Tracking the growth of *Trichoderma reesei* during HFBII production; CO₂-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 2,3. 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.

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**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 medium 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 ab83894). 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).

**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 low, 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. HFBII 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 medium.

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 SDS fermentation broth sparged with air or by CO₂ were compared (T.2). The foamability of the HFBII broth is increased when the samples are sparged by CO₂, which may be explained by the hydrophobicity of CO₂ in comparison with air. In the vicinity of the hydrophobic patch, the number of aggregated CO₂ molecules per Å² of accessible surface area is twice that of the rest of the protein surface.

**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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**References:**