

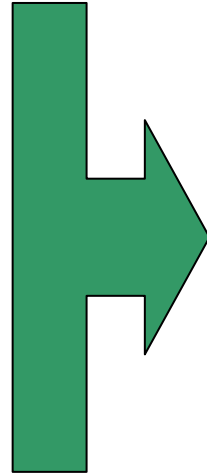
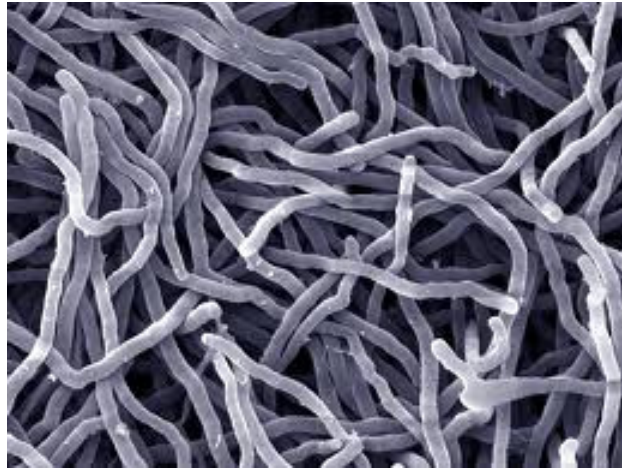


# **IMPLEMENTATION OF A METAL STRUCTURED PACKING IN A FUNGAL BIOFILM REACTOR FOR THE PRODUCTION OF A RECOMBINANT PROTEIN BY ASPERGILLUS ORYZAE**

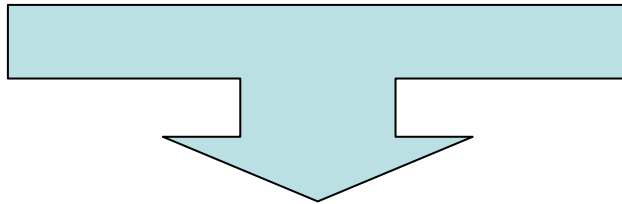
Quentin Zune (Thesis funded by FRIA)

Promotor : Frank Delvigne

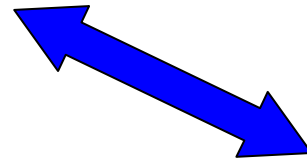
# Filamentous fungi : *Aspergillus sp.*, *Trichoderma sp.*, etc.



- Fine chemicals (organic acids)
- Secondary metabolites (enzymes, antibiotics, etc.)

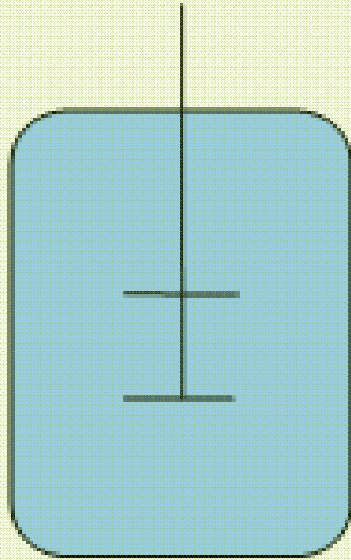


- Recombinant protein
- high secretive power
- post-translational modifications



**Fermentation  
industry**

## Submerged culture



Stirred tank reactor (STR)

→ **free mycelial hyphae**  
= "unwrapped ball of wool"

(+) simple implementation

(-) high viscosity, shear stress

or

## Solid-state culture



Trays with **organic solid substrate**

Muti-stage vessel

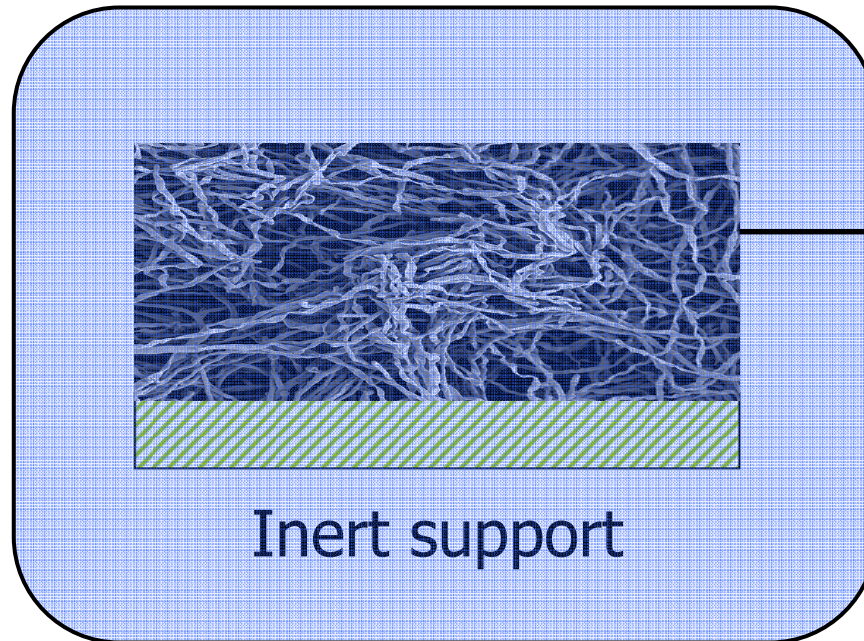
→ **aggregated mycelial hyphae**  
= "wool carpet"

(++) enhancement of metabolites  
secretion and high productivity

(-) heat removal, downstream process

**Fine chemical and pharmaceutical industry**

Fungal biofilm reactors → combined (+)  
from submerged and solid-state culture



Fungal biofilm  
= "structured wool carpet"

Liquid medium

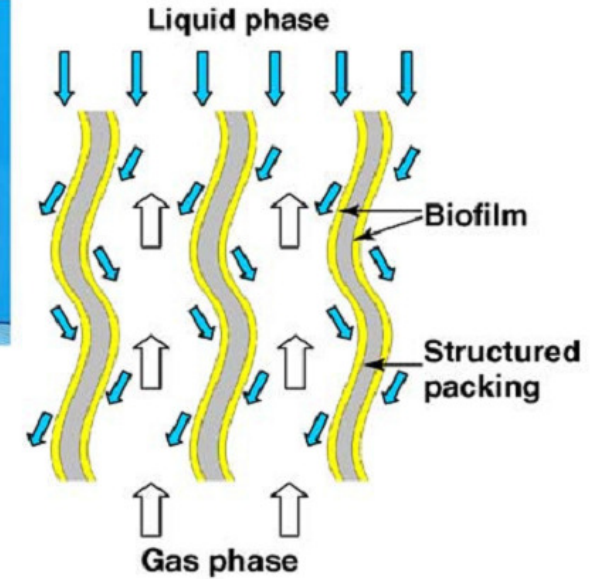
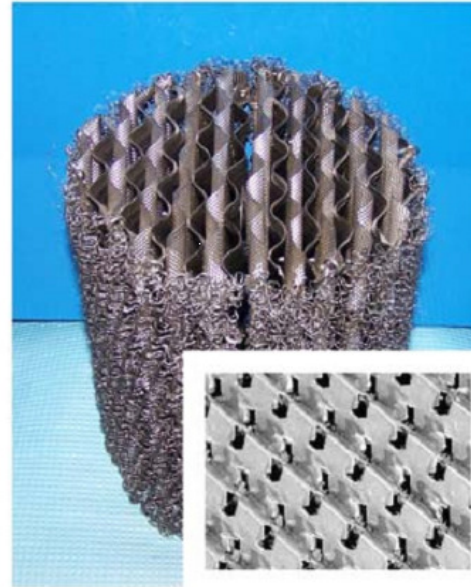
- Enhances metabolites secretion  
→ alpha-pyrone, hydrophobins,  
ligninases, cellulases, etc.

## Objective

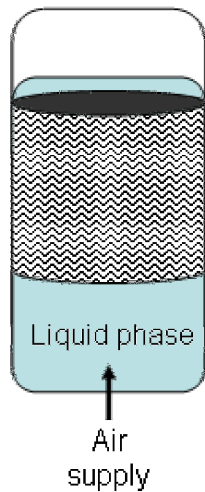
Design of a fungal  
biofilm reactor

Production of a  
recombinant protein

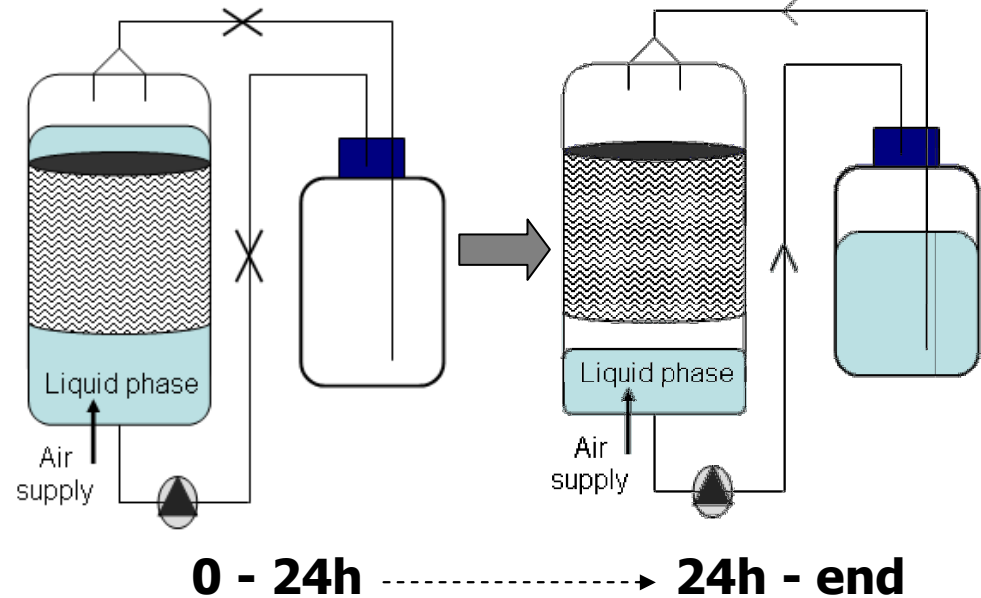
## Metal structured packing (750 m<sup>2</sup>/m<sup>3</sup>)



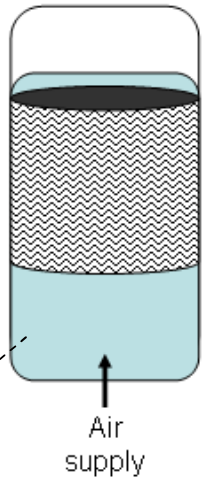
### Immerged conditions



### Aspersed conditions



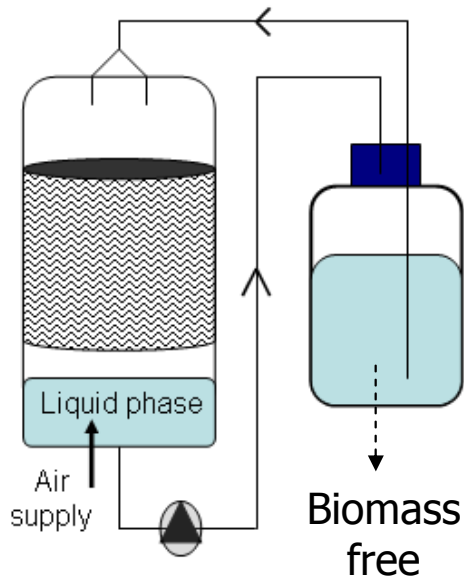
Immersed



Biomass free

Air supply

Aspersed



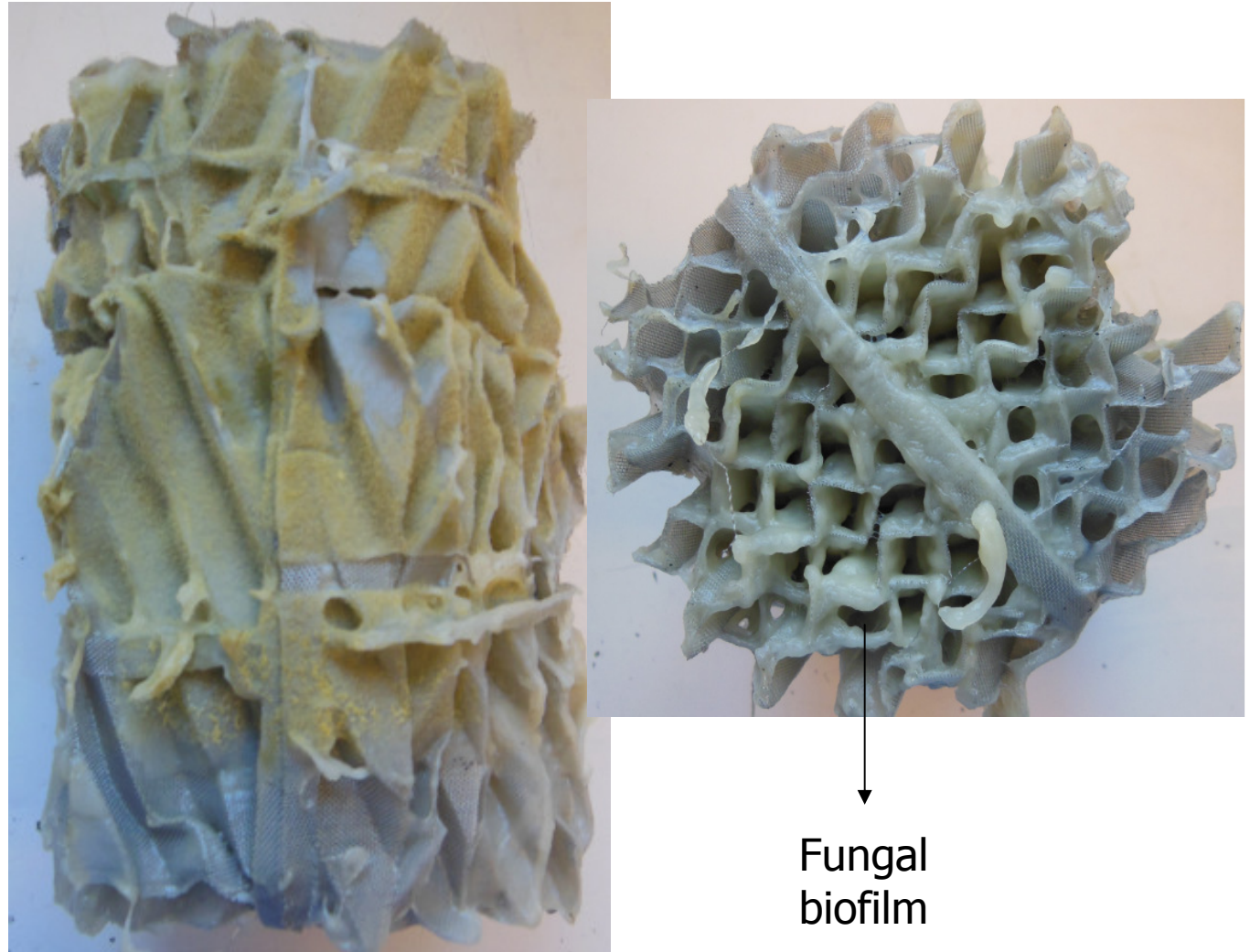
Liquid phase

Air supply

Biomass free

1<sup>st</sup> step = spores adhesion

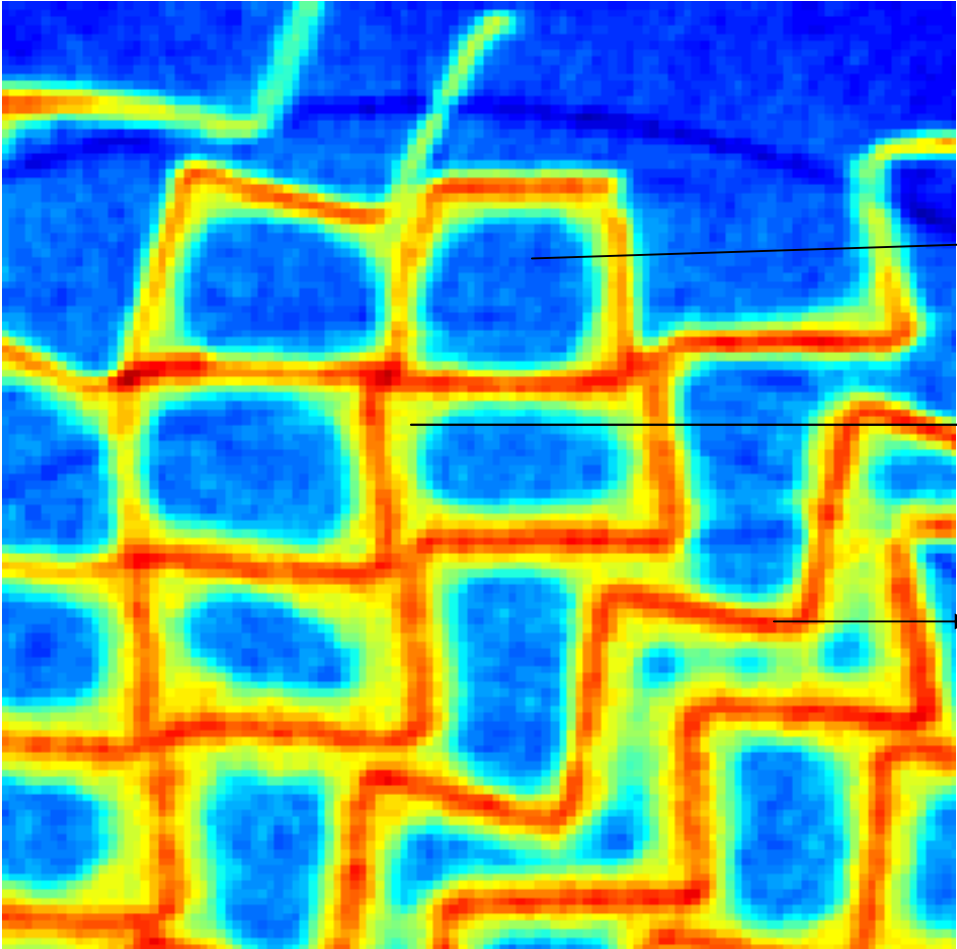
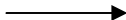
2<sup>nd</sup> step = germination and colonization on the form of a fungal biofilm



Fungal biofilm

Colonized packing after 3 days of culture

# Fungal biofilm visualization by X-ray tomography analysis



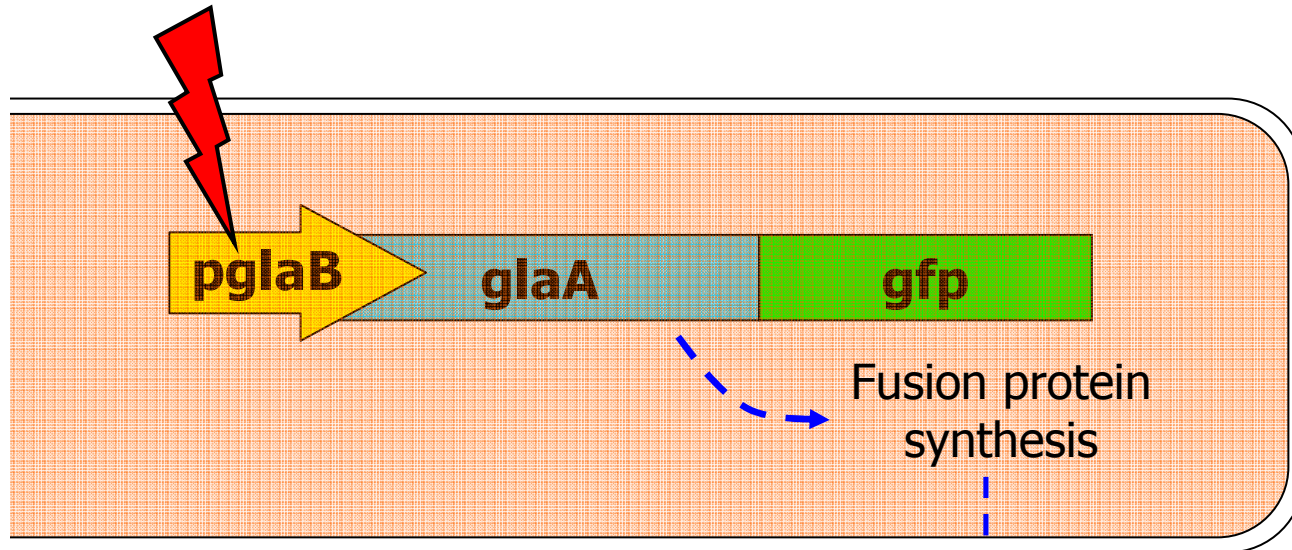
Void (Blue pixels)

Mycelium (yellow pixels)

Packing (red pixels)

# *Aspergillus oryzae* modified strain

Solid-state and biofilm conditions

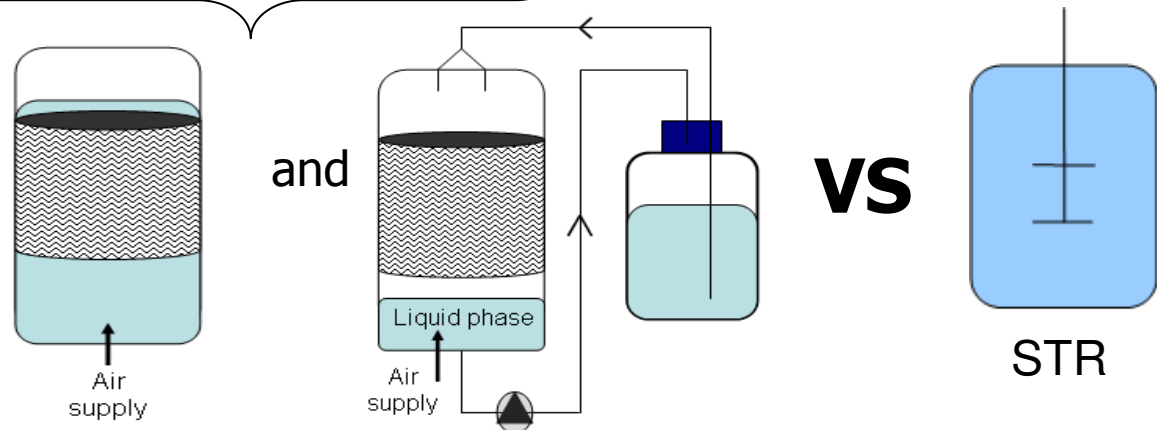


Mycelial hyphae

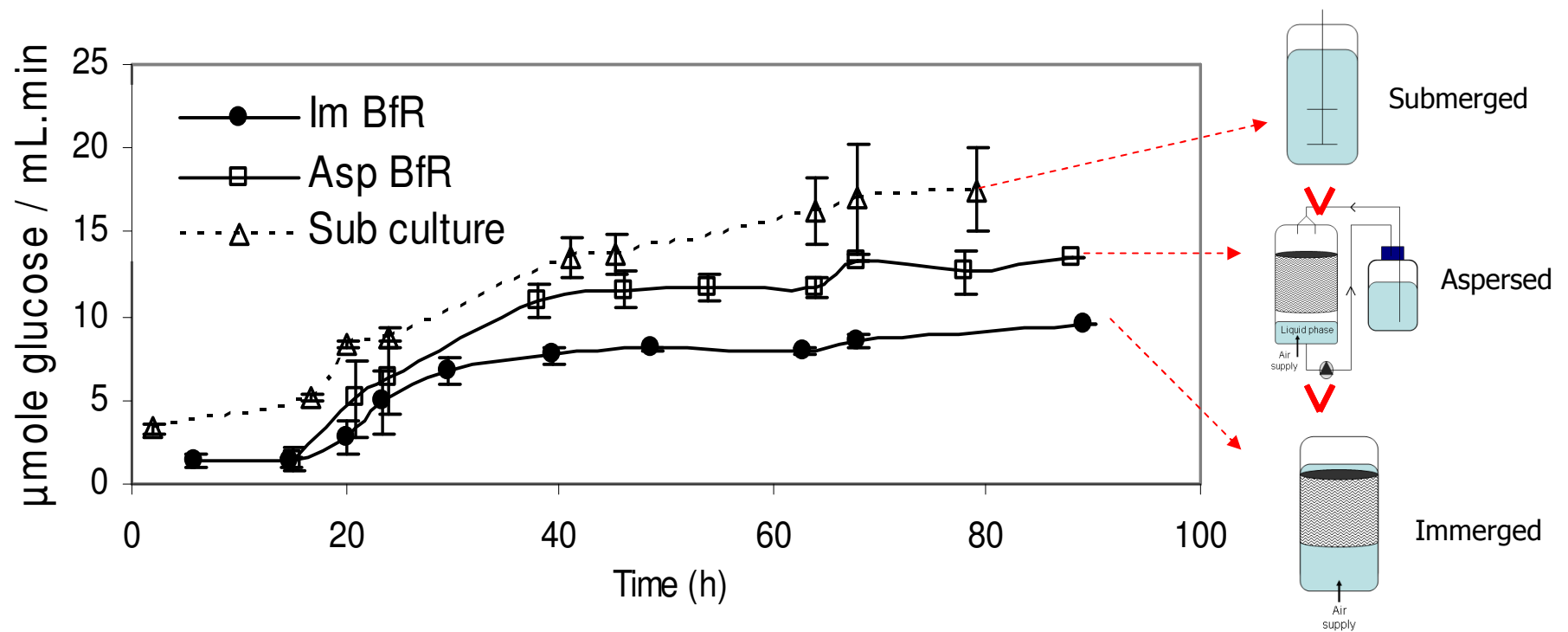
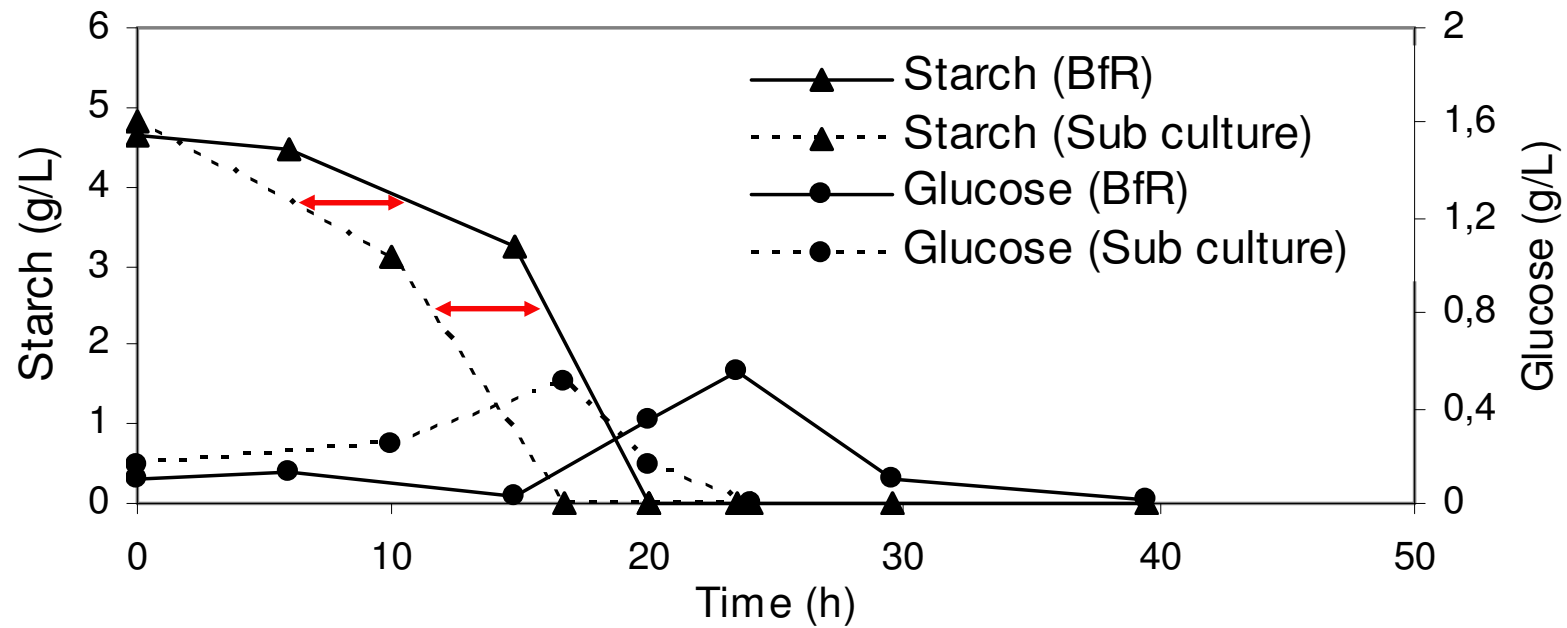
Spectrofluorimetry and Western blot

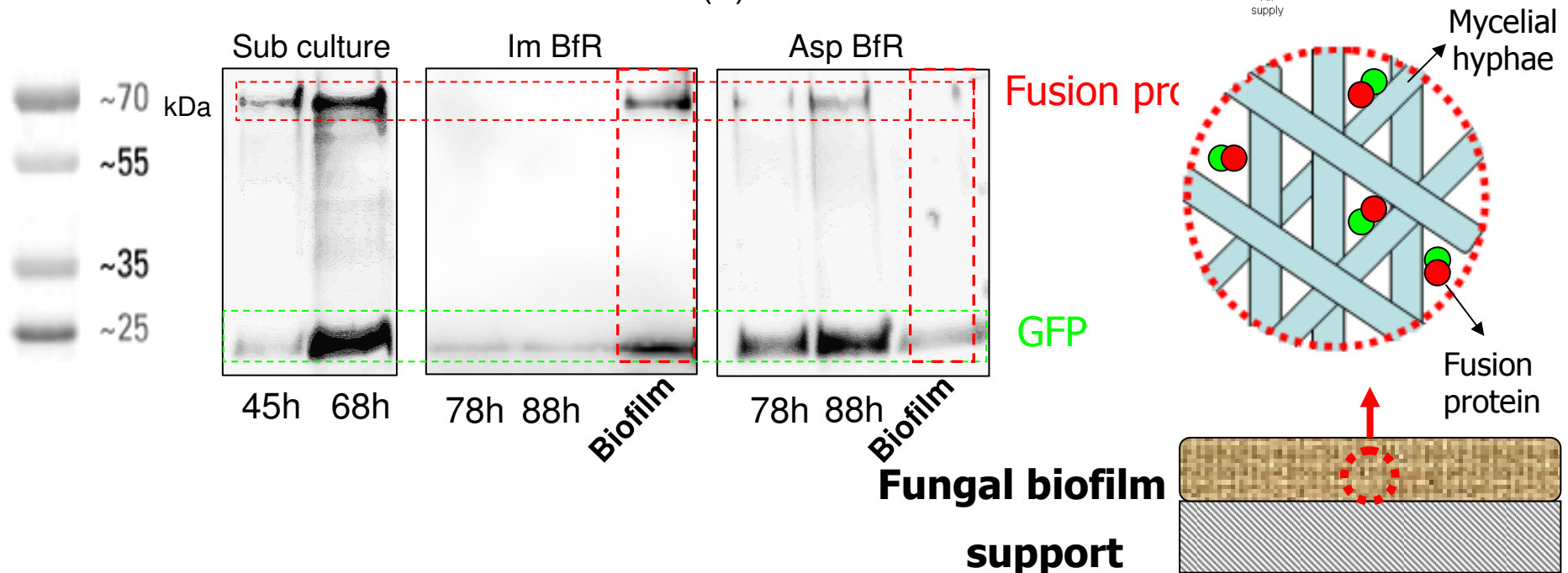
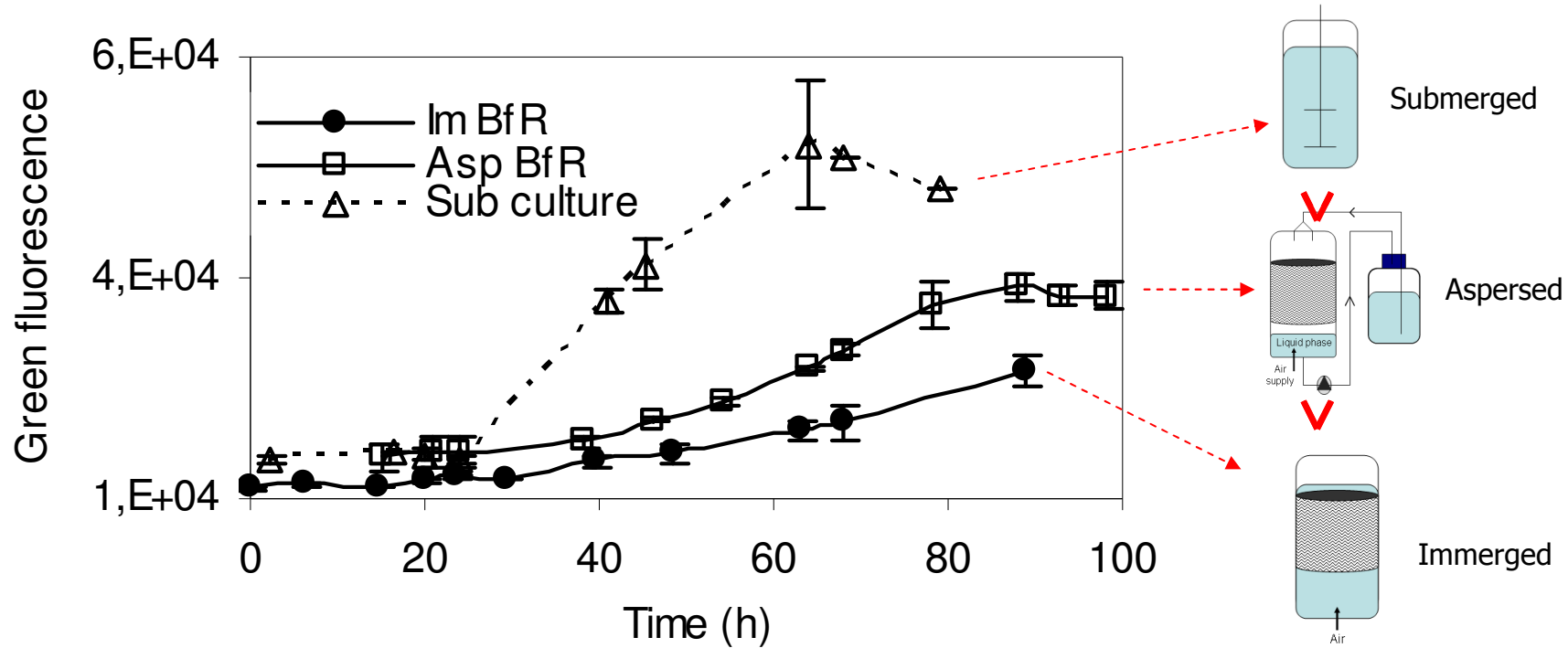
Fusion protein secretion in extracellular environment

→ Performances of recombinant protein secretion









# Discussion

- Lower production yields in the biofilm reactor

- lower mass transfer in biofilm reactor
- diffusion constraints in fungal biofilm

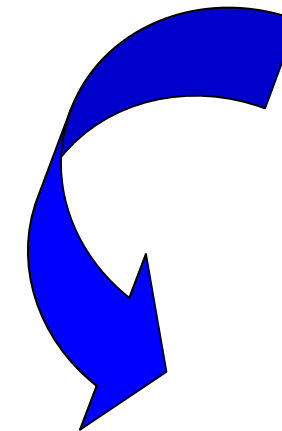
**Physico-chemical effect**

- choice of the promoter ?
- protease effect ?

**Physiological effect**

## Conclusion & Perspectives

- Characterize Protein secretion
- proteomic analysis in each condition



- Low shear effect and fungal biomass retention
- Implementation in a continuous process

- Design improvement
- increase mass transfer