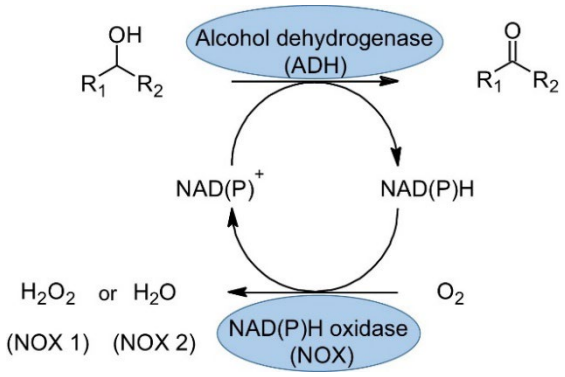
THE EFFECT OF IONIC STRENGTH ON THE KINETIC STABILITY OF NADH OXIDASE IN A BUBBLE COLUMN

Amalie V. Høst, Chemical and Biochemical Engineering, Technical University of Denmark, Kgs Lyngby, Denmark and Sino-Danish Centre (SDC), University of Chinese Academy of Sciences, Beijing, China
AVAHOS@kt.dtu.dk
John M. Woodley, Technical University of Denmark, Kgs Lyngby, Denmark
Andreas S. Bommarius, School of Chemical and Biomolecular Engineering, Krone Engineered Biosystems Building, Georgia Institute of Technology, Atlanta, GA, USA

Key Words: Enzyme stability, gas-liquid interface, ionic strength, bubble column

Despite several advantages of biocatalysis, stability remains an issue, particularly under harsh process conditions [1,2]. Several hypotheses are available for the mechanism of stability loss. In biocatalytic oxidations, where gas is sparged to the system from the bottom of the reactor, deactivation might be caused by a continually added gas-liquid interface [3]. Oxidases are particularly interesting in this problem statement, as they depend on oxygen as a co-substrate. An example is NADH-oxidase (NOX), an enzyme of great interest, as NOX plays an important part in co-factor regeneration. NOX catalyses the reduction of oxygen to either hydrogen peroxide (H_2O_2) or water in a coupled reaction with the oxidation of NADH(P) to NAD(P)+ following scheme 1 [4]. This work investigates the stability of a water-forming NADH-oxidase (NOX) in a bubble column, with the goal of a phenomenological model of NOX stability, which has applications for industrial utility. A bubble column enables good oxygen transfer without drastic enzyme deactivation due to lower shear stress than a stirred tank reactor. Previous hypotheses suggest that the enzyme deactivation is caused by adsorption to the bubble surface, leading to unfolding, aggregation and precipitating, causing deactivation, following a two-stage deactivation trend, which often is explained by dimer dissociation [3]. A significant finding for this work explains the two-stage deactivation as an adsorption stage followed by a deactivation stage.



Scheme 1 – Simplified reaction scheme of NADH-oxidase in a coupled system with alcohol dehydrogenase (ADH) [4]

Additionally, we report a method to determine the transition time by plotting the log of the residual enzyme concentration [5]. The minimum of this curve seems to be where the transition time of the deactivation trend occurs. Several significant findings were made throughout the experimental work. Increasing the buffer molarity appears to have a stabilizing effect on NOX by significantly improving the half-life, which was further improved by adding NaCl to the buffer [5]. Increasing the ionic strength of the buffer decreased the bubble size, which previously has been reported to damages NOX [2]. Thus, the ionic strength's stabilizing effect dominates the small bubbles' damaging effect on NOX [5].

1. Dias Gomes, M.; Bommarius, B.R.; Anderson, S.R.; Feske, B.D.; Woodley, J.M.; Bommarius, A.S. Bubble Column Enables Higher Reaction Rate for Deracemization of (R,S)-1-Phenylethanol with Coupled Alcohol Dehydrogenase/NADH Oxidase System. *Adv. Synth. Catal.* **2019**, *361*, 2574–2581, doi:10.1002/adsc.201900213.
2. Anderson, S.R.; Bommarius, B.R.; Woodley, J.M.; Bommarius, A.S. Sparged but not stirred: Rapid, ADH-NADH oxidase catalyzed deracemization of alcohols in a bubble column. *Chem. Eng. J.* **2021**, *417*, 127909, doi:10.1016/j.cej.2020.127909.
3. Woodley, J.M. Ensuring the Sustainability of Biocatalysis. *ChemSusChem* **2022**, *15*, 1–4, doi:10.1002/cssc.202102683.
4. Riebel, B.R.; Gibbs, P.R.; Wellborn, W.B.; Bommarius, A.S. Cofactor Regeneration of both NAD+ from NADH and NADP + from NADPH:NADH Oxidase from Lactobacillus sanfranciscensis. *Adv. Synth. Catal.* **2003**, *345*, 707–712, doi:10.1002/adsc.200303039.
5. Høst, A.V., Woodley, J.M., Bommarius A.S., Towards a mechanistic model of oxidase deactivation in a bubble column. To be submitted