Chapter 1

Introduction

1.1 Background

The escalating issue of plastic waste, especially polyethylene terephthalate (PET), has become a critical environmental concern. Global plastic production has increased dramatically reaching 400.3 million tons in 2022 and projected to rise to 1,800 million tons by 2050 where packaging industries accounts for 39.1% (Lee et al., 2024). Within this industry, PET is popularly used thermoplastic polymers due to its durability and longevity for packaging purposes. A study from Benyathiar et al (2022) stated that PET bottles contribute 67% of the market share in the beverage industry in London. Despite its popularity, PET poses a threat to the environment due to its stable polymer structure which makes it resistant toward degradation (Anuar et al., 2022). This structure allows PET to persist in landfills and oceans, leading to extensive waste accumulation overtime (Nisticò, 2020). Additionally, a study indicated that 90% of packaging plastic is a single use in which less than 10% packaging waste is recycled (Papadopoulou et al., 2019). As a result, the number of PET waste is uncontrollably increasing and negatively affecting the environment as well as human health. Considering this, the recycling method is insufficient to address the management of PET waste (Gabriel et al., 2023). Thus, an advanced technique is necessary to ease the PET waste problem.

Environment-friendly methods, such as enzymatic degradation, has been studied for its ability to break down PET into its monomers (Lopez-Lorenzo et al., 2024). Cutinase enzyme is particularly known for its capability to depolymerize PET into terephthalic acid (TPA) as the final degradation product, therefore, this enzyme is widely used for PET degradation study. It has a catalytic triad that enables the enzyme to degrade both natural polyesters like cutin as well as synthetic polyesters such as PET (Anuar et al., 2022). With its capability to hydrolyze ester bonds and have a high substrate specificity, it allows it to target the polymer structure of PET. Moreover, the hydrophobicity of the cutinase active site increases its capacity to bind toward the PET surface.

Cutinases, in particular, TfCut2 from *Thermobifida fusca*, have a high thermal stability, enabling them to remain active at high temperatures to promote effective degradation (Sui et al., 2023). However, the degradation process is often limited by low enzyme-substrate binding affinity due to the insufficient hydrophobicity and electrostatic repulsion between the enzyme and PET surfaces, which limit the enzyme adsorption and reduces the catalytic activity (Urbanek et al., 2021). Despite the hydrophobicity of cutinase active site, the area surrounding the active site will also affect the binding between cutinase and PET surface. The area near the active site of TfCut2 is negatively charged which is the same as the PET surface, hence, electrostatic repulsion occurs. So, the enzyme tends to remain in solution and not attached to the PET surfaces, limiting the degradation process to occur. Additionally, the crystallinity characteristic of PET causes low accessibility for enzymatic hydrolysis, thereby limiting the efficiency of attachment and hindering the overall degradation process (Pasula et al., 2022). To overcome these weaknesses, this research focuses on enhancing enzymatic PET degradation through the use of amphiphilic molecules such as an N-terminal modifying reagent known as 1*H*-1,2,3-triazole-4-carbaldehyde (TA4C) reagent developed by Onoda et al. (2020). This approach is highly applicable because the N-terminal of enzymes is solvent-accessible and suitable for precise modification without altering the structure and functionality of the protein (Deng et al., 2020). TA4C is a flexible reagent with a high specificity which enables a precise modification on the N-terminal of the enzyme with a high conversion rate. This facile method will introduce hydrophobic moieties onto the enzymes, thereby improving their binding affinity toward PET surfaces and enhancing catalytic efficiency. The modifications are designed to anchor the enzyme to work more effectively in degrading PET, thus increasing degradation performance.

In this study, TA4C-derived reagents are used to modify cutinase enzymes and investigate their effectiveness in degrading PET. The reagents will covalently attach to the amino group of cutinase, and the effects of these modifications is evaluated on both high-crystallinity PET (hcPET) powder and amorphous PET (A-PET) film. There are three types of TA4C-derived reagents that are used for comparison including C_{12}-TA4C, C_{16}-TA4C and C_{18}-TA4C reagents, to investigate their

effectiveness in degrading PET. The enzymatic activity is quantified by measuring the amount of terephthalic acid (TPA) produced using High-Performance Liquid Chromatography (HPLC). In addition, the most promising reagent on A-PET film degradation is further investigated for enzyme adsorption which is observed through Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and weight loss observation.

1.2 Objective

The research aims to evaluate the N-terminal modified cutinase by TA4C-derived reagents in enhancing PET degradation by quantifying terephthalic acid (TPA) production

1.3 Hypothesis

H_0 : The enzymatic PET degradation efficiency of cutinase is not significantly enhanced via N-terminal modification by TA4C-derived reagents as surfactant

H_1 : The enzymatic PET degradation efficiency of cutinase is significantly enhanced via N-terminal modification by TA4C-derived reagents as surfactant