CHAPTER I

INTRODUCTION

1.1 Background

Indonesia is an archipelagic country occupied by 17.508 islands with such great potential for aquaculture. Due to its location in fish-rich waters, Indonesia became one of the biggest seafood producers in Southeast Asia. Indonesia's aquaculture commodities include various type of fish, seaweed and also shrimp. Shrimp represent an important commodity in Indonesia's fisheries industry, for both domestic and export consumption. According to IBP (2015), as much as 24% of the domestic shrimp production was exported and it kept increasing every year.

As suggested, the suitable forms for exporting shrimp are headless, peeled and deveined. This condition contributes a problem to the environment as about 40% of the total shrimp mass was discarded as by-products, which is known as shrimp shells (Pruthi, 2000). Formerly, shrimp shells were used only as chicken feed, while the rest was dumped into the sea, which caused pollutants in coastal areas due to their slow degradation (Arbia, *et al*., 2013). Nowadays, a number of attempts have been made to utilize shrimp shells not only to solve environmental problems, but as a waste treatment alternative to produce something useful.

The major components of shrimp shells are proteins, chitin, minerals and carotenoids. Crustacean shell consists mainly of 30-40% protein, 30-50% calcium carbonate and 20-30% chitin (Arbia, *et al*., 2013). Chitin, a major component in the supporting tissues of crustaceans, is a linear polysaccharide with the structural

unit of N-acetylglucosamine linked by β-1,4 bonds and considered as the second most widespread biopolymer in nature after cellulose. Chitin and its derivatives have great economic value because of their biological activities also in industrial and biomedical applications (Brzezinska, *et al.*, 2013).

Chitin is used as a raw material for the production of chitin-derived products such as chitosans, oligosaccharides and glucosamine (Einbu, 2007). Glucosamine can be obtained by direct breakdown of chitin from the shrimp shells using the fermentation process, chemical hydrolysis, enzyme process, or any various combination of these methods (Cahyono, *et al.*, 2014). Glucosamine is an amino sugar which plays an important role as a precursor in the biochemical synthesis of glycosylated proteins and lipids. Glucosamine in the form of glucosamine sulphate, glucosamine hydrochloride, or N-acetylglucosamine is used in the treatment for osteoarthritis, knee pain and back pain.

On laboratory scale, glucosamine is usually produced by using the fermentation method. As mentioned by Krithika and Chellaram (2016), the microorganisms isolated from the shrimp shells could produce chitinase enzyme to produce glucosamine. According to Clark, *et al.* (2014) and Renneberg (2008), producing chitin derivatives from shrimp shells by utilizing enzyme fermentation is more efficient rather than using the whole cells. It is because the enzyme concentration is higher and very stable, resulting in higher and faster yield obtained, as there are no unwanted enzymes are present. In addition, compared to the chemically used production, there is no chemical waste produced that may harm the environment. Previous research done by Josephine (2017), showed that

one of the strains that has high chitinolytic activity was *Providencia stuartii* bacteria.

The enzyme extracted from *Providencia stuartii* may be intracellular or extracellular. The extracellular production has multiple benefits than the intracellular, such that the enzyme is already outside the cell. Thus, it is easier to be isolated and it is also more robust so less likely to be broken down by heat or chemicals (Renneberg, 2008). Furthermore, according to Pomeranz (2012), enzyme can work optimally at certain pH, temperature, substrate concentration and incubation time in order to grow successfully. According to Kokate (2011), the optimum stability and activity of extracellular enzymes are very much close to the optimum conditions for its microbial growth. Therefore, in this research, chitin from the *Penaeus monodon* shells, was degraded fermentatively using the crude and semi pure extracellular enzyme obtained from *Providencia stuartii* to produce N-acetylglucosamine on its optimum condition.

1.2 Research Problem

Shrimp shells are by-products resulted from the head and shells of the shrimp, produced mainly from the frozen shrimp export and from domestic consumption. Formerly, shrimp shells were known only as a pollutant, but after further research, shrimp shells evidently have such a great potential in the applications of biotechnology and medicine. Shrimp shells turned out to have 20-30% of chitin (Arbia, *et al*., 2013). Chitinase that is produced by different microorganisms have received increased attention due to their wide range of

biotechnological applications, especially in the production of N-acetylglucosamine.

Providencia stuartii is known to have high chitinolytic activity from previous research done by Josephine (2017). However, the study of glucosamine production by fermentation with crude and semi pure extracellular enzyme of *Providencia stuartii* has not yet been thoroughly studied. Therefore, this present study was aimed to ferment chitin with crude and semi pure extracellular enzyme obtained from *Providencia stuartii* bacteria, in expectancy to find the optimization of influencing factors towards enzyme activity, such as pH, temperature, substrate concentration and incubation time to produce N-acetylglucosamine.

1.3 Research Objectives

The objectives of this research were divided into general and specific objectives.

1.3.1 General Objectives

The general objectives of this research was to utilize *Penaeus monodon* shells to produce N-acetylglucosamine resulted from the fermentation using crude and semi pure extracellular enzyme obtained from *Providencia stuartii* bacteria.

1.3.2 Specific Objectives

The specific objectives of this research were:

1. To determine the optimum temperature and pH for the crude and semi pure extracellular enzyme obtained from *Providencia stuartii* bacteria to produce N-acetylglucosamine from chitin; and

2. To determine the optimum substrate concentration and incubation time to produce N-acetylglucosamine from the crude and semi pure extracellular enzyme obtained from *Providencia stuartii* bacteria.