## **CHAPTER 1**

## Introduction

## **1.1** Statement and significant of the study

The production of biodiesel has been on an exponential growth curve over the past several years because of dwindling petroleum reserves and the associated environmental impacts from the usage of fossil fuels (Thompson and He, 2006; Fan et al., 2010). Moreover, biodiesel may have the potential to reduce the reliance on petroleum fuel and reduce air pollutant emissions from diesel engines (Hayyan et al., 2010). Biodiesel-derived crude glycerol or crude glycerol is generated as the main byproduct from biodiesel production (Knothe, 2005; Thompson and He, 2006; Xu et al., 2012a). The bioconversion of this low quality glycerol is a big challenge as this glycerol cannot be used in food and cosmetic applications (Pachauri and He, 2006) because of its high impurities (Thompson and He, 2006; Pachauri and He, 2006). Moreover, crude glycerol surplus is dramatically increasing, so the novel applications should be developed. The increasing abundance and attractive pricing of glycerol make crude glycerol are appealing source of chemical and bioconversion to high value added chemicals (Fan et al., 2010). Therefore, the utilization of crude glycerol through biotechnological approaches represents a promising alternative for the effective management of this industrial waste (Chatzifragkou and Papanikolaou, 2012).

In terms of bioconversion methods for crude glycerol utilization, many researchers have focused on the utilization of oleaginous yeasts/red yeasts which are considered as abundant sources of oil and fats. These yeasts could produce high lipids content more than 200 mg/g of their biomass (20% by weight) are identified as "oleaginous yeast" (Meng et al., 2009). Moreover, many types of oleaginous yeasts could accumulate both of lipids and carotenoids with high  $\beta$ -carotene content in their cells, which are designed as the group of "oleaginous red yeasts" (Meng et al., 2009;

Zhang et al., 2011). These oleaginous red yeasts have been considered as a potential oil feedstock for biodiesel production as well as a source of natural carotenoids. The microbial production of carotenoids is an environmental friendly compared to chemical production and able to meet the increasing demand of this natural pigment (Das et al., 2007). In the recent year, microbial lipids/oils from oleaginous microorganisms have been evaluated as feedstock for biodiesel production because they have an appropriate fatty acid profile, do not compete with food supply, short time of fermentation requirement low space demand and independence of location, seasons and climates (Bautista et al., 2012; Zhang et al., 2014). Moreover, the mainly compositions of microbial lipids are triacylglycerol (TAG) which rich in unsaturated fatty acids of oleic acid (C18:1) and linoleic acid (C18:2), similarly to vegetable oils (Nigam and Singh, 2011).

Oleaginous red yeast could grow on waste or low-grade substrate, especially crude glycerol as carbon and energy sources (Bautista et al., 2012). Recently, Manowattana et al. (2012) has reported that crude glycerol from biodiesel production process could use as an effective carbon source for carotenoids and lipids productions by *Sporobolomyces pararoseus* TISTR5213. As well as the report of Saenge et al. (2011) who have reported that crude glycerol enhanced oils and carotenoids contents of *Rhodotorula glutinis* TISTR5159. From the mentioned previous reports, crude glycerol is becoming the high potential carbon source for its bioconversion into the high value chemical products by the effective oleaginous and red yeasts.

The oleaginous red yeast *Sporidiobolus paroroseus* KM281507 (formerly *Sporobolomyces pararoseus* TISTR5213) has been selected for this study. The comparison of medium composition, environmental factors affecting on lipids and carotenoids production operating in both of shaking flask and bioreactor are investigated. Moreover, this study also aims to enhance the production of total carotenoids,  $\beta$ -carotene and lipids operating in an airlift bioreactor. The effect of light irradiation and dissolved oxygen (DO) on the growth of strain KM281507 and the accumulation of total carotenoids,  $\beta$ -carotene and lipids are also studied.

## 1.2 Objectives

- 1.2.1 To screen, identify and select of the effective oleaginous red yeasts which can metabolize crude glycerol for theirs biomass, carotenoids and lipids productions.
- 1.2.2 To optimize the carotenoids and lipids productions using crude glycerol as a sole carbon source by the selected oleaginous red yeast strain, *Sporidiobolus pararoseus* KM281507.
- 1.2.3 To study the up-scale production of carotenoids and lipids in the bioreactor under the optimal condition by strain KM281507.



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