THE GROWING NEED TO ASSESS THE KINETIC STABILITY OF ENZYMES

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Enzyme stability has long been an important topic of great scientific interest[1] as well as of practical importance in the application of enzymes to synthetic problems[2]. Despite great progress in recent years developing protein engineering algorithms for improving stability, this is often assessed solely by measuring increased melting temperature. Nevertheless, industrial reactor conditions can vary greatly and are frequently very different from those found in Nature, meaning that other methods of assessment such as direct kinetic stability measurements are still required. This motivated us to examine the effect of the gas-liquid interface on the kinetic stability of oxidase enzymes, which require molecular oxygen as a second substrate. In order to achieve adequate mass transfer of oxygen from the gas to the liquid phase (where the reaction occurs), bubbled systems are usually employed, with a high gas-liquid interfacial area. Therefore, we explored the kinetic stability of oxidases in an aerated stirred tank. As in previous studies[3], the effect of the interface was found to be inactivating and likewise the damaging effect of oxygen to be more significant than nitrogen. Nevertheless, we have also determined now that the agitation of the solution itself appears to inactivate the enzyme. While the size of the protein is too small for the Kolmogorov scale mixing to affect the enzyme itself[4], it was found that secondary effects of such mixing do have a significant role. This has important implications for the application of enzymes in industrial reactors. In this paper, we present work on kinetic stability measurements of NAD(P)H oxidase (NOX) in an aerated stirred tank using image analysis methods linked with computational tools to clarify the effects of the gas-liquid interface, and thereby differentiate the effects of mixing alone. NOX is an increasingly important enzyme in synthetic applications to allow regeneration of expensive $NAD(P)^+$ cofactors[5], which are necessary for the enzymatic oxidation of alcohols to their corresponding carbonyl compounds using alcohol dehydrogenases. Aside from the specific observations on NOX stability, the results also show the importance of measuring the kinetic stability of enzymes and the impact of enzyme 'lifetime' on reactor design. Furthermore, the wider implications for laboratory testing and process development will be outlined.

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