

Using Optimal Cultivation Strategies for Enhanced Biomass and Lipid Production of an Indigenous *Thraustochytrium* sp. BM2

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Abstract : Biofuel has drawn much attention as a potential substitute to fossil fuels. However, biodiesel from waste oil, oil crops or other oil sources can only satisfy partial existing demands for transportation. Due to the feature of being clean, green and viable for mass production, using microalgae as a feedstock for biodiesel is regarded as a possible solution for a low-carbon and sustainable society. In particular, *Thraustochytrium* sp. BM2, an indigenous heterotrophic microalga, possesses the potential for metabolizing glycerol to produce lipids. Hence, it is being considered as a promising microalgae-based oil source for biodiesel production and other applications. This study was to optimize the culture pH, scale up, assess the feasibility of producing microalgal lipid from crude glycerol and apply operation strategies following optimal results from shake flask system in a 5L stirred-tank fermenter for further enhancing lipid productivities. Cultivation of *Thraustochytrium* sp. BM2 without pH control resulted in the highest lipid production of 3944 mg/L and biomass production of 4.85 g/L. Next, when initial glycerol and corn steep liquor (CSL) concentration increased five times (50 g and 62.5 g, respectively), the overall lipid productivity could reach 124 mg/L/h. However, when using crude glycerol as a sole carbon source, direct addition of crude glycerol could inhibit culture growth. Therefore, acid and metal salt pretreatment methods were utilized to purify the crude glycerol. Crude glycerol pretreated with acid and CaCl_2 had the greatest overall lipid productivity 131 mg/L/h when used as a carbon source and proved to be a better substitute for pure glycerol as carbon source in *Thraustochytrium* sp. BM2 cultivation medium. Engineering operation strategies such as fed-batch and semi-batch operation were applied in the cultivation of *Thraustochytrium* sp. BM2 for the improvement of lipid production. In cultivation of fed-batch operation strategy, harvested biomass 132.60 g and lipid 69.15 g were obtained. Also, lipid yield 0.20 g/g glycerol was same as in batch cultivation, although with poor overall lipid productivity 107 mg/L/h. In cultivation of semi-batch operation strategy, overall lipid productivity could reach 158 mg/L/h due to the shorter cultivation time. Harvested biomass and lipid achieved 232.62 g and 126.61 g respectively. Lipid yield was improved from 0.20 to 0.24 g/g glycerol. Besides, product costs of three kinds of operation strategies were also calculated. The lowest product cost 12.42 \$NTD/g lipid was obtained while employing semi-batch operation strategy and reduced 33% in comparison with batch operation strategy.

Keywords : heterotrophic microalga *Thraustochytrium* sp. BM2, microalgal lipid, crude glycerol, fermentation strategy, biodiesel

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