Characterization of the PET-Depolymerizing Activity Using the Amorphous PET Powder

The PET-depolymerizing activity of the purified enzyme was evaluated using amorphous PET powder as the substrate. Unless otherwise stated, the standard reaction mixture consisted of 30 μg of purified enzyme and 10 mg of amorphous PET powder suspended in 1 mL of 0.1 M phosphate buffer (pH 8.5). Reactions were carried out in 2 mL Eppendorf tubes incubated at 72 °C in a thermomixer with shaking at 800 rpm to ensure homogeneous suspension of the PET particles.

To determine the effects of pH and temperature on enzymatic activity, the reactions were performed under identical conditions but with buffers adjusted to pH 7.0, 7.5, 8.0, 8.5, or 9.0, and at reaction temperatures of 69, 72, 75, 78, 81, and 84 °C, respectively. For time-course analysis, aliquots of the reaction mixture were withdrawn at predetermined intervals. Each reaction was terminated by adding 1 mL of ice-cold acetonitrile, followed by centrifugation at 12,000 × g for 10 min at 4 °C to remove residual PET particles. The supernatant was collected for product quantification.

The concentrations of terephthalic acid (TPA), mono-(2-hydroxyethyl) terephthalate (MHET), and bis-(2-hydroxyethyl) terephthalate (BHET) were quantified by high-performance liquid chromatography (HPLC). HPLC analysis was performed using an

LC-20AT system (Shimadzu, Kyoto, Japan) equipped with dual pump modules, an autosampler, a thermostatted column oven maintained at 40 °C, and a UV detector set at 240 nm. Separation of depolymerization products was achieved on a ZORBAX Extend-C18 column (150 × 4.6 mm, 5 μm, Agilent, USA) using a binary mobile phase consisting of solvent A (0.1% v/v trifluoroacetic acid in water) and solvent B (acetonitrile). The flow rate was maintained at 0.6 mL/min with an isocratic elution program of 80% solvent A and 20% solvent B. Standard calibration curves of TPA, MHET, and BHET were established to ensure accurate quantification.

For kinetic analysis, depolymerization reactions were conducted under optimal conditions (72 °C, pH 8.5) for 30 min using varying enzyme concentrations (0-12 μM) with 10 g/L amorphous PET powder as the substrate. All reactions were performed in triplicate to ensure reproducibility. The initial reaction rates were determined based on the linear increase in product concentrations, and Michaelis–Menten kinetic parameters were calculated using GraphPad Prism software (GraphPad Software, San Diego, CA, USA).