filter paper (mg), B is weight of empty filter paper (mg) and V is sample volume (mL).

2.2.6. Gas chromatography (GC) Measurement

After degradation, the PET wastewater was measured using PerkinElmer Clarus 690 FID-GC (Flame Ionization Detector Gas Chromatography) with Glass/Steel 10% Carbowax 20M on 80/100 Supelcoport/Chromasorb WHP as stationary phase. The degraded product after the degradation process was analyzed.

3. Results and Discussion

3.1. Microorganism Identification

This study only identified coliform and *E. coli* bacteria due to equipment limitations. *E. coli* and coliform bacteria are generally known to degrade simple organic compounds such as glucose, acetate, and amino acids, although other bacteria were not identified. Some studies have shown that coliform and *E. coli* bacteria can degrade other aromatic compounds (9,10,16).

The identification results indicate an increase in coliform bacteria after the degradation process. The increase in total coliform count depicts microbial growth during treatment. This finding aligns with previous studies showing that *E. coli* and coliform can excrete esterase enzymes to digest PET compounds found in wastewater as a carbon source for energy production (17,18).

Table 1. Identification of microorganisms in shrimp waste

Microorganism	Before degradation (CFU/mL)	After degradation (CFU/mL)
<i>E. Coli</i>	TNTC	TNTC
Total Coliform	64	173

3.2. Molecular Docking

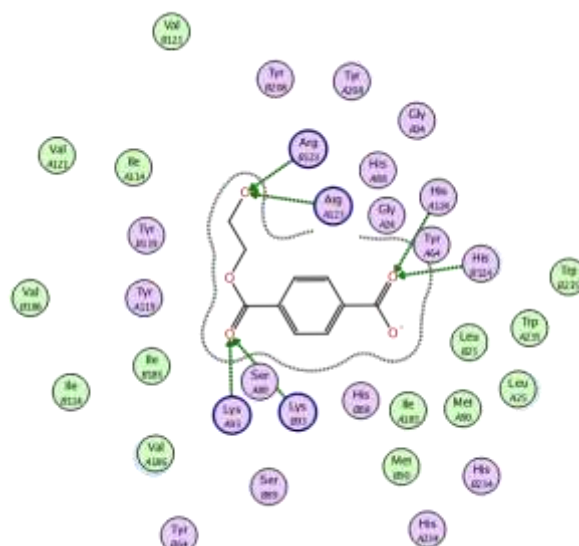


Figure 2. The visualization of PET-Esterase enzyme interaction

Based on these previous results, this study considered the esterase enzymes produced by *E. coli* and coliform in shrimp waste and conducted in silico studies to determine the potential of these microorganisms to degrade PET compounds. Molecular docking studies between the esterase enzyme and positron emission tomography were conducted using the flexible docking method. Flexible docking is a method that assumes that the receptor/enzyme structure is semi-rigid or flexible. The results of molecular docking are shown in Figure and Table 2. The esterase enzyme interacts well with PET, as indicated by a binding energy of -7,742 kcal/mol, indicating its potential for PET plastic degradation. The PET structure interacts nonpolar with the enzyme on the chain of residues Tyr64, Ser89, Lys93, Arg123, and His124 through carbonyl and hydroxyl groups (7).

Table 2. The molecular docking result of PET with the esterase enzyme

Enzyme ID	Binding Energy	RMSD	Residue Chain Interaction
3BF7	-7.742 kcal/mol	1.208	Tyr64, Ser89, Lys93, Arg123, and His124

3.3. COD, BOD, and TSS of PET Wastewater After Degradation

The degradation treatment was performed in a prototype flow activated sludge reactor with an activated sludge system for 2, 4, 6, 8, and 10 h, with a ratio of 10,000 mL of activated sludge to 5,000 mL of wastewater. Table 3 shows that the operating time can reduce the COD concentration in deteriorated wastewater by 90.35% to 97.5%. The higher the operating time in wastewater treatment, the lower the COD concentration. Several other studies have found a similar tendency, with longer operating times removing more COD. This is consistent with the findings of Thapa et al. (2022), who found that increasing the operating time from 1 to 12 h can minimize COD by up to 93% (19).

Table 3. COD measurement results of PET wastewater treatment

Time (hours)	COD before degradation (mg/L)	COD after degradation (mg/L)	COD reduction efficiency (%)
2	1400	135	90.35
4	1400	91	93.5
6	1400	84	94.0
8	1400	57	95.9
10	1400	35	97.5

The BOD measurement results are presented in Table 4. Wastewater samples were collected from shrimp waste inoculum before and after degradation with activated sludge and evaluated using the Winkler titration method. According to the results, activated sludge from shrimp waste inoculum successfully reduced the BOD

content from 76.4% to 84.8%. The findings of this study are consistent with those of a previous study (20), which found that wastewater treatment using activated sludge on a laboratory scale with an 8–12 h residence period can reduce BOD by 72%–93% (21).

Table 4. BOD measurement results of PET wastewater treatment

Time (hours)	BOD before degradation (mg/L)	BOD after degradation (mg/L)	BOD reduction efficiency (%)
2	250	59	76.4
4	250	54	78.4
6	250	49	80.4
8	250	42	83.2
10	250	38	84.8

Furthermore, the TSS parameter was examined to determine the efficacy of activated sludge inoculated with shrimp waste microbes in reducing suspended solids in wastewater. When discharged directly into the environment, TSS is a useful indicator of wastewater quality because excessive levels of suspended particles can induce turbidity, restrict light penetration, increase organic matter load, and disturb aquatic ecosystems.

Table 5. TSS measurement results of PET wastewater treatment

Time (hours)	TSS before degradation (mg/L)	TSS after degradation (mg/L)	TSS reduction efficiency (%)
2	405	70	82.7
4	405	40	90.1
6	405	35	91.3
8	405	25	93.8
10	405	20	95.1

Based on these findings, TSS was significantly reduced at each operating time, with the highest efficiency of 95.06% at 10 h. This decrease imply that the microorganisms in the activated sludge are excellent in assisting the flocculation and sedimentation processes. This result is consistent with that of Wang et al. (18), who reported that microorganisms capable of creating extracellular polymeric substances (EPS) operate as natural bio flocculants, speeding up the sedimentation process.

3.4. GC Analysis of PET Degraded Product

GC analysis was performed to identify specific degradation products produced from the PET degradation process by shrimp waste. Figure 3 shows the GC analysis results of the presence of benzyl alcohol (BA) and diethylene glycol (DEG) in the samples after PET degradation, which showed peak of 5.50 and 6.76 retention time, respectively. Benzyl alcohol may be detected in GC analysis because its residue may persist in the polymer matrix during PET processing or react with PET to form benzyl ester derivatives (dibenzyl terephthalate). Subsequent hydrolysis of these esters would release benzyl alcohol as a degradation product (22). While, DEG is a compound with the chemical formula $\text{HO-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-OH}$, consisting of two EG units connected by an ether bond (23). The identification of DEG as a degradation may indicated PET degradation. This finding is consistent with previous PET degradation studies reporting the formation of various oligomers and byproducts besides the main monomers (24).

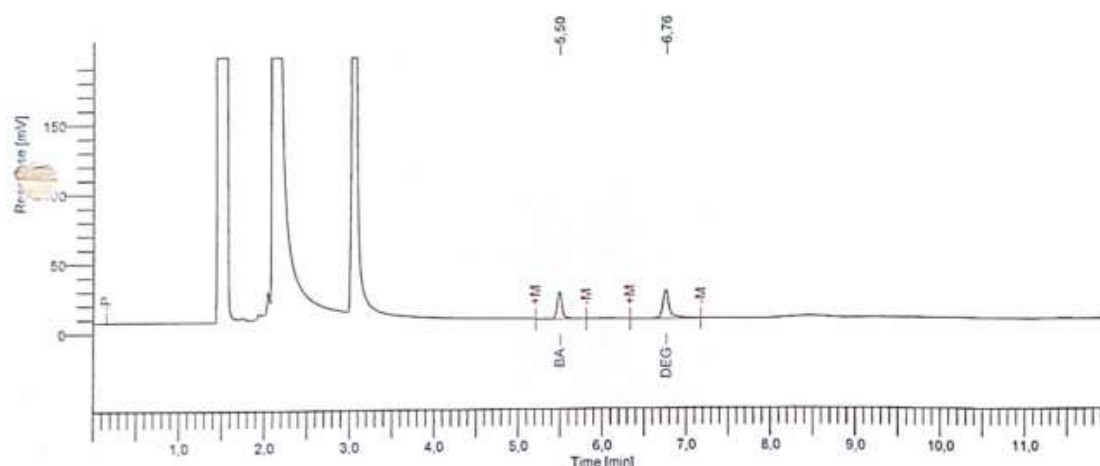


Figure 3. GC measurement results of PET wastewater after degradation treatment

4. Conclusions

Nevertheless, several limitations should be acknowledged. The present study was conducted using a laboratory scale treatment system and a relatively short treatment duration of 10 h. In addition, the experimental conditions were controlled and do not fully represent the complexity and variability of full-scale industrial wastewater. The absence of PET specific mass balance measurements and comprehensive identification of degradation intermediates also limits the interpretation of the biodegradation mechanism. Further studies should therefore evaluate longer treatment periods, independent reactor replicates, PET specific degradation products, and pilot-scale continuous operation before industrial scale application and techno-economic assessment are considered.

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