Degradation experiment

Experimental Materials and Instruments

Materials :

Polymer samples: PBCF film and PBFGA film (thickness ~0.3 mm, initial mass ~100 mg/sample).

Enzyme: Porcine pancreatic lipase (≥100 U/mg, source specified; activity tested before use).

Buffer: 0.1 M phosphate-buffered saline (PBS, pH 7.4; prepared with $Na_2HPO_4 \cdot 12H_2O$ 10.86 g/L, KH_2PO_4 3.87 g/L in ultrapure water; sterilized by autoclaving at 121°C for 20 min).

Other: Anhydrous ethanol (for sample cleaning), ultrapure water (resistivity ≥18.2 MΩ·cm).

Instruments :

Constant-temperature incubator (±0.5°C precision, 37°C).

Vacuum dryer (temperature range 30–60°C, vacuum ≤10 Pa).

Analytical balance (0.0001 g precision).

pH meter (±0.01 precision).

Sterile culture bottles (250 mL, airtight caps).

Ultrasonic cleaner (for sample preprocessing).

2. Pre-Experimental Preparation

2.1 Sample Pretreatment :

Cut polymer films into uniform size (e.g., 2 cm × 2 cm) using a punch.

Rinse samples 3 times with deionized water (5 min each) to remove processing residues.

Soak in anhydrous ethanol for 10 min (remove organic contaminants), then rinse with ultrapure water until neutral (pH test paper).

Dry at 60°C under vacuum to constant weight (two consecutive weighings differ by ≤0.0002 g); record initial dry mass (m_0 , 0.0001 g precision).

2.2 Enzyme Solution Preparation :

Dissolve porcine pancreatic lipase in 0.1 M PBS (pH 7.4) to prepare a 0.1 mg/mL solution (prepare fresh; avoid repeated freeze-thaw cycles).

Test enzyme activity (e.g., olive oil hydrolysis assay) to confirm activity ≥80 U/mg.

2.3 Control and Experimental Groups :

Experimental group (enzyme-mediated degradation) : 3 parallel samples per polymer, placed in 200 mL of 0.1 mg/mL lipase-containing PBS (volume sufficient to fully submerge samples).

Control group (pure hydrolysis) : 3 parallel samples per polymer, placed in 200 mL of enzyme-free 0.1 M PBS.

Label all culture bottles; incubate in a 37°C incubator (avoid light to prevent photo-oxidation).

3. Degradation Process

3.1 Film Degradation (Sampling Every 4 Days) :

Day 0 : Complete pretreatment; record m_0 ; place samples in labeled culture bottles and seal.

Days 4, 8, 12, etc. (until mass stabilizes) :

(1) Sampling : Use forceps to gently remove samples (avoid mechanical damage); transfer to pre-weighed sterile Petri dishes (dried at 60°C for 30 min).

(2) Cleaning : Rinse samples 2 times with ice-cold 0.1 M PBS (30 s each) to remove residual enzyme and degradation products.

(3) Drying : Dry samples + Petri dishes in a vacuum dryer (40°C, ≤10 Pa) for 24 h; cool in a desiccator (with silica gel) for 30 min.

(4) Weighing : Measure mass of dried samples + Petri dishes (m_1) with an analytical balance (average of 3 readings).

(5) Medium replacement : Discard old medium; rinse culture bottles 2 times with sterile PBS; add 200 mL of fresh enzyme-containing (experimental) or enzyme-

free (control) PBS; return samples to incubation.

3.2 Film Degradation (Sampling Every 7 Days) :

Follow the same procedure as PBCF, but adjust sampling interval to Days 0, 7, 14, etc.

4. Data Recording and Analysis

Degradation Rate Calculation :

Residual mass fraction (R) : R(%)=(m0 mt)×100, where mt = average dry mass at time *t*, m0 = initial dry mass.

Mass loss rate (D) : D(%)=[1−(m0 mt)]×100.

Degradation rate constant (k) : If following first-order kinetics (ln(R)=−kt+ln(R0)), fit linear regression to calculate *k* (slope; R0 =100%).

5. Notes

Sterility : Sterilize buffer/enzyme solutions and seal culture bottles to prevent microbial contamination (critical for controls).

Mechanical Protection : Handle samples gently with forceps; avoid friction during rinsing to prevent film damage.

Constant Weight : Confirm dry mass stability (≤0.0002 g difference between two weighings).

Temperature Control : Calibrate incubator regularly to maintain 37°C ± 0.5°C.

Sample Consistency : Ensure thickness/size variations ≤5% for parallel samples to minimize bias.

6. Safety and Maintenance

Dispose of enzyme-containing waste according to institutional guidelines.

Clean culture bottles and instruments after use to avoid cross-contamination.

Regularly calibrate the analytical balance and pH meter for accuracy.