Production of biosurfactant by fermentation with integral foam fractionation

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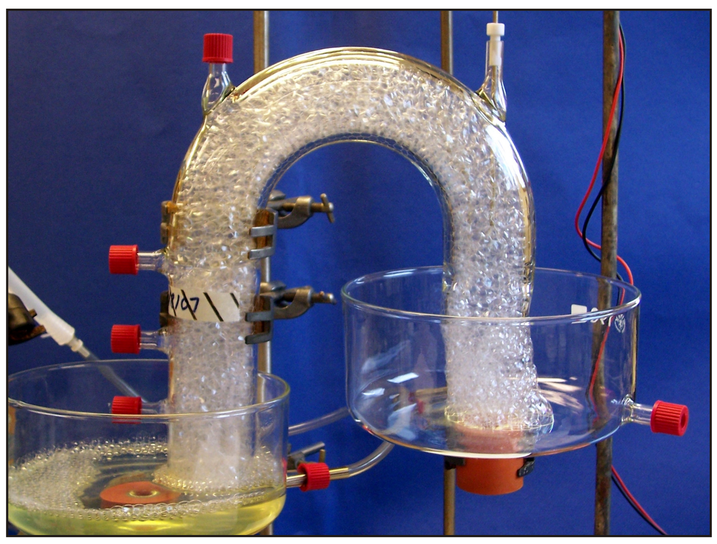
BACKGROUND

Surfactants, surface active molecules which "like" both water and oil, have a broad range of applications, from everyday tasks such as washing the dishes to advanced oil recovery operations. The majority of surfactants currently available are made from non-renewable oil-based feedstocks. An alternative route of surfactant production exists in nature in the form of microbes which can produce surfactants. Microbially produced biosurfactants are characterised by both their chemical composition and microbial origin[1] and can perform many tasks for which traditional petroleum or fat derived surfactants are currently used. Biosurfactants also have uses in other fields such as environmental bioremediation, food-processing and pharmaceuticals[2].

The biosurfactant HFBII, a hydrophobin protein, is the subject of this research project, which has been running for 18 months and comes under the Chemistry for Product Design priority. The production and subsequent separation of HFBII using a process called foam fractionation is being investigated.



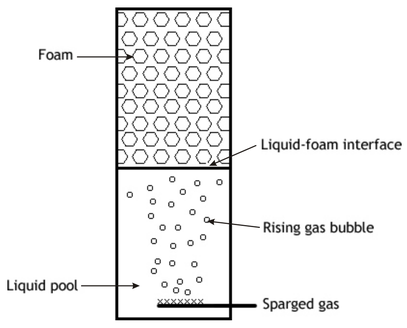
Foam fractionation apparatus.

TECHNIQUES

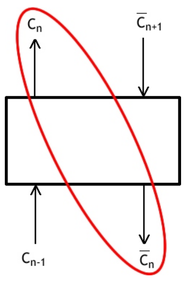
A process for the combined production and recovery of HFBII is being developed.

Foam fractionation is used for primary recovery and increasing the concentration of HFBII in solution.

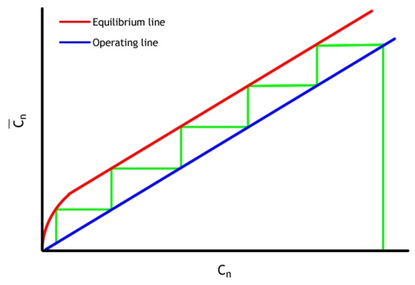
As sparged gas bubbles rise through the liquid pool surface active molecules, HFBII in this case, stick to their surface. At the top of the liquid reservoir foam is formed which constantly overflows from the top of the system. Liquid drains from the overflowing foam as it travels up the column. When the foam is collapsed with a foam breaker at the top of the column a solution with an increased concentration of surface active molecules, known as foamate, is obtained.



Foam fractionation column.



An equilibrium stage. C_n is the concentration of surfactant in the upflowing stream and $\bar{C}_n$ is that of the draining (downflowing) interstitial liquid.

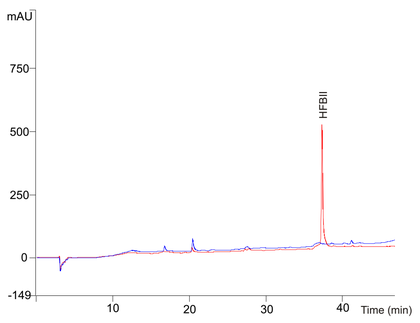


McCabe-Thiele diagram for a fractionation column.

An analogy can be made between foam fractionation and distillation, and the chemical engineering concept of an equilibrium stage applied to foam column design.

The high stability of HFBII foams may be a virtue for food ingredient applications, but is a problem in the foam fractionation process, where foam breaking is a practical difficulty. Non-standard methods of foam breaking, such as ultrasound, may prove to be effective.

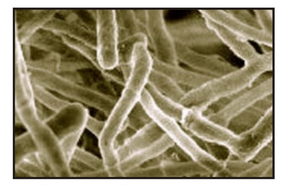
HPLC (High Pressure Liquid Chromatography) is used to determine the concentration of HFBII in a solution. Liquid solvents, water and acetonitrile, are used to wash sample from a silica packed column. HFBII concentration is determined by comparing peak area to that of a standard of a known concentration.



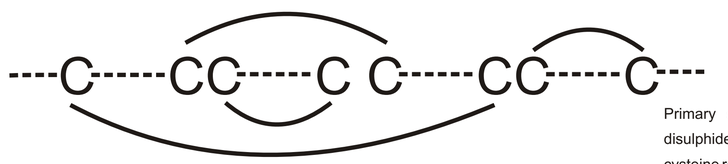
WHAT ARE HYDROPHOBIN PROTEINS?

Hydrophobins are naturallly occuring proteins which are produced by fungi. The hydrophobin protein HFBII is produced by the fungi *Trichoderma reesei.*

The HFBII molecule is composed of seventy one amino acids, including eight conserved cysteine residues. Four disulphide bonds are formed between the sulphur atoms in the amino acid, as shown below.

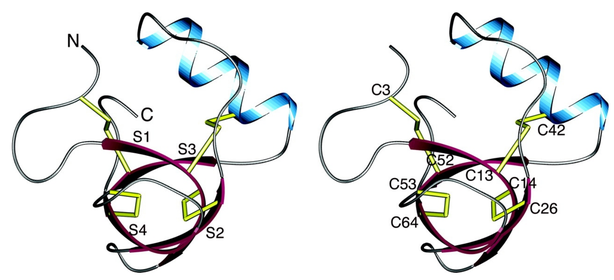


The fungus *Trichoderma reesei.*

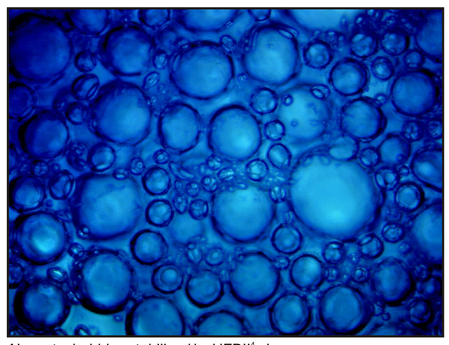


Primary structure of HFBII showing disulphide bonds(curved lines) between cysteine residues.

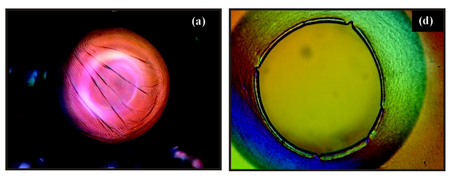
The secondary structure of HFBII contains a β sheet. The two β-hairpins (shown in red) contain all of the conserved and exposed hydrophobic amino acid residues in the protein, eleven in total. These residues form a water "hating" patch which give the protein its amphiphilic nature and accounts for some of its properties.



Secondary structure of HFBII, with the disulphide bridges shown in yellow[3]. Image: Hakanpää, J., *et al.* (2004).



Air- water bubbles stabilised by HFBII[4]. Images: Cox, A. R., *et al.* (2007).



Some interesting properties of HFBII:

Tendency to join together (self assemble) at an interface, e.g. air-water interface.

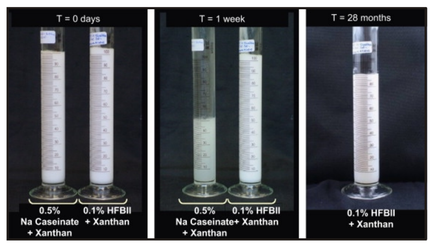
Lowers the surface tension of the air-water interface to around 35 mNm^{-1} at a quite low concentration of 30 μM[4].

High surface elasticity.

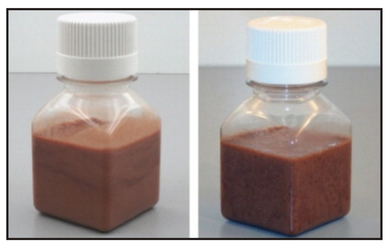
All of these properties result in HFBII being able to form extremely stable foam, which gives HFBII great potential as an ingredient in aerated foods.

THE APPLICATION

The exceptional stability of liquid HFBII foams could allow food manufacturers to create more stable aerated foods such as milkshakes. Currently the shelf life of aerated foods is limited by the rate at which foam destabilisation processes such as the growth of large bubbles at the expense of smaller ones due gas to diffusion (disproportionation). A foam created from a 0.1% wt HFBII solution has been shown by Cox *et al*. to be stable for twenty eight months.



A comparison of the ability of liquid foams of sodium caseinate and HFBII to retain their gas volume over time[5]. Image: Cox, A. R., *et al.* (2008).



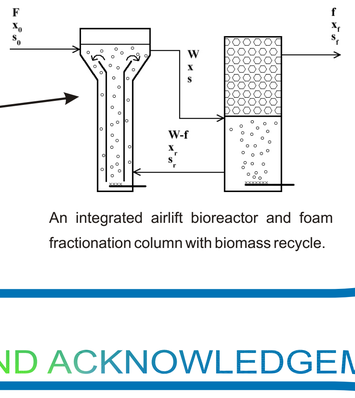
A chocolate milkshake with an initial air fraction of 0.4, stabilised by HFBII. Left hand image, freshly prepared product. Right hand image, milkshake after refrigerated storage for five weeks[5]. Image: Cox, A. R., *et al.* (2008).

The use of HFBII as a food ingredient could also lead to novel food products with interesting new textures or a lower calorific density.

MORE THINGS TO DO...

Devise a method to integrate the HFBII production stage with the foam fractionation separation stage. Maybe something like this

There is also scope for the application of an integrated foam fractionation process to the production of other biosurfactants.



An integrated airlift bioreactor and foam fractionation column with biomass recycle.

REFERENCES AND ACKNOWLEDGEMENTS

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[4]Cox, A. R., Cagnol, F., *et al.* (2007). Surface properties of class II hydrophobins from Trichoderma reesei and influence on bubble stability. *Langmuir* **23**(15): 7995-8002.

[5]Cox, A. R., Aldred, D. L. and Russell, A. B. (2008). Exceptional stability of food foams using class II hydrophobin HFBII. *Food Hydrocolloids* **23** (2): 366-376.

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