# 中文摘要

*Rhodotorula glutinis* (BCRC 22360)是一株可累積高油脂的酵母菌，其中不飽和脂肪酸所佔的含量高，且類似於植物油，適合作為製造生質柴油的油脂。除了生產油脂外也富含高價值的β-胡蘿蔔素，β-胡蘿蔔素是天然的抗氧化劑，利用分子中含有多個雙鍵能與具有不成對電子的自由基結合來中斷脂質過氧化連鎖反應，進而保護細胞不被破壞。*R. glutinis*細胞生長迅速、可達高密度細胞培養，因此具有相當高發展潛力。在綠色能源意識高漲下，使得生質柴油產量大幅提升，同時累積大量的副產物粗甘油，而粗甘油中含有未反應的脂肪酸、醇類及催化劑等，在純化的製程上較為複雜且高成本，因此使用粗甘油作為培養基的碳源可作為解決粗甘油的方法。本研究使用粗甘油做為碳源並利用氣舉式發酵槽作為反應器以及探討不同發酵策略來提升*Rhodotorula glutinis*生長、油脂含量及β-胡蘿蔔素含量，進而提高整體產物之生產速率。

在搖瓶實驗中，分別做培養基組成、環境因子、添加誘導因子的影響，藉此增加β-胡蘿蔔素的提升，其中在照白光實驗中，以38.18μmol/m2/s光強度進行照射48 hr，能夠明顯提升*R. glutinis*濃度4.50 g/L至6.67 g/L及β-胡蘿蔔素含量從2.22 mg/g提升至2.44 mg/g；在培養0小時添加0.5 mM過氧化氫相較於未添加者，可明顯提升β-胡蘿蔔素含量從2.11 mg/g 提升至2.64 mg/g；在改變培養基介質為海洋深層水的實驗中發現含有有機氮所得到的菌體濃度是不含有機氮組的2倍，達到5.0 g/L，然而在累積油脂上，不含有機氮組能明顯提高20 %，高達44.11 %。

放大實驗中分成三個部分探討，(1) 5 L氣舉式發酵槽批次的實驗中，改變通氣量的影響，結果得到生物量生產速率與通氣量有正關係，而以2.5 vvm通氣量下可得到最大生物產率高達0.46 g/L/hr、最大油脂生產速率0.22 g/L/hr以及最大β-胡蘿蔔素產率1.1 mg/L/hr。(2) 為了提升槽體內溶氧量並能節省空壓機供應氣體所消耗的能源，本實驗使用5 L氣舉式發酵槽，並在排氣處設置閥，利用出口閥開合大小改變槽壓的影響，結果顯示出，增加槽體壓力對於菌種濃度及β-胡蘿蔔素的含量之間沒有直接關係。故對於*R. glutinis*的培養，在操作及成本考量上，氣升式生物反應器相對於傳統的攪拌生物反應器是較合適的培養系統。(3) 使用50 L氣舉式發酵槽，探討饋料策略的不同對於*R. glutinis*生長的影響，分別以一次性饋料、等速度饋料 (0.3 L/hr)、指數型速度饋料；指數型饋料利用方程式來計算該時間點的生物含量所需的碳源濃度，此能有效提升生物濃度，而以微生物油脂生產速率與β-胡蘿蔔素生產速率的影響上，以一次性饋料的策略則能得到最佳結果，分別為0.45 g/L/hr、0.82 mg/L/hr。

關鍵字：*R. glutinis*、β-胡蘿蔔素、通氣量、發酵策略

# Abstract

*Rhodotorula glutinis* (BCRC 22360) is an oleaginous yeast that can accumulate high content of total lipid, which is suitable as a microbial oil producer for the production of oil feedstock converted to biodiesel. In addition to total lipids production, *R. glutinis* was well known to have high content of β-carotene, which is a natural antioxidants to protect cells from being damaged by free radical through the interruption of lipid peroxidation chain reaction. Even though, the production cost was still a problem inhibiting the commercialized biodiesel production by using the microbial oils as the feedstock In this study, the effects of fermentation strategy on cell growth and β-carotene production in airlift bioreactor by using crude glycerol as carbon source were investigated.

The effects of medium ingredients and environmental cultivation conditions on the β-carotene content and total lipid production were evaluated. It was found that the irradiation for 48 hours can significantly enhance the biomass from 4.50 g/L to 6.67 g/L and β-carotene content from 2.22 mg/g to 2.44 mg/g. By adding 0.5 mM of peroxide can significantly enhance the β-carotene content from 2.11 mg/g to 2.64 mg/g. The experiments by using deep ocean mineral water with organic nitrogen could have two times of biomass higher than the medium with inorganic nitrogen. However, for the lipid content, the medium with inorganic nitrogen could significantly increase by 20%, up to the value of 44.11%.

Three parts were discussed in the trial of scale up experiment, including (1) Aeration rate of 1 vvm, 1.5 vvm, 2 vvm and 2.5 vvm were performed respectively in a 5 L airlift bioreactor. It seems that the increase of aeration rate can increase the cells growth rate. The batch with 2.5 vvm could achieve the maximum cells productivity of 0.46 g/L/hr, the maximum lipid production rate of 0.22 g/L/hr and the maximum β-carotene production rate of 1.1 mg/L/hr. (2) By controlling the outlet valve to increase the pressure of 5 L airlift bioreactor, which could potentially avoid the high pump power consumption and enhance the dissolved oxygen. The results show that there is no significant effect on the improvement of cells growth and β-carotene content. Conclusively, this study indicated that airlift bioreactor can be a suitable system for the cultivation of oleaginous *R. glutinis* due to its relatively simple operation cost as compared to the conventional agitation bioreactor. (3) To enhance the β-carotene productivity, high cell concentration and high cell productivity that controlling the specific growth rate of cells would be the critical points. In this study, varied feeding criteria including pulse feeding, the feeding with constant rate (0.3 L/hr) and the exponential feeding were tested in the 50 L airlift bioreactor. The fed-batch operation with the exponential feeding could effectively enhance the biomass productivity. Nevertheless, one-time feeding could have the positive effects on lipid production and β-carotene production which was 0.45 g/L/hr and 0.82 mg/L/hr respectively.

Keywords: *Rhodotorula glutinis*、β-carotene、biodiesel、lipid、fermenter strategy