Enhancement of recombinant protein excretion with *Aspergillus oryzae* fermentation in semi-solid conditions

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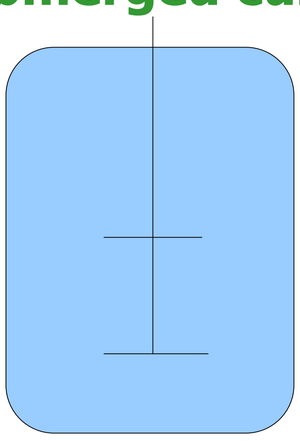
Context

Secondary metabolites

Production at the industrial scale

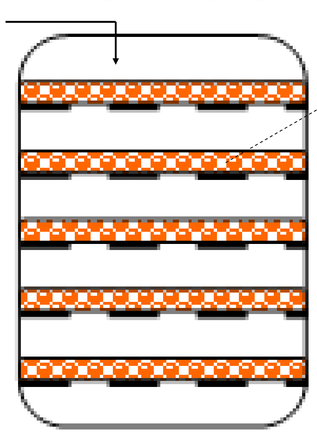
Submerged culture



(+) simple implementation
(-) viscosity, shear stress

OR

Solid state culture



(+) productivity, low effective volume

(-) mass transfer (metabolites recovery) and heat transfer

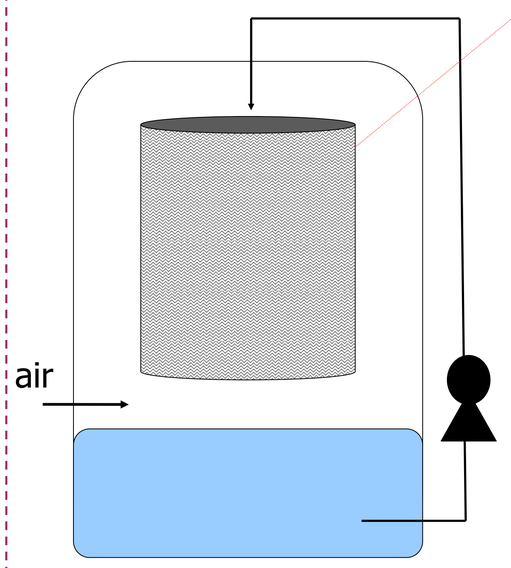
Objectives

• Design a bioreactor combining advantages from submerged and solid state culture

• Compare its performances with a submerged culture in the case of a recombinant protein excretion (GFP) from *Aspergillus oryzae*

Methodology

Semi solid culture



Experimental setting

• metal structured packing (high A_{spec})

• Recirculation of the liquid medium by aspersion on the packing

•*A. oryzae* strain includes a reporter gene (GFP) assimilated as the recombinant protein

Analysis

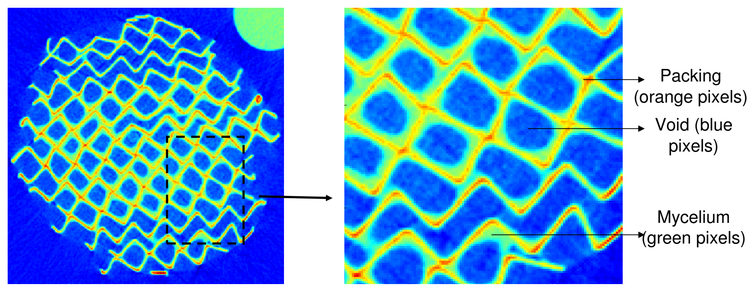
• GFP quantification by immunoblot analysis in the supernatant

• Mycelium visualization inside the metal packing by X-ray tomography

Results & Discussion

Colonized packing (A)

Image of a X-ray tomography analysis of a packing cross section (B)



• Homogeneous distribution of the mycelium on the surface of the corrugated sheets of the metal structured packing (A)

• Mycelium layer is thicker in the center than on edge of the packing (B)

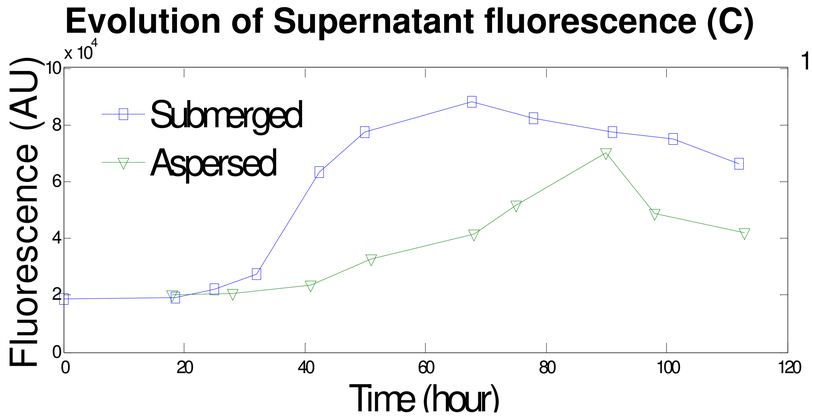
➔ liquid distribution on the packing is not optimal and leads to preferential flow streams

• Liquid medium stays perfectly limpid during all the culture because mycelium grows only on the packing

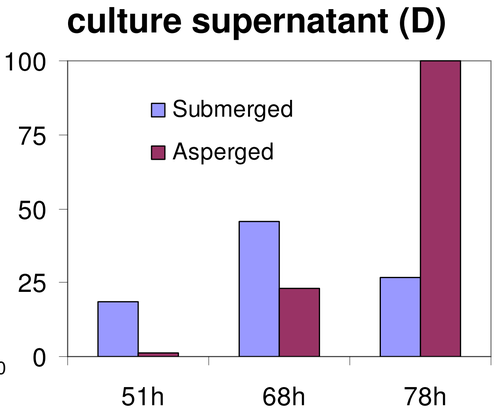
➔ liquid phase keeps a low viscosity

➔ it improves mass transfer and downstream process operations

Evolution of Supernatant fluorescence (C)



Relative abundance of GFP in culture supernatant (D)



• Kinetic of GFP production is better in submerged culture than in semi solid culture (C)

➔ agitation improves dynamic of the process

• Spectrofluorescence does not allow to compare GFP concentration between both conditions because it cannot assay denatured GFP

➔ GFP are quantified by immunoblot analysis (D)

• Relative abundance of GFP is higher in semi solid culture than in submerged culture (2 fold)

➔ promotor *pglaB* monitoring reporter gene transcription (GFP) is specifically induced in solid state conditions

➔ The wrong pH regulation could be the cause of denatured GFP in the semi solid culture. New experiments are running with optimal pH regulation in order to confirm our results

CONCLUSION

In this study, design of a new bioreactor combining advantages from submerged culture and solid state culture was experimented for the production of a recombinant protein (GFP) from A. oryzae. The semi solid conditions achieved in this bioreactor induce the growth of the fungi in a physiology state of solid medium and improve recombinant protein excretion compared with a submerged culture.

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