

Filamenteous fungi (*Trichoderma harzianum*) behaviour in biofilm culture for 6-pentyl-α-pyrone production as secondary metabolite with coconut aroma



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INTRODUCTION

This study examines the morphology and the production of the 6-pentyl- α -pyrone (6-PP), a compound which has a strong coconut-like aroma, by *Trichoderma* spp. species in biofilm bioreactors. It gives the aspect of fungus on inert support and in the liquid during fermentation. The dextrose and castor oil were used as carbon source when culture are conducted in flask bioreactor, and only oil in stirred bioreactor, with castor oil being considered as a precursor in lactones bioconversion processes.

DISADVANTAGE / ADVANTAGE

The effect of *Trichoderma* spp. secondary metabolites like 6-pentyl- α -pyrone on mycelial growth causing inhibition and reducing the production of compounds needed.

The process used in this case gives the possibility to enhance the biomass and secondary metabolite produced by a micro-organism.

EXPERIMENT PERFORMED

The experiment was carried out in a flask and an adapted 20 l bioreactor (Biolafitte, France) with a structured support Sulzer using *Trichoderma* with recirculation system. The carbon substrate was dextrose (Roquette Frères, Lestrem, France) 30 gl⁻¹ and castor oil Alfa, Aesar GmbH & CoKG, Germany) 20 gl⁻¹.

The culture medium composition was (g l-1): NaNO₃ (Grauwmeer, Belgium) 2, KH₂PO₄ (Merck, KGaA, Germany) 1, KCl (Merck, KGaA, Germany) 0.5, MgSO₄.7H₂O (Merck, KGaA, Germany) 0.5, CaCl₂.6H₂O (VWR, Belgium) 0.008, ZnSO₄.7H₂O (VWR, Belgium) 0.001, FeSO₄.7H₂O (Vel, Belgium) 0.01, yeast extract 1.0, peptone 2.0 and 20 castor oil (Alfa, Aesar GmbH & CoKG, Germany) or dextrose (Roquette Frères, Lestrem, France) 30. The pH was adjusted to 6. The agitation was maintain at 200 rpm and aeration at 1 v/v/m.

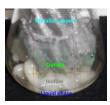
The volatile extraction was performed by a liquid–liquid (1:1) method using diethyl ether (Merck, KGaA, Germany). After that, the ether layer was recovered and dehydrated by anhydrous Na₂SO₄ (Sigma-Aldrich, GmbH, Germany), filtered using Pall acrodisc filter with nylon membrane 0.2 μm Millipore on glass syringe in vials for GC FID analysis.

RESULTS

The effect of culture system on production biomass and 6-pentyl- α -pyone by *T.harzianum* was evaluted on biofilm culture performed in flask and stirred bioreactor. In liquid phase free mycelia (Fig. 1) and pellet are often observed while biofilm and conidia (Fig. 2) are accumulated on the support (Sulzer packing) (Fig. 3) used for the culture







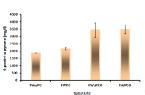






Fig. 1. Microscopic view of *Trichoderma harzianum* when free mycelia and conidia are observed.

Fig. 2. Aspect of biofilm culture in flask, production of 6-pentyl-c-pyrone by *Trichoderma* harzanum during 96 h with 50 ml as working volume and adapted 250 ml flask by making a packing to put into bioreactor, or without support.

Fig. 3. Aspect of biofilm culture in bioreactor with Sulzer packing and secondary metabolite produce

The biomass are accumulated on the support used [1] and they are production of secondary metabolite (Fig.3)

CONCLUSIONS

The results obtained suggest that three forms are observed during the culture and accumulation of biomass on the support used confirm the immobilization of the micro-organism on it. The secondary metabolites are also produced.

REFERENCES