UNDERSTANDING THE EFFECT OF AIR-LIQUID INTERFACE ON ENZYME STABILITY IN THE PRESENCE OF HYDROPHOBINS

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Downstream process optimization of enzyme production is one of the most important steps towards having active biocatalysts with high productivity.[1] Expressing enzymes efficiently in bacterial hosts such as *E.coli* remains a problematic issue in many cases, and issues such as protein solubility require still further optimization.[2] Oxidoreductases are important enzymes for applications in industrial chemistry, but many current studies focus on the chemical aspects of these enzymes rather than trying to understand how the enzyme behaves under different reactor conditions, such as exposure to air-liquid interface. Here, we report on a eukaryotic hydrophobin protein, DewA,[3] which is known to be very hydrophobic and insoluble in *E. coli*, and express it with the solubility-increasing peptide P17[4] and Trx tag on its N-termini. Then we express, as an example oxidoreductase, the enzyme galactose oxidase[5] fused with Trx_P17_DewA protein using the Pet32a plasmid. The proteins are expressed in specific SHuffle® T7 Express lysY cells which facilitates the correct folding of proteins with multiple disulfide bonds. The process optimization is carried out investigating different induction times, growth temperatures, and extraction steps. Finally, following the enzyme downstream process optimization, the hydrophobin, hydrophobin-galactose oxidase, and galactose oxidase are in turn exposed to different air-liquid interfaces in various bioreactors, and the effect of the interface on enzyme stability is investigated using colorimetric assays, Gas Chromatography, nanoDSF, as well as DLS.

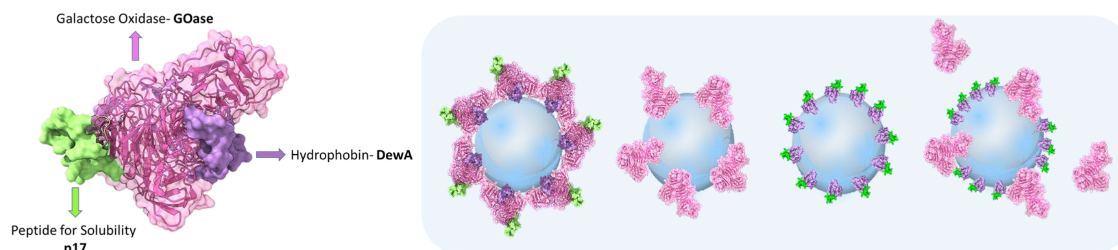

Figure 1 - Effect of air-liquid interfaces on enzyme stability in the presence of Hydrophobins. In a reactor sparged with different gases, Hydrophobins are expected to cover the hydrophobic surface of the bubble while preventing/limiting the access of enzyme to the air-liquid interface. In addition, by using different ratios of hydrophobins, the exposure level of enzymes to the air-liquid surface will be tuned.

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