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An integrated system of autotrophic *Chlorella vulgaris* cultivation using CO_2 from the aerobic cultivation process of *Rhodotorula glutinis*

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ABSTRACT

The production of microbial oils from oleaginous *Rhodotorula glutinis* is especially attractive with regard to the production of biodiesel. Nevertheless, a considerable amount of CO_2 is generated during the aerobic cultivation process, which can have negative effects on the environment. An integrated system of autotrophic microalgae cultivation (*Chlorella vulgaris*) using CO_2 from the aerobic *R. glutinis* bioreactor can efficiently reduce CO_2 emissions in this regard. The results indicated about $2.5 \pm 0.1\%$ and $0.5 \pm 0.15\%$ of CO_2 in the flue gas streams from the aerobic tank and from the photosynthesis bioreactor, respectively. It is estimated that about 80% of the CO_2 generated in the cultivation of *R. glutinis* was fixed by *C. vulgaris*, with the integrated system producing biomass of about 20 g/L of *R. glutinis* and 1.2 g/L of *C. vulgaris*. The results of this work indicate that this integrated system can produce microbial oils without the high CO_2 emissions seen with the standard cultivation system. Also, the high value compound produced in the microalgal biomass (e.g. lutein) can compensate the microbial oils production cost, which makes the commercialization of this integrated process more feasible.

1. Introduction

The limited supply of fossil-based energy resources and the pollutions associated with the consumption of such fuels has motivated researchers to explore the development of alternative, renewable energy resources, in addition to the processes of carbon capture and utilization. Among other options, biomass-derived fuels can play an important role in diversifying the energy supply and enhancing its security. One such fuel is biodiesel, which can be produced through the transesterificaiton of several oil-containing feedstocks, such as conventional oil crops, waste kitchen oils and microbial oils. Among all potential feedstocks, microbial oils from oleaginous microorganisms are especially attractive, as they can avoid the problem of competition with arable land use while using non-food carbon sources for the accumulation of microbial oils. Oleaginous microorganisms are defined as those species for which the dry biomass contains more than 20% of lipid content [1]. Numerous oleaginous yeasts and microalgae have been reported to be capable of accumulating large amounts of lipids, with some studies reporting a content of more than 70% [2–4]. The majority of these lipids are triacylglycerol (TAG) containing long-chain fatty acids, and the fatty acid profiles of most oleaginous microorganisms are comparable to those of conventional oil crops, which are quite suitable for use as the feedstock for biodiesel production. More specifically, *Rhodotorula glutinis* is especially suitable for microbial oil production, due to its characteristics of rapid growth and high lipid content when using various carbon sources, such as crude glycerol and cellulosic hydrolysate [5,6]. Nevertheless, the cultivation of aerobic *R. glutinis* produces considerable CO_2 emissions, working against the core concept of reducing such emissions by using renewable biofuels. One way to overcome this issue it to use photosynthetic microalgae to reduce the amount of CO_2 emitted from the aerobic fermentation process.

CO_2 fixation by using microalgae has been widely explored in literature [7–9], and in addition to this property that microalgae are also a potential source of biofuels and numerous high value compounds, such as lutein and long chain fatty acids. Liquid fuels, such as biodiesel, diesel, gasoline, and jet fuel, can also be produced from microalgal oils using existing technology. The source of CO_2 needed to carry out photosynthesis is another factor affecting the growth rate of microalgae. The provision of sufficient light intensity and dissolved CO_2 in the medium are both essential to attaining a high yield of microalgal biomass under autotrophic conditions. Many reports have explored the effects of the CO_2 flow rate and percentage of CO_2 in the inlet gas on the cultivation of microalgae [10,11]. In general, 2%–20% of CO_2 is regarded as a suitable amount in the inlet gas for the cultivation of microalgae [12–14]. It was reported that an integrated system composed of *Dunaliella tertiolecta* microalgae using CO_2 from the brewing fermentation

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process, and the effects of this CO_2 on the production of lipid and carotenoid-rich *D. tertiolecta* were examined [15]. The results showed that the values from this system were almost twice as high as those observed for control cultivations in which atmospheric CO_2 was used. This earlier work thus demonstrated that the integration of yeast fermenters and microalgae photo-bioreactors is an interesting approach to improve both biomass formation and product contents [15].

The purpose of the current study is to examine the effects of using CO_2 from the aerobic cultivation of *R. glutinis* for the growth of *C. vulgaris* under autotrophic conditions. The growth of *C. vulgaris* using CO_2 from the aerobic fermenter is compared to that seen with the batches using various CO_2 percentages in the inlet air gas. The CO_2 production profile in the aerobic fermentation process of *R. glutinis* is also examined.

2. Materials and methods

2.1. Microorganisms and medium

The microalgae *Chlorella vulgaris* was generously provided by Prof. Jo-Shu Chang (National Cheng Kung University, Taiwan). A basal medium was used for autotrophic cultivation in flasks. Details of the basal medium preparation have been described in a previous work [16]. Freeze-dried *Rhodotorula glutinis* BCRC 21418 was obtained from the Bioresource Collection and Research Center, Taiwan (BCRC). The seed medium composition and the cultivation methods followed the suggestions provided by the BCRC.

2.2. Cultivation methods

The batch autotrophic cultivation of *C. vulgaris* was performed at 25 ± 1°C, bubbled with 1 vvm air and supplemented with set CO_2 percentages in a glass photosynthesis bioreactor (PBR) containing 3 l of medium. The glass column was continuously illuminated with regular fluorescent lights or white LED lights (model MR 16 with 7 Watts, Shianyih Electronic Industry Co., Taiwan) to provide sufficient light. The light intensity on the surface of the flask was measured by a light meter (LI 250, LI-COR, Inc., Lincoln, NE, USA), giving a value of 340 μmol/m^2 s.

Batch fermentation of *R. glutinis* was carried out in a 5-L agitation tank with a working volume of 3 L. All experiments were controlled at 24 °C and the pH was controlled at 5.5 by using 1 N NaOH solution. The agitation rate was in the range of 100–300 rpm to keep the DO over 20%, and the aeration rate was fixed at 1.0 vvm. The fermentation medium (per liter) comprised defined amounts of crude glycerol, 2 g of yeast extract, 2 g of $(NH_4)_2SO_4$, 1 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of $CaCl_2$ and 0.1 g of NaCl [17]. The integrated system comprised an agitation tank for the cultivation of *R. glutinis* and an autotrophic tank for the growth of *C. vulgaris*. The flue gas from the tank of *R. glutinis* was completely directed to the PBR of *C. vulgaris*, which achieved 1 vvm for the aeration rate in both tanks. A 0.45 μm sterilized air filter was installed in the air connection line between the agitation tank and the autotrophic PBR to avoid contamination of *C. vulgaris* cultivation.

2.3. Analytical methods

An infrared balance (Denver Instrument, IR 35) was adopted to rapidly measure the biomass concentration. Five ml of broth was centrifuged at 7000 rpm for 10 min. After removing the supernatant, about an equal volume of distilled water was added to eliminate impurities. This washing procedure was performed several times, and the final liquor was dried using the infrared balance at 150 °C to evaporate the water content.

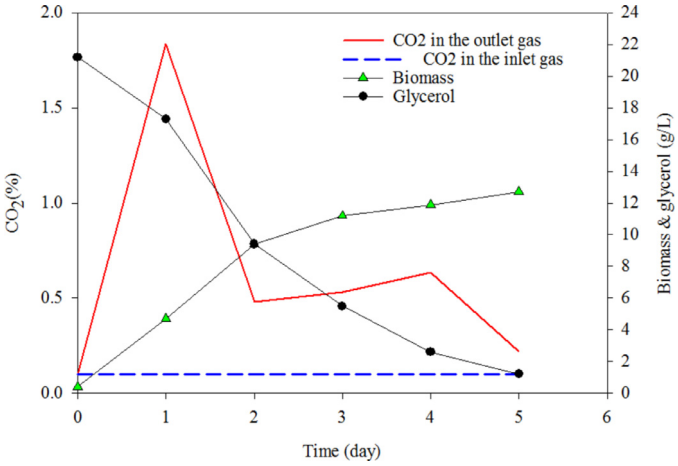

Fig. 1. Time course of the percentage of CO_2 and biomass growth of *R. glutinis* in a 5 L agitation fermenter.

The dry biomass was first ground into a fine powder; 0.05 g of the powder was then blended with 5 ml chloroform/methanol (2:1), and subsequently agitated for 20 min at room temperature in an orbital shaker. The solvent phase was recovered by centrifugation at 7000 rpm for 10 min. The same process was repeated twice, and the whole solvent was evaporated and dried under vacuum conditions.

The glycerol concentration was measured by HPLC (Agilent series 1100, Agilent Technologies, Santa Clara, CA) with a refractive index detector, while the analysis was performed in a C-18 column (Vercopak N5ODS, 250 mm ×4.6 mm, Taiwan). The mobile phase was composed of 0.01 N H_2SO_4 with a flow rate of 0.4 ml/min [18]. All shaker conditions were performed in triplicate to obtain the mean ± standard deviation.

3. Results and discussion

3.1. Time course of CO_2 generation in the batch of R. glutinis

The cultivation of *R. glutinis* is a typical aerobic fermentation process, and requires a considerable amount of dissolved oxygen to obtain a large amount of high biomass [19,20]. The use of crude glycerol for the cultivation of *R. glutinis* was performed in an agitation tank at the aeration rate of 1 vvm. The time courses of the biomass productions, glycerol consumption and CO_2 generation are shown in Fig. 1. It can be seen that the maximum biomass was 12.4 g/L after 5 days' cultivation. The initial glycerol was about 21 g/L, and this decreased to less than 1 g/L after 5 days' cultivation. The maximum biomass growth rate was about 4.7 g/L day, which led to the maximum CO_2 generation percentage of 1.8% observed in the flue gas. During the exponential growth phase, the rapid growth of biomass lead to the maximum CO_2 percentage observed in the flue gas. The CO_2 generation curve reached its maximum after 1 day cultivation, and fell to a stable level of about 0.5% while the cell growth entered the stationary stage. The results indicated that the CO_2 generation was strongly related to the growth of biomass. A higher biomass would produce a higher CO_2 percentage in the flue gas. To confirm this, the effects of biomass on CO_2 generation were examined by controlling the dissolved oxygen (DO) at high (>30%) and low (<30%) levels. The DO control strategy was achieved by automatically adjusting the agitation rate to provide the required DO level, and the results are shown in Fig. 2. It can be seen that the batch with high DO (denoted as high) produced more biomass than the low DO batch (denoted as low). Besides the higher biomass obtained in the high batch, a higher CO_2