CHAPTER I

INTRODUCTION

1.1 Background

The development of shrimp processing industry in Indonesia does not only contribute to the large national income (Wati, 2018), yet it also offers a downside in regards to the drastic increase of shrimp waste production. About 40-48% by weight of shrimp raw material is discarded as waste, depending on the species (Kandra *et al*., 2012). The increase of shrimp waste production that is accompanied with a limited utilizing technology, will pose a risk of environmental problem. Shrimp waste contains high level of protein, which has been acknowledged to negatively impact the environment when discarded with no further handling (Kandra *et al*., 2012; Henchion *et al*., 2017). Shrimp waste consists of the head, as well as the shells of shrimp. Shrimp shells contain 40% protein, 35% minerals, 14-30% chitin and carotenoid pigments that are considered as bioactive compounds with a wide range of application in medical, therapeutic, biotechnology, textile, as well as food sectors (Kandra *et al*., 2012).

Chitin is the second most abundant, biodegradable polysaccharide in nature that is primarily present in crustacean exoskeletons. Its antibacterial and wound-healing properties are the main reasons for its application in various fields, such as food technology, material science, biomedical, wastewater treatment and many others (Kandra *et al*., 2012). The monomer of chitin, namely β-(1,4) linked N-acetyl-D-glucosamine, is an acetylated derivative of glucosamine (Sitanggang *et*

al., 2012; Liu *et al*., 2013). Glucosamine is an amino sugar biochemically synthesized by all organisms, including human. In human body, glucosamine presents at high concentration in the joint tissues to serve as a precursor of glycosaminoglycans biochemical synthesis. Consequently, it is extensively used as a dietary supplement for treating osteoarthritis, as well as knee and back pain (Liu *et al*., 2013; Benavente *et al*., 2015).

As the basic compound that constructs chitin, glucosamine can be produced by either the hydrolysis or fermentation of chitin. Acid hydrolysis of chitin is the most widely used method to obtain glucosamine, yet it is known to result in low yield, as well as high amount of acid wastes (Sashiwa *et al*., 2002). Another hydrolysis method is conducted by using crude enzymes. Unfortunately, these crude enzymes are not efficient to be used directly for hydrolyzing the chitin derived from crab or shrimp shells. Moreover, the high cost and instability of the crude enzymes should also be accounted. To overcome the complications of the two previously mentioned methods, fermentation using filamentous fungi and bacteria to produce glucosamine, in form of N-acetylglucosamine, was developed. This method has attracted increasing attention due to its relatively low production cost, as well as environmentally friendly process (Liu *et al*., 2013).

Fermentation method of glucosamine production is predominantly conducted using bacteria or fungi with the ability of producing chitinase enzyme, which is responsible for degrading chitin into its simpler basic compound form, namely glucosamine (Sitanggang *et al*., 2012; Benavente *et al*., 2015). *Providencia stuartii* is one of the bacteria that can produce chitinase enzyme, hence it is capable

of breaking down chitin into glucosamine (Josephine, 2018). Despite its superiority compared to chemical and enzymatic hydrolysis methods, the fermentation method also has a disadvantage regarding the usage of free microorganism cells, which are generally low in stability and viability due to their high sensitivity to the environmental changes (Pazzetto *et al*., 2012). Therefore, a specific technique that can protect the free microorganism cells from environmental changes in order to preserve their stability and viability, is essential to be conducted for optimizing the fermentation of chitin into glucosamine.

Cell immobilization is one of the simplest techniques that can be utilized with the aim of preserving cells in stable and viable form. Immobilized cells are significantly decreased in the sensitivity towards temperature and pH changes, therefore they are protected against morphological, physiological and genetic changes, as well as maintained in stability and viability. Moreover, it also offers a relatively low cost of preservation (Pazzetto *et al*., 2012; Elakkiya *et al*., 2016). The simplest and safest cell immobilization method to be used is the entrapment of cells on natural or synthetic matrices (Elakkiya *et al*., 2016). Synthetic sponge, one of which is polyurethane foam, is one of the most widely used matrices for entrapment of cells that is not only inexpensive and easily obtained, but also has inert nature, hence it does not interfere with the cell properties (Delani *et al*., 2012).

The fermentation of chitin for glucosamine production using either immobilized cells or chitinase enzyme of *Providencia stuartii* by entrapment method on natural gels, have been conducted. However, a research on cell immobilization of *Providencia stuartii* by entrapment method on polyurethane

foam as well as its application on the fermentation of chitin for glucosamine production, has not been conducted.

1.2 Research Problem

N-acetylglucosamine, which is the monomer of chitin, has many benefits in pharmaceutical field. The production of N-acetylglucosamine by microbial fermentation of chitin is an alternative method, which is able to overcome the complications in acid and enzymatic hydrolysis of chitin. *Providencia stuartii* is one example of bacteria that has strong chitinolytic activity based on the previous research by Josephine (2018). The instability of the free microorganism cells used in the fermentation process can be overcome by the application of cell immobilization, which allows a continuous and repeated use of the immobilized cells, as well as to reduce the risk of contamination (Delani *et al*., 2012; Rouf *et al.*, 2017). Polyurethane foam is one of the most commonly used matrices for cell immobilization with entrapment method, due to its inert nature, low cost and availability. However, a research that utilizes the immobilized cells of *Providencia stuartii* using entrapment on polyurethane foam, for N-acetylglucosamine production by fermentation of chitin in shrimp shells has not been conducted.

1.3 Objectives

The objectives of this research include the general and specific objectives.

1.3.1 General Objective

The general objective of this research was to immobilize *Providencia stuartii* cells using polyurethane foam and to apply it in N-acetylglucosamine production by fermentation using shrimp shells powder.

1.3.2 Specific Objective

The specific objectives of this research were:

1. To determine the optimum size of polyurethane foam used to immobilize *Providencia stuartii* cells for the production of N-acetylglucosamine by shrimp shells powder fermentation.
2. To determine the optimum ratio (w/v) between polyurethane foam and growth medium used to immobilize *Providencia stuartii* cells for the production of N-acetylglucosamine by shrimp shells powder fermentation.
3. To determine the optimum fermentation cycle of the immobilized *Providencia stuartii* cells for the production of N-acetylglucosamine by shrimp shells powder fermentation.