



Enhanced single cell oil production by mixed culture of *Chlorella pyrenoidosa* and *Rhodotorula glutinis* using cassava bagasse hydrolysate as carbon source

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ABSTRACT

The single cell oil (SCO) production by the mono and mixed culture of microalgae *Chlorella pyrenoidosa* and red yeast *Rhodotorula glutinis* was investigated using non-detoxified cassava bagasse hydrolysate (CBH) as carbon source. The results suggested that the two strains were able to tolerate and even degrade some byproducts presented in the CBH, and the mixed culture approach enhanced the degradation of certain byproducts. Biomass (20.37 ± 0.38 g/L) and lipid yield (10.42 ± 1.21 g/L) of the mixed culture achieved in the batch culture were significantly higher than that of the mono-cultures ($p < 0.05$). The fed-batch culture further raised the biomass and lipid yield to 31.45 ± 4.93 g/L and 18.47 ± 3.25 g/L, respectively. The lipids mainly composed of oleic acid and palmitic acid, suggesting the potential applications such as biofuel feedstock, cosmetics, food additives and lubricant. This study provided new insights for the integration of the economical SCO production with agro-industrial waste disposal.

1. Introduction

Single cell oil (SCO) produced by oleaginous microorganisms can potentially be one of the sustainable and promising alternatives to traditional fossil fuel in the future. Various types of oleaginous microorganisms (e.g., yeast, molds and microalgae) have been documented to have the ability to accumulate large amounts of lipids when cultivated in nitrogen-limited and carbon-rich medium (Liu et al., 2016; Sun et al., 2014). Carbon sources, such as glucose and pentose which are commonly used for microbial cultivation, accounted for most of the production costs. The high costs have largely limited the economic feasibility of microbial lipid production processes, which prevented the technology from wider applicability (Xu et al., 2012). Hence, much effort has gone into utilizing alternative low-cost and renewable materials as potential feedstocks for cost-efficient and environmental-friendly SCO production.

In the last decade, there has been an increasing trend towards efficient utilization of agro-industrial wastes and residues (e.g., hydrolysate of sugarcane, corn and cassava) as substrate for the production of microbial oils, which could help solve the waste disposal problems as well as alleviate the worldwide fuel crisis (Yu et al., 2011; Zhang et al., 2016). Cassava (*Manihot esculenta* Cranz), the world's sixth most important crop, is widely planted in many countries and regions including

China, Africa, Thailand, and Latin America, and mainly utilized for the industrial production of cassava starch (Li et al., 2010; Lu et al., 2012a; Xia et al., 2014). During the industrial processing of starch extraction from cassava, a considerable amount of cassava bagasse (CB) is generated and disposed into landfills directly or utilized as low-value animal feed. However, high residue contents of carbohydrate components such as cellulose, hemicellulose and starch in CB can be converted to fermentable sugar (e.g., glucose, pentose and hexoses) largely required for the microbial growth and production of value-added products (Shi et al., 2014; Yu et al., 2011). Bioconversion of cassava bagasse to the production of biodiesel, bio-ethanol and succinic acid were carried out in the lab scale decades ago, which have been demonstrated to be economical and environmental-friendly (Wang et al., 2012).

Mixed culture techniques, taking advantage of the microbial interactions among two or more preselected species by synchronously culturing them in the same medium, have been reported to have myriad applications in wastewater treatment, bioactive compounds production, and the degradation of halogens and hydrocarbons (Goers et al., 2014; Magdouli et al., 2016). Successful microalgae-yeast combinations with beneficial relationship of gas exchange and pH adjustment were documented in several studies involving combinations of microalgae and yeasts (e.g., *Spirulina platensis* and *Rhodotorula glutinis*, *Scenedesmus obliquus* and *Candida tropicalis*) (Wang et al., 2015; Xue et al., 2010). In

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