Production of Astaxanthin by *Xanthophyllomyces dendrorhous* ZJUT46 with Fed-Batch Fermentation in 2.0 M^3 Fermentor

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Summary

Astaxanthin is a main carotenoid pigment. Fed-batch fermentation bioprocesses of astaxanthin in 2.0 M^3 fermentor have been investigated. Moreover, pH shift control was employed in fed-batch fermentation at 2.0 M^3 scale. The results of astaxanthin fermentation showed that the fed-batch fermentation with pH shift strategy was the best among all the experiments, and the maximum astaxanthin concentration of 39.47 mg/L was achieved at 132 h.

Key words: astaxanthin, fed-batch fermentation, carotenoid, *Xanthophyllomyces dendrorhous*

Introduction

Astaxanthin is an orange-pink carotenoid pigment commonly found in marine animals (*1,2*). It has a strong antioxidant activity (*3*) and some essential biological functions, including protection against UV-light effects (*4*) and enhancing immune response (*5*).

Astaxanthin is important for animal feeding (especially in the aquaculture industry), pharmaceuticals, cosmetics and the food industry (*6*). The red yeast, *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*) (*7*), has been considered as a good source of astaxanthin (*8*), and is being considered for industrial production of astaxanthin (*9*).

Many recently published works report astaxanthin fermentation by *X. dendrorhous* at lab scale (*10–13*). Some of them have studied the optimization of astaxanthin production, either analyzing the influence of the operational conditions or investigating carbon sources and other components. Moreover, sugar-feeding strategy and pH control strategy on astaxanthin production by *X. dendrorhous* at lab scale were investigated in our previous works (*14,15*). Present study reports the production of astaxanthin by *X. dendrorhous* in 2.0 M^3 fermentor.

Materials and Methods

Microorganism

The microorganism *X. dendrorhous* ZJUT46, employed in this study, was maintained at 4 °C on YM agar slants with composition (in g/L) of glucose 10, malt extract 3, yeast extract 3, peptone 5, agar 20 and 1 L of water, and transferred monthly (*14,15*).

Seed preparation

The seed culture was prepared in the mechanically stirred fermentor that had a total volume of 200 L and a ratio of height-to-diameter of 3.0, and had three impellers on the vertical shaft. The type of impeller was the disc turbine with six flat blades. Temperature was controlled by water pumped through jackets on the vessels.

The inoculum was first grown in 500-mL flasks each containing 50 mL of sterile inoculum medium, which was composed of 20 g of glucose, 8 g of yeast extract and 2 g of peptone per litre. Then the flasks were kept on rotary shakers with 200 rpm for 48 h at 20 °C. This culture was transferred to 200-litre fermentor containing 130 L of inoculum medium, to which 15 mL of polyethylene glycol were added as antifoam agent.

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Temperature, pH, dissolved oxygen and agitation speed were measured online. The main operation conditions of seed culture were as follows: temperature was 20 °C, pH profile was maintained at 5.0 with 2.0 M NaOH and 2.0 M HCl, and the aeration ratio and agitation speed were controlled as follows: the aeration ratio and agitation speed were 0.5 and 1.0 (by volume per volume per minute) and 300 and 350 rpm for 0–24 and 24–48 h, respectively. The course of seed culture was constantly monitored by off-line sampling and determined at 12-hour intervals. Each sample was subjected to the test for sterility, reducing sugars, total sugars and biomass content.

After 48 h, the seed culture was transferred to the 2.0-M^3 fermentor with working volume of 1.5 M^3. An inoculum ratio of 5 % (by volume) was used during the process.

Batch fermentation procedure

All fermentation experiments were carried out in the mechanically stirred fermentor. It had a total volume of 2.0 M^3 and was of the same structure as the 200-litre fermentor that was used in seed culture.

The medium for batch fermentation contained 40 g of glucose, 8 g of yeast extract, 8 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of KNO_3, 0.5 g of K_2HPO_4 and 0.1 mL of polyethylene glycol per litre, and polyethylene glycol was added as antifoam agent.

Temperature, pH, dissolved oxygen and agitation speed were measured online. The main operating conditions were as follows: temperature 20 °C, pH profiles maintained at 5.0 with 2.0 M NaOH and 2.0 M HCl, and the aeration ratio and agitation speed controlled with the following method: the aeration ratio and agitation speed were 0.3, 0.4 and 0.5 (by volume per volume per minute) and 100, 140 and 180 rpm for 0–48, 48–96 and from 96 h until the end of fermentation, respectively. The course of fermentation was constantly monitored by off-line sampling at 12-hour intervals, each sample was subjected to the test for sterility, reducing sugars, biomass content and astaxanthin concentration.

Three batches were repeated in each experiment.

Fed-batch fermentation procedure

The basal medium for fed-batch fermentation was composed of 40 g of glucose, 8 g of yeast extract, 8 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of KNO_3, 0.5 g of K_2HPO_4 and 0.1 mL of polyethylene glycol per litre.

Glucose and $(NH_4)_2SO_4$ were sterilized separately. The fermentation media were autoclaved for 30 min at 121 °C.

The stock solution of glucose (50 %) was used as the feeding substrate in the fed-batch process.

The fed-batch fermentation was first carried out in the batch model with initial glucose until the glucose concentration dropped to around 5 g/L. Glucose was then added in three feedings of 22.5, 22.5 and 15 kg of sugar during the experiments. The final total glucose, including initial glucose in the basal medium and the glucose added in three feeding processes, made total concentration in all experiments of 80 g/L. Moreover, the main

operating conditions of fed-batch fermentation were the same as those of the batch fermentation procedure.

Fed-batch fermentation procedure with pH-shift control

In this experiment, the conditions of temperature, dissolved oxygen and agitation speed were the same as those of fed-batch fermentation. However, the pH-shift control strategy was as follows: first, the culture pH was maintained at pH=6.0 around 80 h with 2.0 M NaOH and 2.0 M HCl, then it was shifted slowly to pH=4.0 with 2.0 M HCl.

Analytical methods

Samples were withdrawn from the fermentor every 12 h, and each sample was split in two aliquots. One of them was centrifuged (5000 × *g*, 15 min) and the pellet was collected, washed twice with distilled water, and dried at 105 °C until constant mass, which was taken as the biomass content of the culture. On the other hand, the supernatant was used to determine the reducing sugars. 3,5-Dinitrosalicylate method was used for quantitative analysis of reducing sugars (*16*). The other aliquot was used for quantification of astaxanthin according to *Calo et al.* (*17*).

The cell yield ($Y_{x/s}$), the production yield ($Y_{p/s}$) and the production formation rate (Q_p) were calculated as follows:

$$Y_{x/s}=(X_f-X_i)/(S_i-S_f) \qquad /1/$$

$$Y_{p/s}=(P_f-P_i)/(S_i-S_f) \qquad /2/$$

$$Q_p=(P_f-P_i)/T \qquad /3/$$

where X_i, S_i, and P_i are the initial cell, substrate (glucose), and astaxanthin concentration, respectively; X_f, S_f, and P_f are the final cell, substrate (glucose), and astaxanthin concentration, respectively; and T is the fermentation time (*18*).

Results and Discussion

Batch and fed-batch fermentation at 2.0 M^3 scale

To establish the optimal conditions for the production of astaxanthin at 2.0 M^3 scale, the batch and fed-batch fermentation experiments were carried out in 30-litre fermentor scale. Moreover, the sugar-feeding strategies were performed (*14*). According to our previous experimental results, the optimal sugar-feeding strategy for astaxanthin fermentation at 30-litre scale was as follows: the initial sugar was 40 g/L with the total sugar of 80 g/L, and 300, 300 and 200 g of glucose were fed at discrete pulse. The same sugar feeding strategy was employed at 2.0 M^3 scale fermentation process.

The time-courses of the batch and fed-batch fermentations are shown in Figs. 1 and 2. In fed-batch fermentation process, with the consumption of sugar, the astaxanthin formation was observed after the beginning of cell growth and continued for about 24 h after the cessation of the cell growth. The maximum biomass (X_{max}) of 17.78 g dry cell/L was achieved at 108 h. It was higher by 5.9 % than that of batch fermentation, and the cell
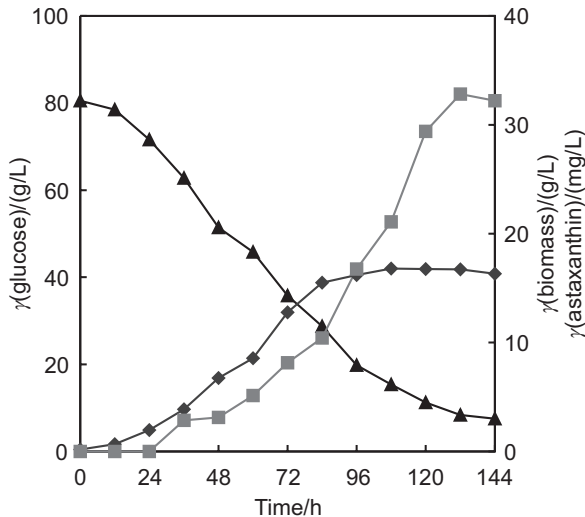
Fig. 1. The time-course of 2.0 M^3 scale batch fermentation of astaxanthin at 20 °C with constant pH=5.0: astaxanthin (■), glucose (▲), biomass (◆)
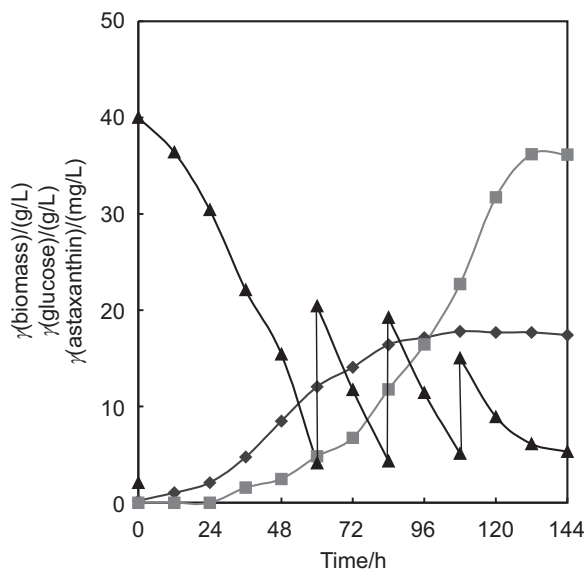

Fig. 2. The time-course of 2.0 M^3 scale fed-batch fermentation of astaxanthin at 20 °C with constant pH=5.0: astaxanthin (■), glucose (▲), biomass (◆)

yield ($Y_{x/s}$) of 0.24 and 0.23 g/g in fed-batch and batch fermentation were obtained, respectively, with only a small discrepancy of 4.3 %. Moreover, the astaxanthin concentration reached the maximum (P_{max}) of 36.21 mg/L, which was higher by 10.3 % than that of batch fermentation, and the overall astaxanthin formation rate (Q_p) of 0.27 mg/(L·h) and the production (astaxanthin) yield ($Y_{p/s}$) of 0.49 mg/g at fed-batch fermentation were obtained, which were higher by 8.0 and 6.5 %, respectively, than that of batch fermentation.

Fed-batch fermentation at 2.0 M^3 scale with pH-shift control strategy

In our previous work, investigation about the effects of pH on astaxanthin production and cell growth in batch fermentation at the lab scale showed that the opti-

mal pH for cell growth and for astaxanthin formation was around 6.0 and 4.0, respectively (*15*). Furthermore, the optimal pH-shift control strategy in batch fermentation process was developed as follows: in the first stage, the culture pH was maintained at pH=6.0 around 80 h for cell growth, it was then shifted to pH=4.0 for astaxanthin production in the secondary stage. In this work, the same pH-shift control strategy was carried out in fed-batch fermentation of astaxanthin at 2.0 M^3 scale, and the results of the experiment are shown in Fig. 3.
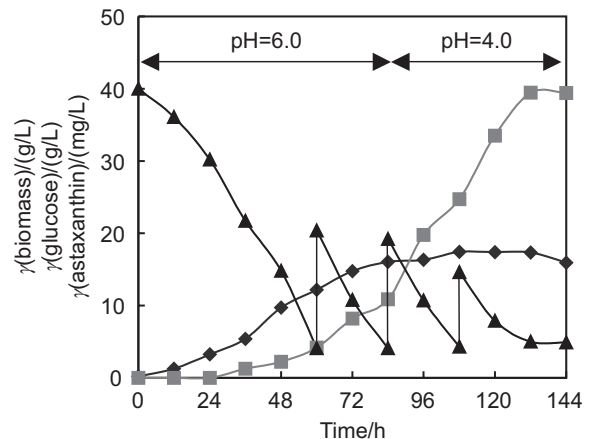

Fig. 3. The time-course of 2.0 M^3 scale fed-batch fermentation of astaxanthin at 20 °C with pH-shift control strategy: astaxanthin (■), glucose (▲), biomass (◆)

The maximum astaxanthin concentration (P_{max}) at fed-batch fermentation with pH-shift control strategy reached 39.47 mg/L at 132 h, and was higher by 20.2 and 9.0 % than that of the batch and fed-batch fermentation, respectively, with constant pH=5.0. Moreover, the overall astaxanthin formation rate (Q_p) of 0.30 mg/(L·h) and $Y_{p/s}$ of 0.53 mg/g were achieved, which were higher by 11.1 and 8.2 %, respectively, than those of fed-batch fermentation with constant pH=5.0. However, the maximal cell density (X_{max}) at fed-batch fermentation with pH-shift control was 17.42 g dry cell/L, and was lower by 2.0 % than that of fed-batch fermentation with constant pH=5.0.

Conclusion

The above results showed that fed-batch fermentation with the pH-shift control strategy is the best among all the experiments in this work. A significant increase (9.0 %) in production of astaxanthin was achieved in 132 h in fed-batch process with pH-shift control strategy, as compared with fed-batch process with constant pH=5.0. By fine tuning of operation conditions, there may be a scope for further enhancement of astaxanthin concentration.

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