Tracking the growth of *Trichoderma reesei* during HFBII production; CO_2-HFBII foam

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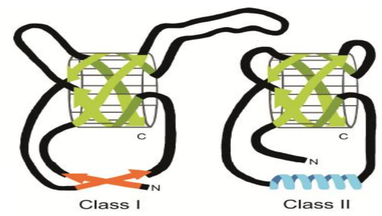
Introduction

Hydrophobins are a family of cysteine-rich amphiphilic fungal proteins with MW in the range of 7-10 kDa **(F.1)** [1]. Unique properties with potential for using as a novel foaming agent make this protein very interesting [2]. In this study, the effects of external conditions on *Trichoderma reesei* during HFBII production in submerged cultures were evaluated. Furthermore, the foam resulted from HFBII was analysed and compared with SDS foam.

Zampieri et al., 2010 [2]



F.1

Materials and methods

HFBII production and purification: *T. reesei* MUCL 44908 was used in this study. The culture medium was prepared according to *Khalesi et al.,* 2013 [3]. The medium was supplemented with either lactose, galactose or glucose at initial concentration of 40 g/L. The growth curves were obtained using Bioscreen C **(F.2)**. Lactose consumption during the fermentation was tracked by the enzymatic Lactose assay kits (Abcam ab83384). To isolate hydrophobin HFBII, the samples were directed to 15RPC liquid chromatography **(F.3)**. The fractions of interest were submitted to MALDI-TOF for confirmation of the HFBII. NanoDrop ND-1000 at 280 nm wavelength was carried out for quantification **(F.4)**.

Foamability analysis: Foamability analysis was conducted using two different methods, the shaking method and the Bikerman test. Sample solutions of SDS and HFBII were prepared; SDS at 6 and 10 mM (below and above the CMC of 8 mM), and HFBII rich fermentation broth (100 mg L^{-1} of HFBII).

Foam stability: The trends of foam ripening over the time were recorded using multiple light scattering [4]. After foaming, the vessels were placed in the Turbiscan **(F.5)**.

Forced foam drainage: The liquid drainage characteristics of foams resulted from HFBII fermentation broth were investigated by conducting forced drainage experiments, using the apparatus shown in **F.6** [5].

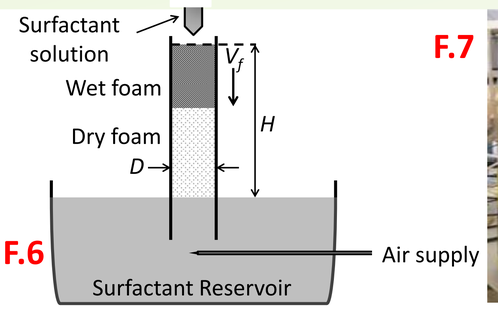
Equilibrium surface tension: The equilibrium surface tension of hydrophobin protein containing fermentation broth was measured using a CAMTEL CDCA-100 tensiometer **(F.7)**.

F.2

F.3

F.4

F.5



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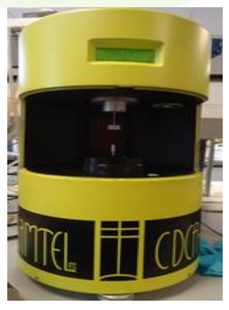


Results and discussion

HFBII production: The production of HFBII occurs mainly when the lactose is completely used and the cell growth has entered the stationary phase **(F.8)**. When the rate of lactose consumption is very high, the production of HFBII starts at a low rate of around 0.12 mg/Lh. When almost all the lactose was consumed the rate of HFBII formation became ten times higher, reaching 1.2 mg/Lh. The highest amount of HFBII was obtained after 4 days of fermentation. Thereafter, starvation of growth substrates may induce some proteolysis of excreted proteins including HFBII. HFHBII was not obtained when galactose or glucose was used as the main carbon source.

The results of MALDI-TOF reveal that only in the presence of lactose the complete molecule of HFBII is obtained in 4 days, thereafter, some molecules of HFBII lose the last amino acid (Phe) due to biodegradation by the fungi.

Foamability1; Shaking method: The results show that the broth with HFBII concentration of 100 mg mL^{-1} foams just slightly less than SDS solution with a concentration close to the CMC value. The surface activity of HFBII protein derived from its structure explains the broth foamability. The results therefore, confirm that the foamability of the HFBII broth is indeed strong even in very low concentrations **(T.1)**.

Foamability2; Bikerman test: The results of foaming HFBII fermentation broth sparged with air or by CO_2 were compared **(T.2)**. The foamability of the HFBII broth is increased when the samples are sparged by CO_2, which may be explained by the hydrophobicity of CO_2 in comparison with air. In the vicinity of the hydrophobic patch, the number of aggregated CO_2 molecules per $Å^2$ of accessible surface area is twice than that of the rest of the protein surface.

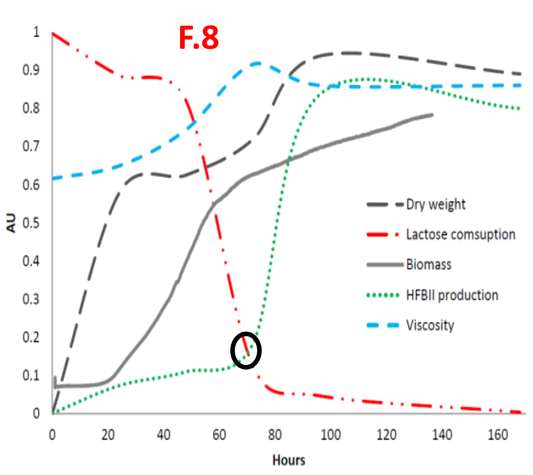
Forced foam drainage: As liquid drained through the foam two distinct regions were observed; a wet zone behind the propagating wet front and a dry zone ahead of the wet front. The velocity of the wet front (V_f) was measured as it travelled down the foam column. The wet front velocity and the superficial drainage velocity (j_d) are related to the liquid fraction (ε) by the following equation: $\varepsilon = j_d / V_f$, V_f was found to increase with increasing j_d. The calculated ε was between 0.016 – 0.020.

SDS-HFBII joint surfactant behaviour: Different samples of SDS-HFBII broth were prepared. The system recorded the trends of foam ripening each 2 h in 80 h. The results show that in first 2 h, high reduction of foam is occurred and later on, the foam becomes really stable. The trends of foam ripening shows that the main part of the foam disappeared in first 2 h and almost all the foam is depleted till the first 24 h. This proposes having a highly stable foam, it's well possible to combine SDS with HFBII-broth **(F.9)**.

CO_2 foam stability: The foam stability of CO_2-HFBII broth was reported to be higher than that of air-HFBII broth. Nevertheless, CO_2-HFBII at environmental condition shows low stability except if we could make nanobubbles with 100 nm diameter, the critical diameter of CO_2 nanobubbles at ATM. The results were compared to the air-HFBII broth and CO_2-air-HFBII broth when the foaming process was carried out by gas sparging **(F.10)**.

Equilibrium surface tension and absorbance behaviour of air-HFBII: When the concentration of the HFBII in broth is equal to 100 mg L^{-1}, the equilibrium surface tension of the sample was found to be equal to 40 mJ m^{-2}. The difference between pure water and the solvent is due to the presence of biosurfactant (*i.e.* HFBII) in the solvent **(F.11)**.

F.8



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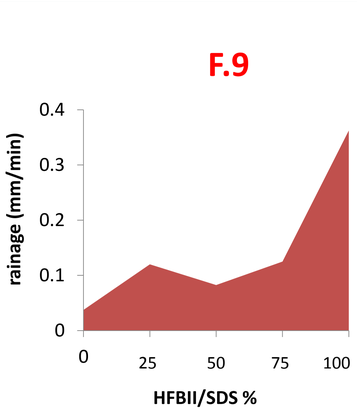

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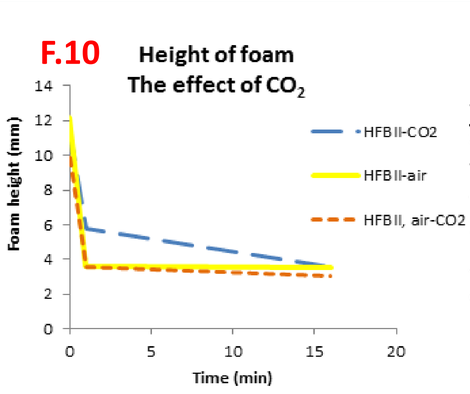

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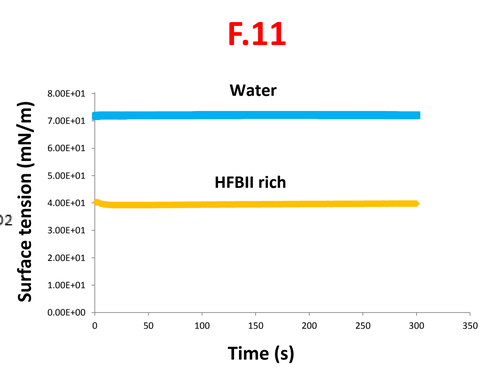

T.1

Surfactant	Foamability (cm)
SDS 10 mM	3.4 ± 0.2
SDS 6 mM	3.0 ± 0.3
HFBII broth	2.4 ± 0.1

T.2

Surfactant	Foamability (cm)
SDS 10 mM (above CMC) + air	14.6 ± 1.3
SDS 6 mM (below CMC) + air	14.3 ± 1.9
HFBII broth + air	9.8 ± 2.1
HFBII broth + CO_2	13.3 ± 1.6

Conclusion

- The production of HFBII occurs mainly when the cell growth has entered the stationary phase. The results states that the formation of lactase promotes the responsible gene for HFBII production, *hfbII*, to be expressed.
- Furthermore, the physical characteristics of HFBII make this protein as a compatible foaming agent for food industries.

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