Chapter 1

Introduction

1.1 Background

Stunting, according to WHO (2015) is defined as an impaired growth of children resulting from chronic malnutrition, repeated infections, and inadequate psychosocial stimulation, and is commonly identified by a height-for-age value below the standard growth reference. In Indonesia, stunting remains as one of the most prevalent health problems that particularly affects children in its early life, where inadequate nutrient intake, such as protein that is essential for tissue growth, enzymatic production, and metabolic functions, would lead to long-term consequences such as reduced cognitive development or decreased productivity in adulthood (Beal et al., 2018). The high prevalence of the issue thus has caused an increase of national demand for affordable and high quality protein sources. The use of animal protein is becoming increasingly unsustainable economically due to the high production cost and limited accessibility as well as environmentally due to greenhouse gas emissions and huge water consumption (Appleton, 2016). Thus this calls for the need of alternative protein sources to sustain the national protein demand that is sustainable environmentally and economically speaking.

When comparing the known alternative protein sources, plant-based protein had long been deemed as a more sustainable option in comparison to animal protein due to its lower ecological footprint. However, it has limited digestibility and an incomplete amino acid profile that limits protein absorption (Coffey et al., 2023). Algae-based proteins are nutritionally rich and environmentally promising, but face challenges for consumer acceptability due to its challenges related to the stronger taste profile. Insect-based protein sources also provide high protein content, but have lower overall consumer acceptability and high pathogenicity risk (Fatima et al., 2023). In comparison, mycoprotein, a protein derived from fungal sources, contains the advantages of high protein levels with favorable amino acid composition, superior digestibility compared to plant proteins, and has better consumer acceptability than algae or insect based proteins, thus making it a promising alternative protein source to address the national protein demand (Pratama et al., 2025).

Commercial mycoproteins such as Quorn (made from *Fusarium venenatum*) are known to be expensive, due to the use of pure glucose as substrate of growth, thus there is still subject of interest for more sustainable mycoprotein source to be developed. Souza-filho et al. (2018) conducted a research on several fungal strains, and of all that have been tested and it has been found out that *Aspergillus oryzae* is known to produce the highest amount of mycoprotein. *Aspergillus oryzae* is known to be able to grow on cheap, soy based waste such as soy whey or okara as shown by Devanthi et al., 2024. In the end, soy whey is shown to have a better protein concentration than okara, and a further optimization by Pratama et al., 2025 have shown that the protein content produced by A. oryzae on an optimized soy whey medium is up to 53% (w/w). However, in comparison to mycoprotein grown on oat flour and starch wastewater, the produced biomass is relatively low at 4.9 g/L.

Preliminary findings from the author's work have shown that molasses added at around 3% glucose equivalent into soy whey medium have produced biomass at 16.05 g/L, yield of over 100 mg/g DW substrate under laboratory-scale batch cultivation. While these results demonstrate the feasibility of using low-cost substrates for mycoprotein production, conventional batch cultivation inherently limits productivity due to the fixed nutrient composition and the unavoidable transition from nutrient excess to depletion during growth. Such conditions restrict process consistency and reduce overall efficiency, particularly when considering scale-up and repeated production cycles (Vrabi et al., 2019). Repeated-batch fermentation offers a practical strategy to overcome these limitations by periodically renewing the cultivation medium while retaining an active fraction of the fungal biomass (Gabrielyan et al., 2026). Through successive cultivation cycles, this approach enables partial restoration of nutrient availability, prolongs the productive growth phase, and reduces downtime associated with complete culture restart.

However, the effectiveness of repeated-batch systems depends strongly on the design of the cycle parameters, including the frequency of medium replacement, the proportion of culture retained between cycles, and the resulting balance between nutrient availability and biomass concentration. Optimization of these parameters is therefore essential to maximize biomass yield, titer, and productivity while minimizing substrate waste, ensuring that mycoprotein can be produced consistently at high levels as a sustainable alternative protein source.

1.2 Objective

To optimize and improve Aspergillus *oryzae* mycoprotein production by using repeated batch fermentation as a cultivation strategy, as well as characterize the following produced biomass and medium to evaluate whether the cultivation strategy used is able to maximize the fungal biomass titer, volumetric productivity, and biomass yield.

1.3 Hypothesis

The null hypothesis (H0) is the use of repeated-batch fermentation as a cultivation strategy has no or minimum impact on the biomass titer and volumetric productivity. While the alternative hypothesis (H1) is the use of repeated-batch fermentation as a cultivation strategy has a significant impact on the biomass titer and volumetric productivity.