CHAPTER I
INTRODUCTION

1.1 Background

Shrimp is a valuable aqua cultural product and is usually processed for the flesh, leaving the shell and head portions as bio-waste or sold to animal feed manufacturers (Rao and Stevens, 2006). In recent years, the production of shrimp waste from shrimp processing industries has undergone dramatic increase. Shrimp industries generate large amount of shrimp bio-waste during processing, contributing to 45-55% of the weight of raw shrimp. However, this bio-waste can be further processed to produce value-added products such as chitin (Lertsutthiwong *et al.*, 2002).

Chitin is a natural biopolymer that is non-toxic, biodegradable and biocompatible in nature (Anitha *et al.*, 2014). The exoskeleton of the black tiger shrimp contains up to 10% chitin, up to 40% protein and 45-50% mineral. The extraction of chitin from shrimp shell involves two steps, including demineralization and deproteinization, which can be conducted by chemical or biological method. The chemical method requires the use of acids and bases, while the biological method involves microorganisms (Arbia *et al.*, 2012). Chitin along with chitosan, the deacetylated chitin derivative, have been widely used for various biological and biomedical applications including treatment for burns, wound dressing and development of artificial skin. They also show antimicrobial, anticholesterol and antitumor activity (Dutta *et al.*, 2004).

Glucosamine is the end hydrolytic product of chitin that can be obtained by chemical and enzymatic hydrolysis. Glucosamine in the form of either glucosamine sulphate, glucosamine hydrochloride, or N-acetyl-glucosamine is extensively consumed as a dietary supplement to treat osteoarthritis, back pain and knee pain. Furthermore, the consumption of glucosamine is safe and does not affect glucose metabolism in the body under normal conditions of use (Benavente *et al.,* 2015). Unfortunately, the chemical hydrolysis can pose some defects including chemical modifications of glucose ring and harsh hydrolysis conditions (Wang *et al.,* 2008). To overcome the problem, glucosamine is obtained by chitin hydrolysis using chitin degrading enzymes found in many organisms including viruses, bacteria, fungi, insects, plants and animals that have strong chitinolytic activities (Akhir *et al.*, 2009). Enzymatic hydrolysis method have advantages over chemical methods, such as mild reaction conditions and high yield of glucosamine without any modification of glucose ring (Pan *et al.,* 2011).

Chitinase is an enzyme capable of hydrolyzing insoluble chitin into its oligomeric and monomeric components. Chitin hydrolysis is performed by three different enzymes, including endochitinase, exochitinase and *N*-acetylglucosaminidase. Endochitinase randomly cleaves the glycosidic bonds of chitin to produce polymers of N-acetylglucosamine, while exochitinase cleaves the chain from nonreducing end to form diacetyl-chitobiose. *N*-acetylglucosaminidase then hydrolyze diacetyl-chitobiose into *N*-acetylglucosamine (Tanaka *et al.,* 2001). Chitinase enzyme activity is affected by factors such as substrate concentration, temperature and pH. Moreover, chitinase enzyme production is affected by

fermentation time (Hao *et al.* 2012). Even though chitinase can be obtained from many sources, chitinases of filamentous fungi have shown to have higher activity levels than those of plants and bacteria (Wang and Yang, 2007). However, in filamentous fungi, chitin is located in the inner layers of the cell wall, close to the plasma membrane. It is therefore not easily accessible from the outer side of the cell wall as its presence is masked by layers of other carbohydrates and proteins (Harti *et al*., 2012).

In this research, chitin was degraded from shrimp shells that were originated from *Penaeus monodon* shrimp. The shrimp shells were degraded through the fermentation of crude and semi-pure intracellular chitinase obtained from *Mucor circinelloides.* The usage of crude and semi-pure chitinase enzyme was to compare if the purification of chitinase enzyme yielded a significantly higher enzyme activity. The optimum condition including pH, temperature, substrate concentration and incubation period of the chitinolytic enzyme were then observed.

1.2 Research Problem

Shrimp shell tend to be thrown away as a waste product. However, shrimp shell can be utilized for its chitin which can be further hydrolyzed to form its derivate which is N-acetylglucosamine. The hydrolysis of chitin using chemical method is less desirable compared to enzymatic process as it can result in chemical modifications of the glucose ring and harsh hydrolysis condition of chitin. Moreover, enzymatic hydrolysis of chitin using chitinase enzyme is faster and give more yield than through fermentation of chitin (Struszczyk, 2002). *Mucor circinelloides* is a fungus that can convert chitin into *N*-acetylglucosamine

(Battaglia *et al.,* 2011). However, the study regarding the production of *N*-acetylglucosamine by using crude and semi-pure intracellular enzyme of *Mucor circinelloides* and the optimum condition of the crude and semi-pure intracellular enzyme to produce *N*-acetylglucosamine is not done yet.

1.3 Objective

1.3.1 General Objective

The general objective of this research was to utilize chitin from shrimp shells (*Penaeus monodon)* to produce N-acetylglucosamine using crude and semi-pure intracellular enzyme from *Mucor circinelloides.*

1.3.2 Specific Objective

The specific objectives of this research were:

1. To determine the optimum pH and temperature for chitinase from *Mucor circinelloides* for the production of N-acetylglucosamine.
2. To determine the optimum incubation period and substrate concentration for chitinase from *Mucor circinelloides* for the production of N-acetylglucosamine.