1. INTRODUCTION

1.1 Motivation and statement of problem

Methanol was the simplest alcohol that contained only one carbon atom (CH$_3$OH). It was known as wood alcohol. Methanol was a colourless liquid, volatile, flammable and poisonous. It can be produced from different feedstock resources, predominately from natural gas and coal. Methanol was used in several industries such as in plastic industry. It also acts as an original feedstock to make polymer. Methanol also had been proved to be as fuel. When it was blended with gasoline in internal combustion engines, 85% of it was methanol and other 15% was gasoline. It also can be produced as pure methanol. Besides that, methanol also served as a raw material to produced chemical products for example formaldehyde, acetic acid, polymers, paints, adhesives, construction material and synthetic chemicals. (Luzia et al., 2011)

Pectin methylesterase (PME) involved in de-esterification of pectin that released methanol and acidic acid. It is a heterogeneous group of enzyme complex that involved in pectin hydrolysis and composed of pectin esterase. The applications of PME were in food industry, textile, wines, pulp and paper industry (Sameer et al., 2013). This enzyme catalysed the hydrolysis of the methyl ester group from pectin. It found in plants and also in pathogenic fungi and bacteria (Gayen S., & Ghosh U., 2011). PME had been purified and characterised from several species of citrus fruits such as orange and lemon (John & Tove, 2002). According to John & Tove (2002), citrus fruits were commercially used for juice extraction. Because of the high content in pectin, it also been used in production of methanol.

In this study, PME were be extracted from the citrus fruit that was lime peels waste. Lime peels were being mixed with pectin solution for enzymatic reaction process. Based on the production of methanol, the best factors were being investigated further in order to increase methanol production. The factors that were varied are temperature, pH, fermentation time, concentration of NaCl and agitation.
Fractional analysis by using two levels Fractional Factorial Designs (FFD) was studied in this research. Two level Fractional Factorial Designs (FFD) are popular experimental design and commonly used in engineering analysis (Don, 2013). In addition, FFD allows possible consideration of multi factors and it can determine the most relevant factors from all of the outcomes. Recently, FFD was the analysis that used to investigate the effect of tested independent variables to the response within the investigation range (Khalil et al., 2011). Hence, FFD was a technique to determine the influence of several variables on the response and also estimating the overall main factor effects and interaction of different factors (Golshani et al., 2013). According to Jawad et al., (2013), FFD sign had been used to study the effect of independent variables and the level of selected factors that been chosen for preliminary experiments.

Usually, methanol was produced from synthesis gas where the main gas used is carbon monoxide and hydrogen. In order to produce methanol in typical plant, natural gas feedstock had to convert into a synthesis gas stream that consist of CO, CO$_2$, H$_2$O and H$_2$. It was usually accomplished by the catalytic reforming of feed gas and stream. Hence, by this synthesis gas method, the methanol produced was highly exothermic and taking place over a catalyst bed at moderate temperature. This condition will generate more energy of electricity and this were increased the capital cost. In this research study, a lime peels were used as the medium to release PME where PME was needed in this research for the production of methanol. They were chosen because it was agricultural or agro-industrial wastes that were abundant, renewable and inexpensive energy source that available in Malaysia. Hence, by PME that extracted from lime peels waste, it provides cost effective and eco-friendly method for the production of methanol on large scale (Patil & Chaundhari, 2010).
1.2 Objectives

The following were the objectives of this research:

- To produce methanol by using pectin methylesterase (PME) from lime peels waste and pectin solution as substrate
- To analyse the factor that affecting the methanol production from pectin methylesterase (PME)

1.3 Scope of this research

This research study was about the biological production of methanol by using pectin methylesterase (PME). Firstly, substrate which is pectin solution was prepared and the PME was extracted from lime peels waste. The fruits were cut into halves and take out the peels. Immediately the peels were treated to extract the pectin enzyme. Then, the preliminary studies were started by varying the factors. Production of methanol based on enzymatic reaction process was analysed by using High Performance Liquid Chromatography (HPLC). Hence, both objectives were completed. In this research study, it was interested to apply optimization tools like Response Surface Methodology (RSM) to improve the production of methanol via Fractional Factorial Design (FFD). FFD can provide clear understanding about the interactions involved between the process variables during the production process (Salleh, H. et al., 2011). The screening process and analysing factors by using FFD that was taking into account were temperature, pH, enzymatic reaction time, agitation and concentration of NaCl.