CHAPTER I

INTRODUCTION

1.1 Background

Shrimp is one of main commodities in fishery industry. The industry only uses mainly flesh of the shrimp and then the head, shell and tails will become a solid waste in the industry. Every year, the industry produces huge waste that could be an environmental hazard. About 75% of the total weight of shrimp was discarded as by-products. Thus, utilizing these wastes could develop added-value products which can be applied in many fields (Al-Hassan, 2016).

Chitin is one of the product that comes from crustacean shell waste. Chitin is a linear polysaccharide and the second most abundant natural polymer after cellulose. Chitin and its derivatives have been used in many applications like pharmaceuticals, textile, food and cosmetics (Junianto, *et al.*, 2013). Glucosamine is an amino sugar which plays an important role as a precursor in the biochemical synthesis of glycosylated proteins and lipids (Cahyono, *et al.*, 2014).

Glucosamine in the form of glucosamine sulphate, glucosamine hydrochloride, or N-acetyl-glucosamine is used in the treatment for ostheoarthritis, knee pain, and back pain (Benavente, *et al.*, 2015). Glucosamine can be obtained by direct breakdown of chitin from the shrimp waste using the fermentation process, chemical hydrolysis, enzyme process or any various combinations of these methods (Cahyono, *et al.*, 2014). Production of glucosamine by chemical hydrolysis has an

environmental issue. Fermentation process has several disadvantages like long process time, high capacity utilisation, high risk of contamination, difficulties in morphology, and challenges for upscaling and reproducibility. However for enzymatic hydrolysis has advantage that the selected enzyme can favour the production of monomer product over oligomer, high chance of reproducibility and potential for side reactions of contamination are minimized (Hodgins, 2001; McNeil, 2013).

Mucor circinelloides is one of the fungi that can convert chitin into N-acetyl-glucosamine (Veronica, 2018). There are some studies about the ability of bacteria and fungi to secrete a wide variety of enzymes. However, the activity of enzyme produces from bacteria and fungi are affected by pH, temperature and substrate concentration (Scanlon, *et al.*, 2018). Enzyme also can be applied in various application like medicine, agriculture, industry and environment (Romagnolo, *et al.*, 2017). Enzyme can be produced from intracellular and extracellular by bacteria and fungi. Extracellular enzyme is an enzyme that produced by bacteria or fungi outside the cell and it can easily harvest without breakdown the cell membrane of bacteria or fungi (Traving, *et al*., 2015). The research about chitinolytic activity from *Mucor circinelloides* has been conducted, however the study of production chitinase enzyme, enzyme activity and glucosamine production by fermentation with extracellular enzyme of *Mucor circinelloides* has not yet been studied.

1.2 Research Problem

Glucosamine can be obtained by direct breakdown of chitin from shrimp waste using fermentation process (Cahyono, *et al*., 2014). Glucosamine is beneficial in the pharmaceutical field, can be produced through chemical hydrolysis, enzyme process, and fermentation process. Chemical hydrolysis results in acidic residues which poses an issue to the environment, low and obstreperous yields. However, fermentation using chitinase will not have issue to the environment and higher yield. Chitinase can be obtained from intracellular and extracellular. Chitinase enzyme has wide-range of application such as producing chitooligosaccharides and N-Acetyl-glucosamine, isolation of protoplast, control of pathogenic fungi and treatment of chitinous waste (Saima and Roohi, 2013). Fermentation-based production by using extracellular chitinase enzyme from fungi is an alternative to produce glucosamine. *Mucor circinelloides* is one of the fungi that can convert chitin into N-acetyl-glucosamine (Calcagno, *et al*., 1997). However, the study on fermentation-based production of glucosamine by extracellular enzyme of *Mucor circinelloides* and the optimization of influencing factors such as pH, temperature, substrate concentration and fermentation time have not yet been thoroughly studied.

1.3 Objectives

The objectives of this research can be divided into two, consists of general objective and specific objectives.

1.3.1 General Objective

The general objective in this research is to produce glucosamine using the extracellular chitinase enzyme produced by *Mucor circinelloides*.

1.3.2 Specific Objectives

The specific objectives of this research were:

1. To determine the optimum temperature and pH for chitinase to produce N-Acetyl-glucosamine from chitin.
2. To determine the optimum substrate concentration and fermentation time to produce N-Acetyl-glucosamine.