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Application of airlift bioreactor for the cultivation of aerobic oleaginous yeast *Rhodotorula glutinis* with different aeration rates

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The high cost of microbial oils produced from oleaginous microorganisms is the major obstacle to commercial production. In this study, the operation of an airlift bioreactor is examined for the cultivation of oleaginous yeast-*Rhodotorula glutinis*, due to the low process cost. The results suggest that the use of a high aeration rate could enhance cell growth. The maximum biomass concentration of 25.40 g/L was observed in the batch with a 2.0 vvm aeration rate. In addition, a higher aeration rate of 2.5 vvm could achieve the maximum growth rate of 0.46 g/L h, about twice the 0.22 g/L h obtained in an agitation tank. However, an increase in tank pressure instead of the aeration rate did not enhance cell growth. The operation of airlift bioreactor described in this work has the advantages of simple operation and low energy consumption, thus making it suitable for the accumulation of microbial oils.

[**Key words:** Microbial oils; Oleaginous; Airlift bioreactor; Feedstock; Aeration rate]

Various renewable lipids have been used for the production of biodiesel, including vegetable oils, animal fats, and kitchen waste oils. However, the current high price of biodiesel, as compared to conventional petroleum diesel, is a major obstacle to its commercialization. This high production cost is mainly due to the cost of raw materials (1), and thus it is necessary to find and develop cheaper alternative raw materials if biodiesel it to go into large scale commercial production. The biological production of single cell oils (SCO) from oleaginous microorganisms is of the main methods of producing biodiesel. Since microbial lipids have many advantages over vegetable oils, such as a short life cycle and no need for agricultural land, they have attracted much interest as a potential non-food feedstock for biodiesel production.

Oleaginous microorganisms have a microbial lipid content in excess of 20% (g/g) (2). Numerous oleaginous yeasts and microalgae have been reported to grow and accumulate significant amounts of lipids. More specifically, the characteristics of rapid growth and the ability to utilize a range of carbon sources mean that *Rhodotorula glutinis* is an attractive candidate for microbial oil production (1,3–5).

The biotechnological processes underlying microbial oil production should be based on the utilization of cheap substrates, in order to make commercialization possible, and for this reason crude glycerol is a particularly appealing material. Crude glycerol is the main byproduct of the biodiesel industry, which produces about 1 kg of glycerol in the transesterification of 10 kg of vegetable oil. Since the worldwide production of biodiesel is increasing, so the production of crude glycerol is also rising, with a consequent decrease in its market price (6). In addition to the cost of the substrates, the operating cost for the accumulation of microbial oils is also a major concern in the commercial viability of microbial oils. Choosing an appropriate bioreactor is a key step in achieving the successful cultivation of oleaginous microorganisms. An airlift bioreactor has the advantages of simple operation and low energy consumption, and thus can be used to grow oleaginous microorganisms in a relatively inexpensive manner, facilitating the commercial production of microbial oils.

While many studies have examined the production of microbial oils from crude glycerol using oleaginous *R. glutinis*, few works have focused on the usage an airlift bioreactor. This may be because the growth of *R. glutinis* requires a large amount of dissolved oxygen. The mass transfer coefficient in the airlift bioreactor must thus be lower than that in the agitation tank, although this means that such bioreactors may not be suitable for cultivation. However, an increase in the aeration rate and the pressurization of the airlift bioreactor might improve the low the mass transfer rate observed in the airlift bioreactor. Therefore, this study investigates the effects of the aeration rate and the pressurization of the airlift bioreactor on the growth of *R. glutinis* and the accumulation of total lipids.

MATERIALS AND METHODS

Microorganism and medium The freeze-dried *R. glutinis* BCRC 22360 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. 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 (7). Sodium hydroxide at 1.0 N or hydrogen chloride at 1.0 N was used to adjust the pH.

Batch fermentation in 5-L agitation fermentor The batch fermentation was operated in a conventional 5-L stirred desk-top fermentor (model BTF-A, Biotop Ltd., Taiwan) of 2-L working volume with the fermentation medium described in the

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above section. The pH level was automatically maintained at 5.5 by automatically feeding NaOH solution (1.0 N). The fermentor was operated at 24°C with dissolved oxygen controlled over 30% by adjusting the agitation rate in the range of 200–500 rpm at 1 vvm of aeration rate.

Batch fermentation in airlift bioreactor Batch fermentation was carried out in a 5-L internal-loop glass airlift bioreactor (30 cm in height, with a 10 cm outer diameter and 7.7 cm inner tube diameter) with a working volume of 3 L. All the experiments were controlled at 24°C and the pH was controlled at 5.5 by using 1 N NaOH solution. Different aeration rates at 1 vvm, 1.5 vvm and 2 vvm were used to explore the effects on cell growth and on the accumulation of total lipids. The airlift bioreactor was pressurized at 0.5 atm to evaluate the potential for using pressurization instead of a high aeration rate to reduce the amount of energy consumed.

Analytical methods Infrared balance was adopted to rapidly measure the biomass concentration. Five ml broth was centrifuged at 7000 rpm for 10 min. After removing the supernatant, about an equal volume of distilled water was added to wash away the impurities. The washing procedure was performed several times, and the final liquor was dried by using infrared balance at 150°C to evaporate the water content.

The total lipid analysis was based on a modification of the procedure used by Bligh and Dyer (8). The dry biomass was ground into a fine powder: 0.05 g of powder was blended with 5 ml chloroform/methanol (2:1), and the mixture was agitated for 20 min at room temperature in an orbital shaker. The solvent phase was recovered by centrifugation. The same process was carried out 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, USA) with a refractive index detector. 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 (9).

Measurement of the volumetric mass transfer coefficient The volumetric oxygen transfer coefficient (K_La) was measured in cell-free reactors by the gassing method, consisting of gassing nitrogen until the oxygen concentration dropped near to zero, at which moment air started being injected into the reactor. The dissolved oxygen concentration (DO) was then monitored by a polarographic electrode and the K_La determined according to Eq. 1 (10).

$$\ln\left[\frac{100 - \mathrm{DO}}{100}\right] = K_La \times t \quad (1)$$

RESULTS AND DISCUSSION

Comparison of *R. glutinis* growth in an agitation bioreactor and in an airlift bioreactor.

R. glutinis is an aerobic microorganism, and it is thus expected that providing sufficient dissolved oxygen can be determined to its growth. In general, conventional agitation tanks are often used for the cultivation of *R. glutinis*, due to the relatively high oxygen transfer coefficient that can be achieved in such devices. Nevertheless, airlift bioreactors are attractive alternatives to agitations tanks, as they are both relatively simple and cheap to operate. The cell growth in an agitation tank and airlift bioreactor was thus compared. The results are shown in Fig. 1, in which it can be seen that the growth of *R. glutinis* in the agitation tank was better than that of in the airlift bioreactor, based on a 1 vvm aeration rate. The greatest biomass in the agitation tank and airlift bioreactor was about 20.8 and 16.6 g/L, respectively. The higher level of dissolved oxygen in the agitation tank greatly enhanced the cell growth rate, up to 0.22 g/L h compared to the 0.12 g/L h that was obtained in the airlift bioreactor. Nevertheless, the rapid cell growth in the agitation tank led to a lower lipid content compared to that seen with the airlift bioreactor. More specifically, the average lipid content in the agitation tank was only about 25 ± 4%, far less than the 45 ± 3% achieved in the airlift bioreactor. However, even though the cell growth rate was much lower in the airlift bioreactor than in the agitation tank, the high lipid content obtained with the former method could partially compensate for the low biomass. Therefore, the total lipids collected from the airlift bioreactor were only slightly less than from the agitation tank. From the perspective of reducing costs, airlift bioreactors deserve further attention to examine how to enhance cell growth by using higher aeration rates than the 1 vvm used in the current work.

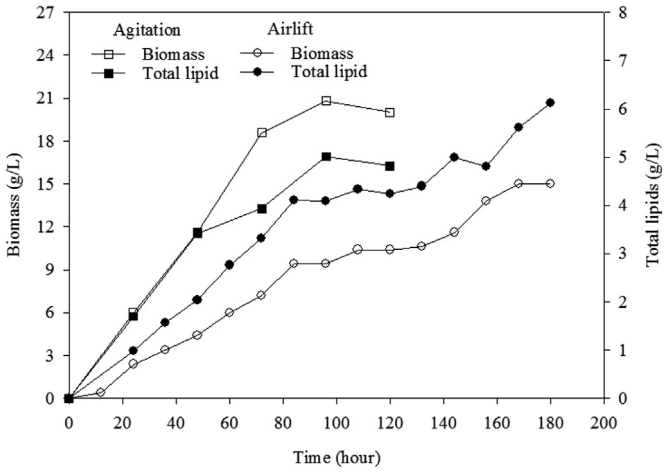

FIG. 1. The cells growth and total lipids production in an agitation tank and in an airlift bioreactor. Open squares, biomass in the agitation tank; closed squares, total lipid in the agitation tank; open circles, biomass in the airlift bioreactor; closed circles, total lipid in the airlift bioreactor.

The effects of aeration rate on cell growth in a 5-L airlift bioreactor The relatively low level of dissolved oxygen in the airlift bioreactor may be the reason for the low level of cell growth reported above. In order to raise the amount of dissolved oxygen in the airlift bioreactor, the aeration rate was increased, with the results for rates of 1.0, 1.5, 2.0 and 2.5 vvm shown in Fig. 2. It can be seen that a higher aeration rate led to a significantly higher cells growth rate. Table 1 compares the kinetic parameters obtained in batches with different aeration rates, and the results suggest that an increase in this rate can successfully enhance cell growth. A maximum biomass growth of 0.46 g/L h was obtained in the batch with an aeration rate of 2.5 vvm, almost four times greater than was obtained with a rate of 1 vvm. The maximum biomass concentration of 25.40 g/L was observed in the batch with the aeration rate of 2.0 vvm. Most oleaginous microorganisms using crude glycerol as the carbon source yield biomass in the range of 10–20 g/L (11), although Xu and his colleagues reported a value of 26.40 g/L when using *Rhodosporidium toruloides* with crude glycerol in an agitation tank, and a maximum lipid content of 69.5% (12). The value obtained with an airlift bioreactor in the current study is very close to the highest value obtained in the

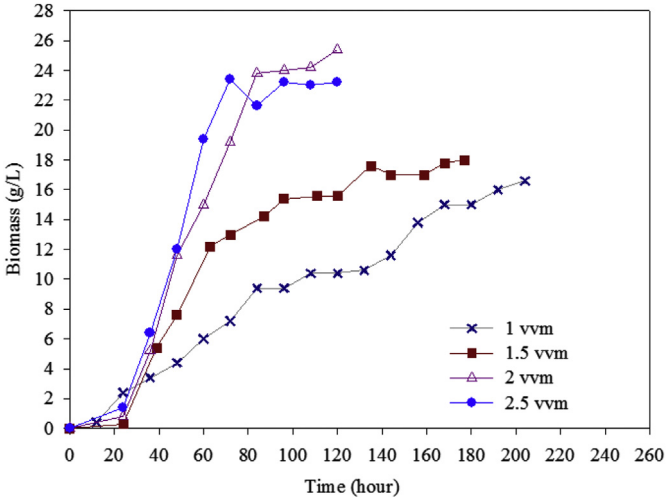

FIG. 2. The effects of aeration rate (1, 1.5, 2.0 and 2.5 vvm) on the cells growth in the airlift bioreactor. Crosses, 1.0 vvm; squares, 1.5 vvm; diamonds, 2.0 vvm; circles, 2.5 vvm.

TABLE 1. The comparison of kinetic parameters obtained in the batches with different aeration rate.

	Airlift				Agitation
	1 vvm	1.5 vvm	2 vvm	2.5 vvm	1.0 vvm
Max biomass (g/L)	16.6	18	25.4	23.4	20.3
Average lipid content (%)	44.8 ± 3.5	53.4 ± 4.1	50.2 ± 4.8	37.6 ± 3.6	25 ± 4
Max biomass growth rate (g/L h)	0.117	0.305	0.383	0.458	0.220
Max total lipids (g/L)	7.4	9.6	12.8	8.8	9.1
Average total lipids productivity (g/L h)	0.052	0.163	0.192	0.172	0.094

agitation tank. As shown in the previous section, the cells grown in the airlift bioreactor could have a higher lipid content than those grown in the agitation tank at 1 vvm. In the batches with different aeration rates, when this was raised to 2.5 vvm the lipid content would fall slightly to 37.6 ± 3.6%. The highest total lipid productivity of 0.19 g/L h was observed in the batch produced with an aeration rate of 2.0 vvm.

The reason for the increase in the cell growth rate with the rise in the aeration rate may be due to greater amount of oxygen provided by the increased air flow rate. Cerri and Badino reported that an increase in the aeration rate (presented as the superficial velocity in their work) in the operation of a concentric tube airlift bioreactor would increase the value of K_La in a proportional manner (13). Similar to their results, the current study found that the K_La values increased proportionally along with the aeration rate, being 0.135, 0.193, 0.268 and 0.272 (1/min) in the batches with 1.0, 1.5, 2.0 and 2.5 vvm, respectively. The regression line of the cell growth rate and the measured K_La is a well-fitted correlation curve, as shown in Fig. 3. The R^2 is about 0.94, which suggests that the cell growth rate in the operation of the airlift bioreactor is strongly dependent on the K_La. It thus seems that an increase in the aeration rate is a simple route that can efficiently enhance both the growth rate and lipid productivity. Nevertheless, an increase in the aeration rate could lead to the significant foaming, which would threaten the fermentation process. In addition, the use of an air compressor to raise the aeration rate also requires energy, and this needs to be considered in the overall cost analysis. Therefore, the maximum aeration rate cannot be increased without limit, and thus another approach to raising the amount of dissolved oxygen is to increase the airlift bioreactor tank pressure.

The effects of pressurization on *R. glutinis* growth in a 5-L airlift bioreactor As shown in the previous section, an increase in the aeration rate can enhance cell growth in the airlift

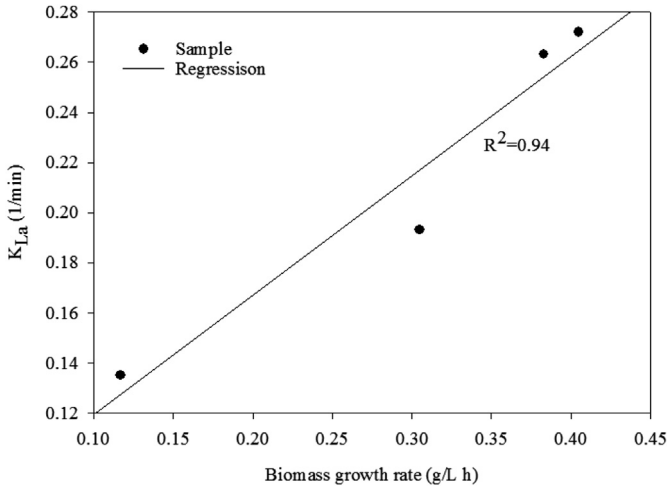

FIG. 3. The correlationship between K_La and the biomass growth rate in the airlift bioreactor. Closed circles, sample data; lines, model regression curve.

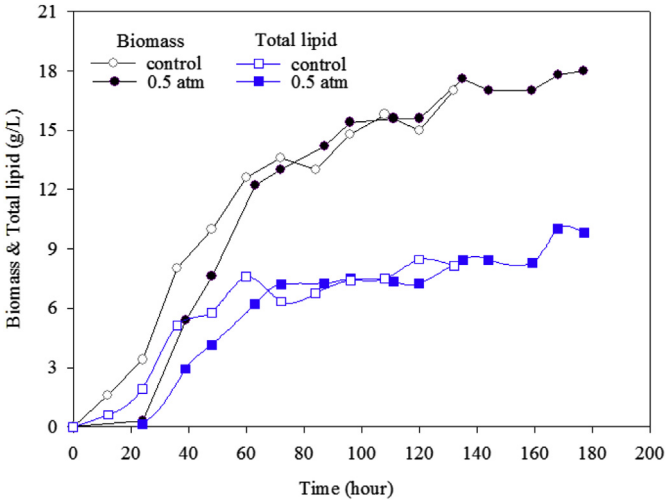

FIG. 4. The effects of pressurization (0.5 atm) on the cells growth and on the total lipids production in the airlift bioreactor. Open circles, biomass in the control batch; closed circles, biomass in the batch with 0.5 atm; open squares, total lipid in the control batch; closed squares, total lipid in the batch with 0.5 atm.

bioreactor. However, increasing the aeration rate requires more energy, which increases the cost of the process. However, increasing the tank pressure is another way that can raise the level of dissolved oxygen. Therefore, the operation of an airlift bioreactor at 0.5 atm and 1.5 vvm was compared with that at 1.5 vvm and atmospheric pressure. The results are shown in Fig. 4, and these indicate that increasing the airlift bioreactor pressure to 0.5 atm did not enhance cell growth compared to the control batch. The lipid contents were also very similar in both batches, which led to a similar total lipid production. The results thus suggest that the aeration rate remains the critical factor controlling the growth of cells. Even though pressurization could slightly increase the amount of dissolved oxygen, it did not have a serious impact on the cell growth rate. Therefore, a more detailed study on the energy balance (air compressor energy input and microbial oil energy recovery) is required to decide the optimum aeration rate for this process. Conclusively, the results of this study indicate that airlift bioreactors might be suitable for use in the cultivation of oleaginous *R. glutinis* for microbial oil production.

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