CHAPTER 1

INTRODUCTION

1.1 Background, motivation and problem statement

Costerton et al., (2005) said that biofilm is a microbial derived sessile community characterized by cells that are irreversibly attached to a substratum or interface to each other, embedded in a matrix of extracellular polymeric substances that they have produced. Wilson and Adams, (2007) found that there is evidence that garlic has properties of antioxidant, anti-inflammatory and antibacterial. Garlic solution can inhibit much more strongly towards bacterial strains compared to antibiotics (Bakri and Douglas, 2004; Lai and Roy, 2004). Lai and Roy, (2004) believed that garlic can inhibit *Bacillus subtilis*, *Escherichia coli*, and *Saccharomyces cerevisiae*.

Bacillus sp. is removed because it may affect the substances in food and pharmaceutical industry by changing the reaction in the substances and can cause disease directly to human by ingestion (Mortensen, 2014; Swichtenberg, 2007). *Bacillus* sp. may cause infectious disease and device-related infections. *Bacillus* sp. can grow on medical devices, living tissues, piping, or natural aquatic system. *Bacillus* sp. was used to represent biofilm because they have been long used as biofilm model to investigate biofilm and their likelihood to biofilm (Vlamakis et al., 2013).

The common type of chemical used to remove microbes are ethylene oxide, phenol, and alcohols (McDonnell and Russell, 2001). However, chemical biocides are toxic, carcinogenic mutagenic, and, pose hazards to personnel and environment (Abdul Khader and Mohandas, 2000). Because of safety issue towards chemical usage, at present, people prefer products that have less chemicals and minimum amount of process (Kerekes et al., 2015). In food industry, the number of usable chemical that can be used is limited

and their usage can cause unpleasant smell (Kerekes et al., 2015). Natural biocide such as crotton seed oil (Abdul Khader and Mohandas, 2000), lavandin essencial (Varona et al., 2009) and the most popular one is garlic (Wilson and Demmig-Adams, 2007) has been found by researchers. Therefore, garlic will be use as agent to remove the biofilms as it has antibacterial, antifungal, antiprotozoal, and antiviral properties and believed that garlic can remove biofilms (Jakobsen, et al., 2012). Factorial analysis via two level factorial is used to allows chemist to study the effect of each factor on response and study the correlation between the factors (Anderson & Whitcomb, 2000).

1.2 Objectives

The following are the objectives of this research:

1) To remove *Bacillus* sp. by using garlic solution.
2) To analyse the factor that affecting the *Bacillus* sp. removal process.

1.3 Scopes of Study

The following are the scopes of this research:

1) The selected factors were ratio of water to garlic (W/G) (1:1 and 1:5), ratio of garlic solution to *Bacillus* sp. (GS/B) (1:1 and 1:3), agitation speed (50-100 rpm) and reaction time between *Bacillus* sp. and garlic (12-24 hours).
2) *Bacillus* sp. was used as biofilm model.
3) Removing *Bacillus* sp. by using garlic solution.
4) Analysed the response of percentage *Bacillus* sp. removal by using garlic solution.
5) Applying of two level factorial analysis in Design Expert software (Version 7) to construct table and analysis data.

CHAPTER 2

LITERATURE REVIEW

2.1 Biofilm

Biofilm is a microbial derived sessile community characterized by cells that are irreversibly attached to a substratum or interface to each other, embedded in a matrix of extracellular polymeric substances (EPS) that they have produced (Costerton et al., 2005). There are many different microbes that can be found in biofilm such as *Bacillus* sp., Listeria monocytogenes, *Staphylococcus* sp., *Escherichia coli* and many more (Lopez et al., 2010).

The main components of EPS are polysaccharides, proteins, phospholipids, teichoic and nucleic acids (Shi and Zhu, 2009). Donlan (2002) discovered that mineral crystals, silt particles and blood components are found in the EPS of biofilms in some special settings. A bio- film community may consist of single and/or multiple species of bacteria and form a single layer or three-dimensional structures (Shi and Zhu, 2009).

Biofilm growth begins with planktonic, or in other words free-swimming, biofilm, which land on a surface and attach themselves. As other organism, formation of biofilm must be at the right conditions of appropriate amount of surface needs, presence of nutrient, and some water (Lehnherr and Miller, 2016). The biofilm grow and reproduce, creating a thick, slimy surface. They can attach on a variety of surfaces, from woods, metals and plastics to living tissues and stagnant water, then grow into a biofilm as in Figure 2.1. Figure 2.2 explain the step of biofilm formation based on Figure 2.1.