

A Comparative Study of *Euglena gracilis* Cultivations for Improving Laminaribiose Phosphorylase Production

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Abstract : Laminaribiose is a beta-1,3-glycoside which is used in the medical field for the treatment of dermatitis and also can be used as a building block for new pharmaceuticals. The conventional process of laminaribiose production is the uneconomical process of hydrolysis of laminarin extracted from natural polysaccharides of plant origin. A more economical approach however is attainable by enzymatically synthesis of laminaribiose via a reverse phosphorylase reaction catalyzed by laminaribiose phosphorylase (LP) from *Euglena gracilis*. Different cultivation methods of *Euglena gracilis* and the effect on LP production have been investigated. Buffered/unbuffered heterotrophic and mixotrophic cultivations of *Euglena gracilis* has been carried out. Changes of biomass and LP production, glucose level and pH, cell count and shape has been monitored in the course of time. The results obtained from experiments each in three repetitions, show that in the heterotrophic cultivation of *Euglena gracilis* not only more biomass is produced compared to mixotrophic cultivation, but also higher specific protein concentration is achieved. Furthermore, the LP activity test showed that the protein extracted from heterotrophically cultured cells has a higher LP activity. It was also observed that the cells develop in a distinctive different shape between these two cultures and have different length to width ratios. Taking the heterotrophic culture as the more efficient cultivation method in LP production, another comparative experiment between buffered and unbuffered heterotrophic culture was carried out that showed the unbuffered culture has advantages over the other one in respect of both LP production and resulting activity. A heterotrophic cultivation of *Euglena gracilis* in a 5L bioreactor with controlled operating conditions showed a distinctive improvement of all the aspects of culture compared to the shaking flask cultivations. Biomass production was improved from 5 to more than 8 g/l (dry weight) which resulted in a specific protein concentration of 45 g/l in the heterotrophic cultivation in the bioreactor. In further attempts to improve LP production, different purification methods were tested and each method was checked through an activity assay. A laminaribiose yield of 35% was achieved which was by far the highest amount amongst different methods tested.

Keywords : *euglena gracilis*, heterotrophic culture, laminaribiose production, mixotrophic culture

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