

## Abstract

Photoautotrophic cultivation of *Euglena gracilis* results in cells with high  $\alpha$ -tocopherol content but the final cell concentration is usually very low due to the difficulty of supplying light efficiently to the photobioreactor. On the other hand, *Euglena* grows heterotrophically to high cell concentrations, using various organic carbon sources, but the  $\alpha$ -tocopherol contents of heterotrophically grown cells are usually very low. Sequential heterotrophic/photoautotrophic cultivation, by which cells are grown heterotrophically to high cell concentrations and then transferred to photoautotrophic culture for accumulation of  $\alpha$ -tocopherol was therefore investigated for efficient  $\alpha$ -tocopherol production. In batch culture, using glucose as the organic carbon source, the cellular  $\alpha$ -tocopherol content increased from  $120 \mu\text{g g}^{-1}$  at the end of heterotrophic phase to more than  $400 \mu\text{g g}^{-1}$  at the end of the photoautotrophic phase. By using ethanol as the organic carbon source during the heterotrophic phase, adding corn steep liquor as a nitrogen source and optimizing light supply during the photoautotrophic phase, the  $\alpha$ -tocopherol content of the cells at the end of the photoautotrophic phase increased to  $1700 \mu\text{g g}^{-1}$ . A system consisting of a mini-jar fermentor (for the heterotrophic phase) and an internally illuminated photobioreactor (for the photoautotrophic phase) was then constructed for continuous sequential heterotrophic/photoautotrophic cultivation. The cells were continuously cultivated heterotrophically in the mini-jar fermentor and the effluent was continuously passed through the photobioreactor for  $\alpha$ -tocopherol accumulation. In this way, it was possible to produce  $7 \text{ gm l}^{-1}$  cells containing about  $1100 \mu\text{g}$   $\alpha$ -tocopherol per g-cell continuously for more than 420 h. The continuous process resulted in  $\alpha$ -tocopherol productivity of  $100 \mu\text{g l}^{-1} \text{ h}^{-1}$  which is about 9.5 and 4.6 times higher than those obtained in batch photoautotrophic culture and batch heterotrophic cultures, respectively.

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