CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Enzyme is a biological catalyst that is used to speed up the biochemical reactions. Nowadays, the uses of enzyme in research and industry applications are increasing due to its ability to increase the rate of reaction. Mostly enzyme is specific and unique according to their function. Thus, each enzyme has its own operating conditions to carry out the biochemical reaction. The operating conditions such as temperature, pH and concentration of substrate have significant effects on enzyme activity. These operating conditions are important to maintain the uses of enzyme as well as to maximize the production of desired product. The range of temperature and pH are different depends on the sources of enzyme. There is enzyme that able to function at high temperature of °C but mostly the enzyme is only function under a moderate temperature of 37°C to 47°C. Moreover, some of enzyme work at acidic and alkaline pHs while others work at neutral condition. The operating conditions can be determine through the characterization and stabilization of enzyme. The identification of optimum conditions of the enzyme helps to increase the efficiency of the enzyme and to obtain the high production of desired product.

Cyclodextrin glycosyltransferase (CGTase) is an enzyme in α-amylase family that produces cyclodextrin (CD) through the degradation of starch (Hirano et al., 2006). CGTase has the ability to catalyze cyclization, disproportionation, hydrolysis and coupling reactions (Veen et al., 2000). However, CGTase is mainly employed in the industrial production of CD through the cyclization reaction (Brena et al., 2013). There are 3 major types of CD that are commonly known which are α-, β-, and γ-CD. The types of CD is depends on the assembly of glucose units in the CD molecules. (Fretas et al., 2012; Frey et al., 2003). These CDs have the ability to protect the fragile substances from
oxygen and UV, to delay the evolution of volatiles and to aid the ancillary processing of dangerous or volatiles substances in powdered form.

CD also generally employed as a complexing agent due to its chemical and physical properties (Brena et al., 2013). Therefore, the uses of CD in the industries is growing in interest as CD can be used in the different applications such as in pharmaceutical, food and cosmetic. The industrial interest is getting wider by years that lead to the high demand in the production of CD. However, nowadays, the production of CD is still in a small scale due to its low yield and time consuming. There are many studies were conducted on characterization and stabilization of CGTase in order to increase the production of CD. In this study, the commercialized CGTase from Bacillus sp. is used and the operating conditions of the enzyme have been identified. The operating conditions such as temperature, pH and concentration of substrate have significant effects on enzyme activity. The characterization and stabilization of the enzymes are important to determine the ideal conditions in order to obtain the high production of desired product. Thus, in order to maximize the production of CD in the future, the characterization and stabilization of CGTase used to produce the CD were carried out.

1.2 Motivation

CGTase is an industrially important group of starch-converting enzyme that synthesises CD (Hirano et al., 2006). CGTase is mainly employed in the industrial production of CD through degradation of starch by cyclization reaction (Brena et al., 2013). The uses of CD in the industrial applications are gaining attentions especially in food, cosmetic and pharmaceutical industry. However, despite of the higher demand, the production of CD nowadays is still in small scale due to the low yield of CD. Thus, in this study, a commercialized CGTase from Bacillus sp. is used to produce CD. The best operating conditions of the CGTase was identified in order to maximize the production of CD in the future.
1.3 Problem Statement

CGTase is an enzyme that is used to produce CD from degradation of starch by cyclization reaction. In recent years, the demand for the production of CD is increasing due to the applications in industrial. However, the production of CD is low due to the unstable enzyme at certain condition. Thus, the characterization and stabilization of enzyme must be carried out in order to maximize the production of CD in the future.

1.4 Objectives

The objectives of this research are:

1) To determine the best operating conditions on characterization of CGTase for the future CD production.
2) To investigate the best operating conditions on stabilization of CGTase for the future CD production.

1.5 Scopes of Study

The scopes of this research are:

1) Study the effect of starch concentration (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0% (w/v)) on characterization of CGTase.
2) Study the effect of pH (5, 6, 7, 8, 9 and 10) on characterization of CGTase.
3) Study the effect of pH (5, 6, 7, 8, 9 and 10) on stabilization of CGTase.
4) Study the effect of temperature (20, 30, 40, 50, 60 and 70°C) on stabilization of CGTase.