Improvement of gluco-amylase B excretion by *Aspergillus oryzae* in a biofilm reactor

Quentin Zune[1], Romain Kinet[1] Dominique Toye[2], Philippe Thonart[1], Peter J. Punt[3], Frank Delvigne[1]

Context and objectives

The glucoamylase B gene (*glaB*) from *Aspergillus oryzae* is known to be highly expressed in solid-state fermentation conditions (SSF) compared with classical submerged fermentation (SmF). This work aims to design a bioreactor reproducing SSF conditions in order to enhance glucoamylase production from *A. oryzae*. The experimental setting involves a stainless steel structured packing (*SSP*) immerged in a culture medium. Dynamic of excretion of the glucoamylase B in the experimental setting has been compared with a SmF culture and biofilm repartition inside the support was visualized by X-ray tomography. The study was carried out at the lab-scale (flask) and at the pilot scale (20L bioreactor).

Methodology

• At the lab-scale, the experimental setting (Fig 1A) contains an adapted *SSP* (Fig 1B) partially submerged in 100 mL of liquid medium. Fermentation run has been compared with a shake flask without support.

• At the bioreactor scale, the experimental setting (Fig 1C) contains a *SSP* (Fig 1D) fully immerged in 14L of liquid medium. Air injection allows for medium homogenization. Fermentation was compared with a culture performed in a stirred tank bioreactor.

• *A. oryzae* strain used for glucoamylase production contains an expression vector composed of the promoter region of the *glaB* gene linked to a gene sequence (GLA::GFP) carrying the encoding gene of the green fluorescent protein (GFP) as marker gene to monitor *glaB* expression. Quantification of the gene of interest was assessed by measuring the amount of GFP in the culture supernatant by using spectrofluorimetry and western blot analysis.

•X-ray tomography analysis at the end of the fermentation allows for the visualization of biofilm distribution inside the packing. Collected data from the X-ray tomography permit the reconstruction of an image in 2-D corresponding to a given cross sectional area of the *SSP* (Fig 1E).

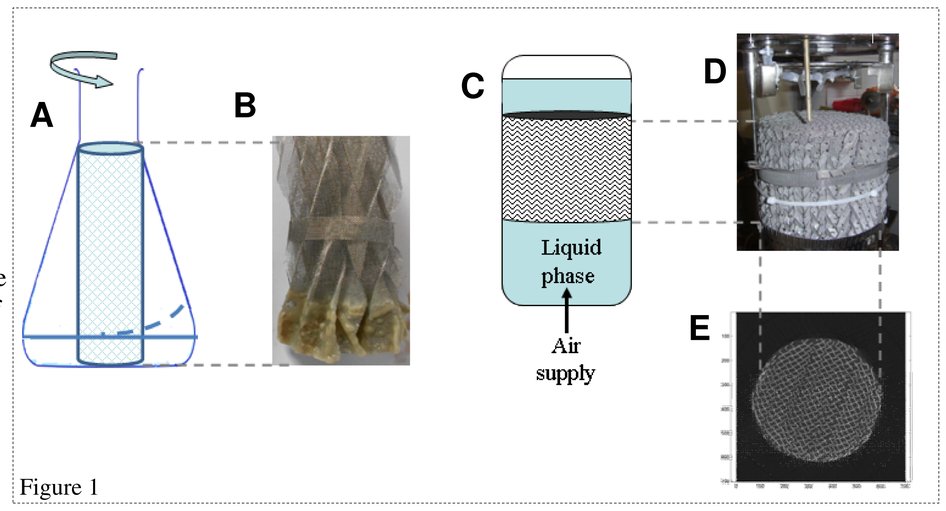

Figure 1

Results → Discussion

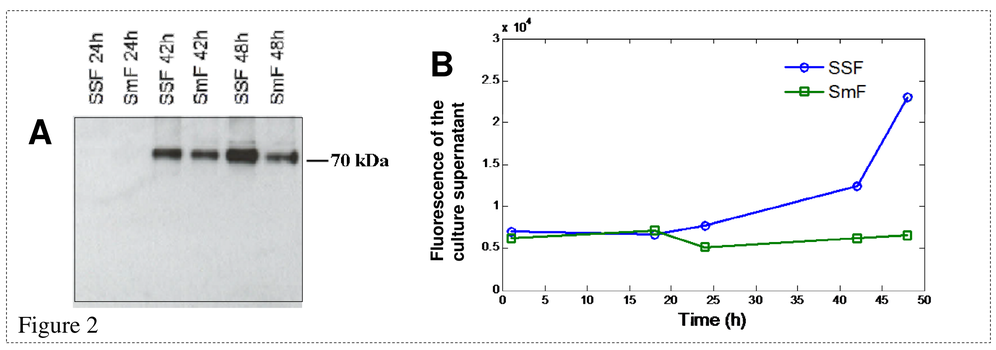

Figure 2

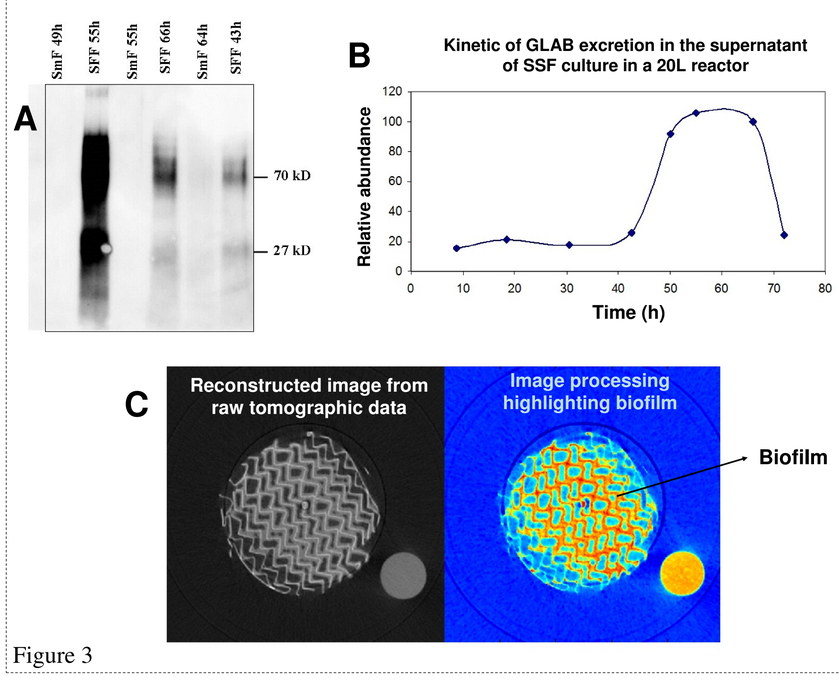

Figure 3

• Figure 2 summarizes the results gained from culture carried out at the lab-scale (flask). Previous experiment demonstrated the reliability of the fluorescent reporter system to be used as a physiological tracer to assess GLAB excretion in the culture supernatant (results not shown).

• The evolution of the fluorescence of the culture supernatant, given in Fig 2D, shows an increase from the 40th hour until the end of the run during SSF fermentation. This observation supports the fact that SSF-like conditions promote *glaB* expression compared with SmF-like conditions.

• In order to take into account degradation or denaturation of GFP in the culture supernatant, immunoblot analysis with an anti-GFP antibody has been performed(Fig 2A). Analysis reveals the presence of a major protein with a molecular weight around 70 kDa. The presence of *SSP* seems to enhance excretion and stability of the fusion protein (GLA::GFP) compared with the submerged culture.

• Figure 3 summarizes the results gained from culture performed in the 20L bioreactor. In this case gfp has only ben detected in the bioreactor equipped with the SSP (Fig 3A). This result highlights the difference between both bioprocesses. The first band at 70 kDa corresponds to the fusion protein (GLA::GFP) used as the physiological tracer and the second band at 27 kDa corresponds to a truncated GFP.

• Relative abundance of the GLA::GFP fusion protein during SSF culture (Fig 3B) shows an increase from the 42nd hour with a maxima at the 58th hour. Protein reconsumption by the mold could possibly explain the decrease of the GLA::GFP fusion protein at the end of the fermentation.

• During the culture, the mold forms a constant layer of mycelium (biofilm) at the surface of the corrugated sheets from the *SSP*. X-ray tomography image highlights biofilm distribution inside the support (Fig 3C).

CONCLUSION

An experimental setting reproducing conditions of solid-state fermentation has been used to enhance excretion of the glucoamylase *B* gene (*glaB*). The stainless steel structured packing (*SSP*) allows the immobilization and growth on the form of a biofilm. These SSF-like conditions lead to the enhancement of *glaB* expression. X-ray tomography proved to be a relevant tool to characterize non-invasively microbial growth inside the support. Further experimentations will be focus at on the physico-chemical drivers affecting the performances of excretion of the *glaB* gene in the SSF-like conditions such as hydrodynamic or medium composition.

[1]Univ. Liege- Gembloux Agro-Bio Tech. Bio-Industries Unit. Passage des Déportés, 2. B-5030 Gembloux (Belgium).
[2]Univ. Liege. Chemical Engineering Laboratory. Allée de la Chimie, 3/6c. B-4000 Liege (Belgium).
[3]Wageningen Centre for Food Sciences, P.O. Box 557, 6700 AN Wageningen (The Netherlands)

Contact : f.delvigne@ulg.ac.be