Bioprocess design for microbial glycosylation of Azidothymidine

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Abstract

This work shows the glycosylation of azidothymidine (AZT) by whole cells and biofilms in different reaction scales.

Introduction

Antiretroviral drug azidothymidine is an endogenous thymidine analogue which has azido (N3) in the ribose, was synthesized initially as anticancer agent[1,4]. Glycosylation is one way of obtaining derivatives that provide a better solubility, bioactivities and stability to organic compounds[5]. Bioprocesses on a large scale with the use of whole cells, although few described in the literature, are favored by mass transfer, metabolism and cell growth.[2] Biofilms are successful microbial communities, which ensure protection against environmental stresses and antimicrobial agents to cells embedded in the matrix and can be produced in a multitude of surfaces[3].The present work aimed to obtain derivatives of azidothymidine in microplate, semi preparative scale, and scale-up in bioreactor of whole cell and biofilm.

Results and Discussion

The assays were performed with *Cunningamella echinulata* ATCC 9245 in whole free cells in micro scale, semi-preparative scale, in 100 ml of medium in erlenmeyer, and scale-up on a bench bioreactor with 3L culture medium. The biofilm assays were carried out in semi-preparative scale on stainless steel springs. In all bioprocesses, azidothymidine was added at a concentration of 0.5 mg / ml. After addition of the AZT, each 24 hours, aliquots of the reaction medium were taken for HPLC e TLC analysis. In this work was observed a reduction of production of the derivatives when the scale was increased and that in the bioreactor there was the absence of a derivative. The mass transfer and oxygen may be impaired with increasing workload, showing necessity for culture conditions adjustments for the scaling of these reactions (Table 1). With biofilm were observed the presence of same derivatives of semi-preparative scale with whole cells, but there was decrease in yield of extracted compounds (Figure 1).

Table 1. Derivatives obtained in different processes monitored by HPLC. MS – micro scale, SPS - semi-preparative scale, SUB – Scale-up bioreactor, BFM-Biofilm. Mobile phase: water: (methanol: water 50:50) 50:50.

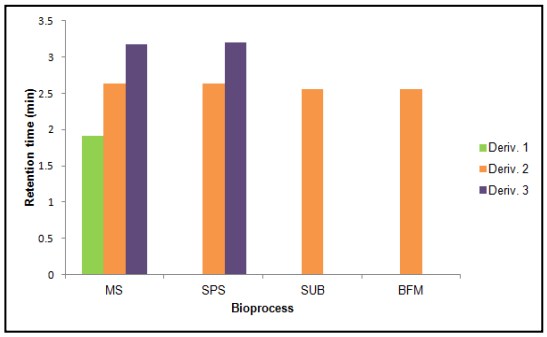

Figure 1. Yield rate compounds extracted with ethyl acetate and morphology of microorganism in different process.

Bioprocess	Yield	Morphology
Whole cells	79.78%	
Biofilm	33.7%	

Conclusion

This work suggests that the reaction medium volume and process type may lead to qualitatively and quantitatively different yield.

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