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Heterotrophic Mass Cultures of *Chlorella Vulgaris* with Glucose Feeding in Fermenters*

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Abstract: *Chlorella vulgaris* was heterotrophically cultured in 5, 50, 200, 800, and 4 000 L stirred tank fermenters with glucose feeding, which gave significant improvement in cell density and productivity compared to batch culture. The highest cell dry weight mass concentration reached 43.31 g/L. The specific growth rate was 0.069 h^{-1}. The cell productivity was 0.62 g/(L·h). The results suggest *Chlorella vulgaris* can be heterotrophically produced in stirred tank fermenters for commercial use.

Key words: *Chlorella vulgaris*; stirred tank fermenter; heterotrophic mass culture

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0 Introduction

Microalgae have been produced as health food, and have found applications in agri-culture, wastewater treatment and aquaculture and for the production of bioactives[1, 2]. So there are considerable demands in market.

The production of microalgae has mainly employed photoautotrophic culture system. There are some drawbacks in such system, such as difficulty of maintaining monoculture, light limitation and lack of control of important environmental factors. Technical devel-opments and improvements in photobioreactor design may overcome these problems, but this invariably requires significant extra initial capital investment[1, 3].

Heterotrophic culture system, which can overcome the above problems encountered in the photoautotrophic system and improve growth and productivity, may be used as a substitute for photoautotrophic culture system. Up to now, several kinds of microalgae strains with heterotrophic function and higher yield have been screened, among which *Chlorella vulgaris* is a typical one[4]. This paper reports the application of heterotrophic fed-batch culture of *Chlorella vulgaris* as a means for increasing

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cell density and produ-ctivity.

1 Materials and Methods

1.1 Microorganism and Medium

The strains used were *Chlorella vulgaris* HKU-1 and 4 and the mutants generated in our laboratory. Cells were cultured in basal medium with 10 g/L glucose[5].

1.2 Fed-batch Cultures with Glucose Feeding

The preculture broth was inoculated into fermenters (5 L Biostat B, 50, 200, 800 and 4 000 L were equipment of Guangdong Jiangmen Centre for Biotechnology). When the residual glucose mass concentration decreased to 3 ~ 5 g/L, glucose was provided. The nutrients except for glucose were supplied at the same time. Dissolved oxygen concentration was controlled by increasing agitation speed and airflow. Aeration rate and the agitation speed were variable and initially set at 0.8 L/(L·min) (0.8vvm) and 300 r/min, respectively. Temperature was controlled (30 ± 1) ℃.

1.3 Analytical Methods

Biomass concentration was determined by centrifuging a fixed volume of the culture, washing the pellet twice by resuspending and recentrifuging, and drying the final cell pellet to constant weight at 105 ℃.

Glucose was analyzed with the method of 3,5-disalicylic acid[6].

2 Results and Discussion

2.1 Fed-batch Culture in 5 L Fermenter

According to the result of flask culture, scale-up culture of *Chlorella vulgaris* was performed in 5 L fermenter. The preculture broth (300 mL) was inoculated into 5 L fermenter containing 2.7 L of the medium. When the residual glucose mass concentration decreased to 3 ~ 5 g/L after 18 h, 600 g/L glucose was batch-fed.

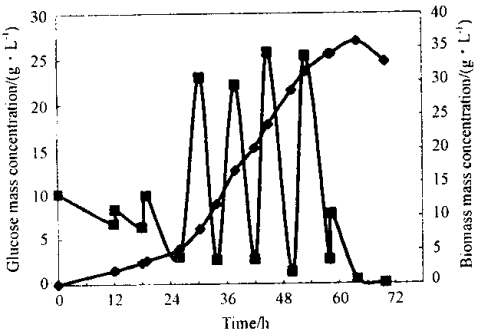

Fig. 1 Growth and glucose consumption curve of the *Chlorella vulgaris* in 5 L stirred tank reactor culture

■—Glucose; ◆—Biomass.

As shown in Fig. 1, with the fed-batch culture by glucose feeding, the cell growth reached maximum value 35.84 g/L in 64 h culture, which decreased to 32.87 g/L in the subsequent 6 h culture. The maximum specific growth rate was 0.057 h^{-1}. The productivity was 0.56 g/(L·h). The growth yield coefficient $Y_{X/S}$ based on glucose was 0.41, which was comparable to other microbial systems.

2.2 Fed-batch Cultures in 50 L and 200 L Fermenter

The good results stimulated us to perform cultures in 50 L and 200 L fermenter. The results of the cultures were shown in Fig.2 and Fig.3.

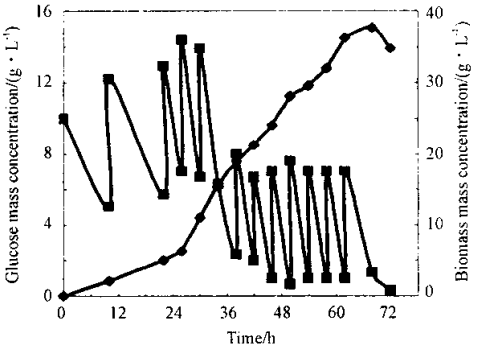

Fig.2 Growth and glucose consumption curve of the *Chlorella vulgaris* in 50 L stirred tank reactor culture

■—Glucose; ◆—Biomass.

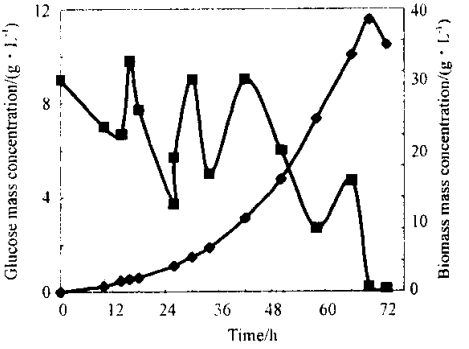

Fig.3 Growth and glucose consumption curve of the *Chlorella vulgaris* in 200 L stirred tank reactor culture

■—Glucose; ◆—Biomass.

As shown in Fig.2, the growth reached 37.6 g/L in 68 h culture in 50 L bioreactor, which decreased to 34.75 g/L in the subsequent 4 h culture. The maximum specific growth rate was 0.049 h^{-1}. The productivity was 0.55 g/(L·h). The results cultured in 50 L bioreactor were comparable to that of 5 L bioreactor. The growth yield coefficient $Y_{x/s}$ based on glucose was 0.47.

As shown in Fig.3, though the maximum specific growth rate was 0.069 h^{-1} which was lower than that of 5 L and 50 L bioreactor, the cell dry weight reached 43.31 g/L in 70 h culture which was higher than that of 5 L and 50 L bioreactor. The cell dry weight decreased to 39.31 g/L in the 4 h culture subsequently. The productivity was 0.62 g/(L·h), which was higher than that of 5 L and 50 L bioreactor. The growth yield coefficient $Y_{x/s}$ based on glucose reached 0.48.

The results indicated that heterotrophic culture of *Chlorella vulgaris* was promising.

2.3 Mass Cultures in 800 L and 4 000 L Fermenter

As shown in Fig.4, cell mass concentration reached 32.42 g/L in 67 h culture, which was slightly lower than that of 5, 50 and 200 L. The maximum specific growth rate was 0.047 h^{-1}. The productivity was 0.48 g/(L·h). The growth yield coefficient $Y_{x/s}$ based on glucose was 0.40.

As shown in Fig.5, cell mass concentration reached only 30.62 g/L in 63 h culture. The maximum specific growth rate was 0.045 h^{-1}. The productivity was 0.37 g/(L·h) and the growth yield coefficient $Y_{x/s}$ based on glucose was only 0.33, which was slightly lower than that of 5, 50, 200 and 800 L. It might resulted from inconvenient controls of the important environmental factors. Lower as the cell concentration was, it was still practical in large-scale industrial production.

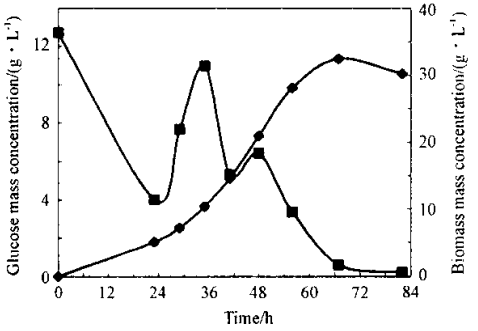

Fig. 4 Growth and glucose consumption curve of the *Chlorella vulgaris* in 800 L stirred tank reactor culture

■—Glucose ; ◆—Biomass.

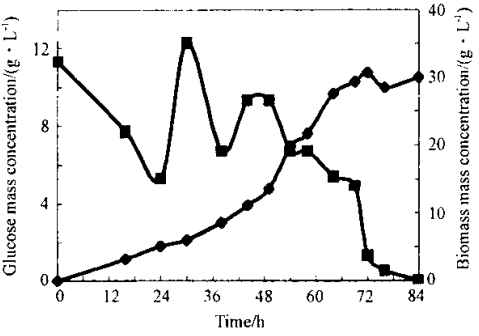

Fig. 5 Growth and glucose consumption curve of the *Chlorella vulgaris* in 4 000 L stirred tank reactor culture

■—Glucose ; ◆—Biomass.

3 Conclusion

The heterotrophic culture of *Chlorella vulgaris* in 5, 50, 200, 800, and 4 000 L stirred tank reactors was successful and promising. The results demonstrate that the heterotrophic fed-batch culture of *Chlorella vulgaris* can increase the alga cell mass concentration and productivity on a large-scale. A cost-effective fermentation process has been established through high cell density strategies for the growth of selected *Chlorella vulgaris*. The results suggest that the development of industrial heterotrophic process for the production of *Chlorella vulgaris* is practical.

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发酵罐葡萄糖流加大规模异养培养小球藻*

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摘 要：应用流加工艺在5，50，200，800，4 000 L机械搅拌发酵罐中大规模异养培养小球藻. 与间歇异养或光照自养相比，流加培养大大地提高了细胞密度和生产率. 最高细胞质量浓度达到43.31 g/L，比生长速率达到0.069 h^{-1}，细胞生产率达到0.62 g/(L·h). 结果表明，利用传统的机械搅拌发酵罐大规模生产小球藻有良好的商业化前景.

关键词：小球藻；搅拌发酵罐；大规模异养培养
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发酵罐葡萄糖流加大规模异养培养小球藻

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