CHAPTER I

INTRODUCTION

1.1 Background

Indonesia is an agricultural country who has various natural resources. Despite Indonesia's wide variation of natural resources, not all of them have been harnessed optimally. Shrimp is one example of Indonesian natural resources which gives high capital income through its exports. Indonesia can be considered as one of several countries that exports shrimp and also has the potential to become the biggest shrimp exporting country. According to MAI (2018), Indonesia has wide range of coastline which is 592.219 km and can be utilized as shrimp farm.

Although Indonesia has high amount of shrimp production, its utilization is only as a source of food while the shrimp shells are thrown away, causing a drastic increase in shrimp waste production (Kandra, *et al.*, 2011). Continued production of this biomaterial without the development of utilizing technology has resulted in waste collection, disposal and pollution problems. Shrimp shells contain many bioactive compounds such as pigments, amino acids, fatty acids and chitin which can be used in wide range of application in medical, therapies, cosmetics, paper, pulp and textile industries, biotechnology and food applications (Kandra, *et al.*, 2011)

Chitin is a copolymer of N-acetyl-D-glucosamine and D-glucosamine units linked with β-(1-4) glycosidic bond, where N-acetyl-D-glucosamine units are

predominant in the polymeric chain. The deacetylated form of chitin refers to chitosan (Muzzarelli, 2013). Chitin and chitosan are known to be natural polymers and they are non-toxic, biodegradable and biocompatible. Both chitin and chitosan can be found as supporting materials in many aquatic organisms, terrestrial organisms, and some microorganisms (Kim, 2010).

Chitinases, a group of enzymes capable of degrading chitin directly to low molecular weight products producing glucosamine (GlcN). Glucosamine is an amino monosaccharide utilized for the biosynthesis of glycoproteins and glycosaminoglycan. It has been promoted for use alone and in combination with chondroitin sulfate (CS) to treat and prevent osteoarthritis. Glucosamine is claimed to increase cartilage formation and CS is commonly advocated to decrease cartilage breakdown (Sardesai, 2011).

Providencia stuartii is one of chitinase producing bacteria. The chitinase enzyme later can be used for breaking down chitin into its derivatives, one of them is N-acetylglucosamine (Josephine, 2018). Semi-pure intracellular chitinase produced by *Providencia stuartii* has optimum condition at 40^{o}C and at pH 5 (Teja, 2018). Enzymatic method has several advantages compared to chemical method or fermentation by micro-organisms method. Breaking down substrate using enzyme is much faster and more efficient compared to other methods. Chemical method is usually more expensive, while fermentation method can take days or even weeks to finish the breaking down process. Although enzymatic method is faster and efficient, but enzymes can be only used once (Torchilin, 2012).

Recovery of an enzyme after its application is an important thing to be concerned for efficiency aspect. Thus, immobilization is considered as the solution for saving the enzyme for reuse. There are many techniques to immobilize enzyme, such as adsorption, covalent, encapsulation, entrapment and cross linking (Salman, *et al*., 2008). Covalent bonds are stronger due to the constant sharing of electrons between atoms. Alginates which is a biopolymer is commercially available, has diverse features, and available at a reasonable cost, which make alginates a good candidate for entrapment method of enzyme immobilization (Hugerth, *et al.*, 1997).

Some studies have researched the immobilization of chitinase enzyme and continuous production of N-acetylglucosamine with the immobilized enzyme, however the study of immobilization of chitinase enzyme using calcium alginate and its application for producing N-acetylglucosamine continuously has not yet been done.

1.2 Research Problem

N-acetylglucosamine can be very useful for building cartilage. Cartilage is a flexible, tough connective tissue found in several parts of the body (Kim, 2010). There are several methods which can produce N-acetylglucosamine from its polymer, chitin. Chemical hydrolysis results in acidic residues and low yield, which may also cause environmental issues; fermentation method requires longer time because of the breakdown process which need days or even weeks; while enzyme method is relatively quick and efficient but enzymes can be only used once (Taherzadeh and Karimi, 2007). Thus, immobilization of enzyme was expected to increase the efficiency of enzymatic process/reaction. Chitinase is an enzyme which

can breakdown chitin into its monomer, N-acetylglucosamine. *Providencia stuartii* is a bacterial strain which can produce chitinase enzyme. However, the study on production of N-acetylglucosamine by immobilized chitinase extracted from *Providencia stuartii* has not yet been thoroughly studied.

1.3 Objectives

1.3.1 General Objectives

The general objectives of this research were to immobilize semi pure intracellular chitinase from *Providencia stuartii* and apply it in N-acetylglucosamine production by entrapment method using calcium alginate.

1.3.2 Specific Objectives

The specific objectives for this research were:

1. To prepare enzyme immobilization with sodium alginate.
2. To determine the effect of number of fermentation cycles on N-acetylglucosamine production using the immobilized enzyme.