Chapter 1

Introduction

1.1 Background

Keratin is a water-insoluble, fibrous protein that is hard to degrade by proteolytic enzymes due to the cystine disulfide bond in its structure (Qiu et al., 2020). Keratin can be classified into α-keratin or β-keratin; α-keratin consists of α-helix-coil, which assembles into filaments, while β-keratin is rich in β-pleated sheet. Chicken feathers typically consist mainly of β-keratin (Ghaffar et al., 2018). This characteristic makes keratin-rich waste, such as poultry feathers, challenging to break down using conventional proteolytic enzymes. Annually, the poultry industry produces approximately 7 million tons, creating a severe solid-waste problem. These feathers are usually discarded or burned as waste and cause air pollution. Hence, a more sustainable disposal of chicken feathers is a significant constraint for the poultry industry. Past studies have researched a more sustainable approach to degrading or recycling poultry waste containing keratin using microorganisms. Hydrolyzed keratin that is produced through the microbial enzymatic breakdown is a highly valuable material due to its properties that are rich in amino acid composition, These amino acids are beneficial in industries as raw material to produce animal feed or cosmetics (Shestakova et al., 2021).

Bacillus species produce keratinase, an enzyme that breaks down keratin by breaking the disulfide bond, resulting in a soluble protein (Sunilson et al., 2020). The bacterium secretes keratinase enzymes under nutrient deficiency stress conditions that effectively break down keratin within 17-48 hours (Sridharan et al., 2021; He et al., 2018). Past studies have shown that *B. subtilis* demonstrates superior feather degradation potential to other bacteria, such as *Bacillus licheniformis* (Veenayohini & Sageetha, 2016). *B. subtillis* has been shown to secrete keratinase, an enzyme capable of degrading keratin-rich materials like feathers (Mousavi et al., 2013; Cai et al., 2008). There are several factors that may affect efficiency of microbial degradation of chicken feather. These includes difference in microbial strain, medium nutrient content, temperature, pH, and fermentation duration. Among

these factors, fermentation time plays an important role. The duration of fermentation determines enzyme secretion activity, state of feather degradation, and accumulation of degradation products. If the duration of fermentation is too short, keratin may not be degraded properly, while prolonged fermentation may lead to keratin degraded completely into simple amino acids, discarding the keratin hydrolysate completely. This factor is even more important as different strain of bacteria have different degradation speed (Cai et al., 2008).

There are 5 strain of *Bacillus* species that are known to produce keratinase from past project at i3L, 5 of them are *Bacillus licheniformis, B. subtillis (*N5NA), *B. subtillis* (N10NR), *B. subtillis* (N5NC), *B. subtillis* (N10ND), these strain will be tested at 37°C condition for 3 days to determine which strain has the highest feather degradation efficiency. The optimal conditions for keratinase production by *B. subtilis* vary, with a study reporting maximum enzyme activity at 40°C and pH 11 (Mousavi et al., 2013), while others found more optimal conditions at 23°C and pH 7.5 (Cai et al., 2008). Another factor in the optimization of keratin production is fermentation duration. Fermentation duration significantly impacts enzyme production and activity, with longer fermentation times generally resulting in higher enzyme activity, with 6 days yielding optimal results for mannanase, protease, and cellulase production (Mirnawati et al., 2019).

1.2 Objective

This experiment aims to evaluate the effect of different fermentation durations on keratin yield from chicken feathers using *B. subtilis*, to determine the optimal period for maximizing keratin extraction efficiency under submerged fermentation conditions.

1.3 Hypothesis

H_0: Fermentation duration has no significant effect on the amount of keratin extracted from chicken feathers using *B. subtilis.*

H_1: Fermentation duration significantly affects the amount of keratin extracted from chicken feathers using *B. subtilis*.