Stability of Enzymes under Industrial Process Conditions

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INTRODUCTION & MOTIVATION

One of the **major drawbacks of many biocatalysts is their poor stability under industrial process conditions**. Interesting examples are biooxidation reactions, catalysed by oxidases or oxygenases, that require the supply of molecular oxygen to the reaction medium (done by sparging air into the reactor)[1].

Stirring is essential to ensure sufficient oxygen transfer from the gas to the liquid phase by dispersing the air bubbles and increasing their residence time in the reactor. Studies indicate that **sparging and stirring strongly enhance enzyme deactivation** by denaturing of protein which then readily aggregates and tends to precipitate[2,3].

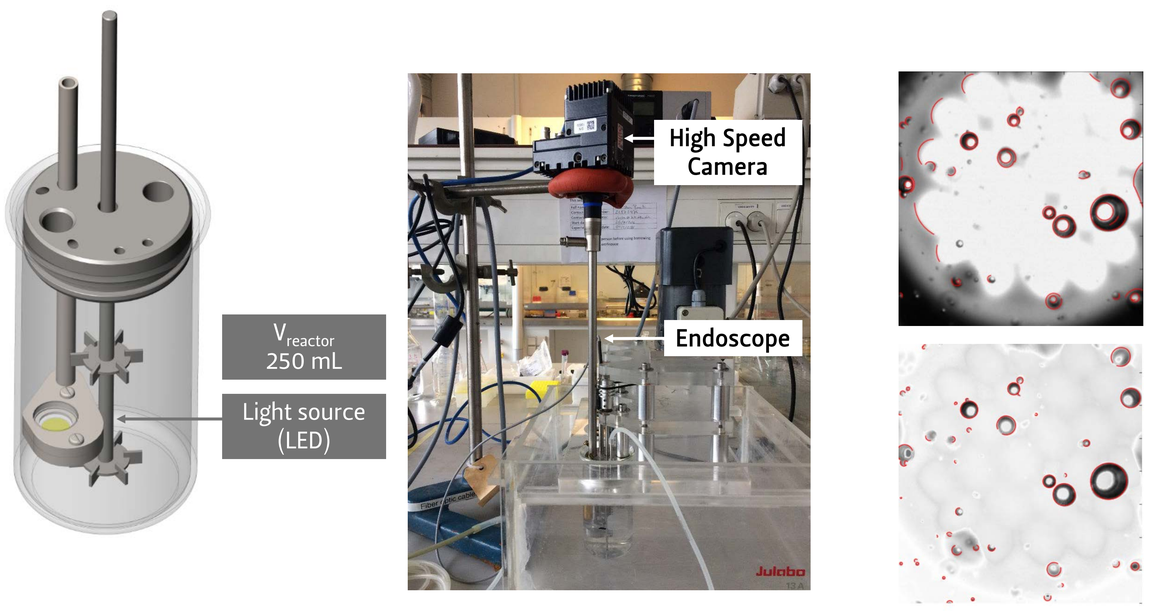
In the present work, NAD(P)H oxidase (NOX) stability was investigated under industrially relevant conditions such as different stirring speeds and gas composition, in order to better understand the correlation of these conditions and enzyme deactivation.

CONCLUSIONS

- NOX deactivation correlates with the gas–liquid interfacial area – faster deactivation at higher gas–liquid interfacial areas.
- The new optical method for bubble size determination can quantify the interfacial area and can be used to predict enzyme stability in large scale bioreactors.
- In the regime of low interfacial areas, the presence of oxygen decreases the enzyme stability indicating that the protein deactivation can be caused by overoxidation of certain amino acids.
- In the presence of higher gas–liquid interfacial areas, the dominating factor for inactivation appears to be the stirring, which indicates that there are different effects causing protein deactivation.

METHODS

Experimental optical method to quantify the gas–liquid interfacial area.

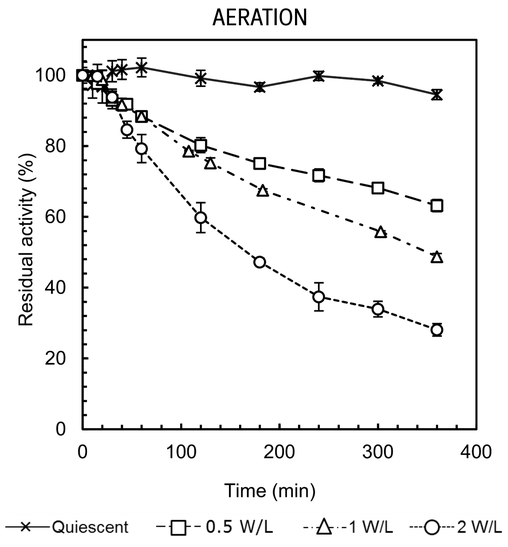


1. NOX* was incubated under different stirring conditions (power input to the reactor) for six hours.
2. Samples from the reactor were taken over time and enzyme solutions assayed in a spectrophotometer*[1] for residual activity.
3. Video image samples taken over time (same time as enzyme) and image analysis executed for bubble size determination.

* Water-forming NAD(P)H oxidase from *Streptococcus mutans*[4]
*[1] NADPH consumption at 340 nm at 25 ° C in 1 mL of 50 mM potassium buffer, pH 7.5, 0.25 mM NADPH and 0.05 g CFE/L

RESULTS

Kinetic stability of NOX decreases with the increase of power input.



$$Residual\ activity(\%) = \frac{E}{E_0} \times 100$$

Experimental Conditions	
Liquid volume	150 mL
Gas flow rate	1 vvm
Power input (P/V)	0.5 to 5 W/L
NOX concentration	1 g_{CFE}/L
KPi buffer	50mM pH 7.5
Gas phase	Air (21% oxygen)
Temperature	RT

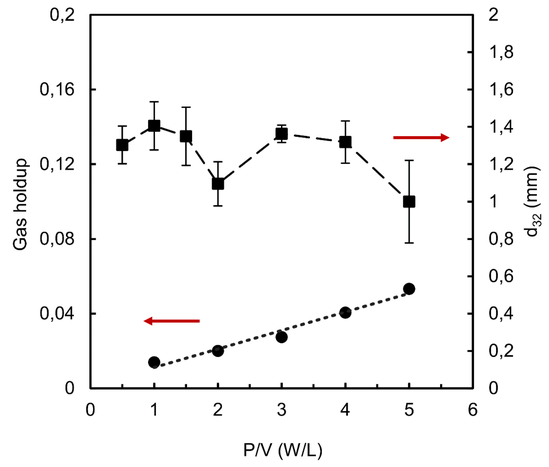
First-order deactivation kinetics

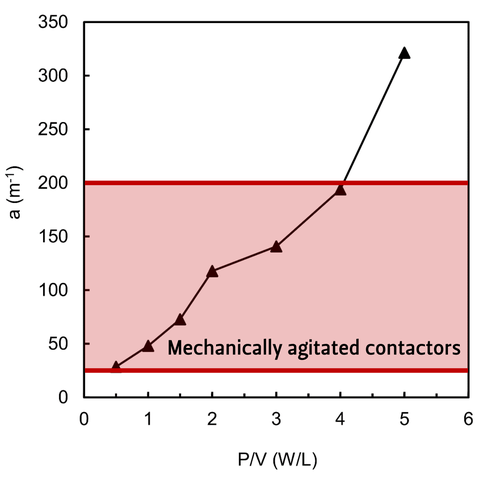
$$E \xrightarrow{k_d} E_d$$

$$Residual\ activity = \frac{E}{E_0} = exp^{-k_d t}$$

P/V [W/L]	k_d [1/min]
0.5	- 0.0014 ± 4.1 x 10^{-5}
1	- 0.0020 ± 2.4 x 10^{-5}
2	- 0.0037 ± 7.2 x 10^{-5}

Experimental determination of the bubble size diameter and gas holdup and correlation of specific interfacial area with power input to the reactor.



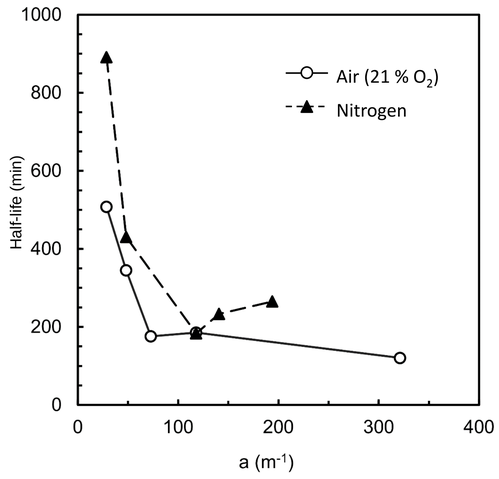


$$Sauter\ mean\ diameter\ (d_{32}) = \frac{\sum n_i d_{B,i}^3}{\sum n_i d_{B,i}^2}$$

$$Gas\ holdup\ (\varepsilon) = \frac{V_g}{V_d}$$

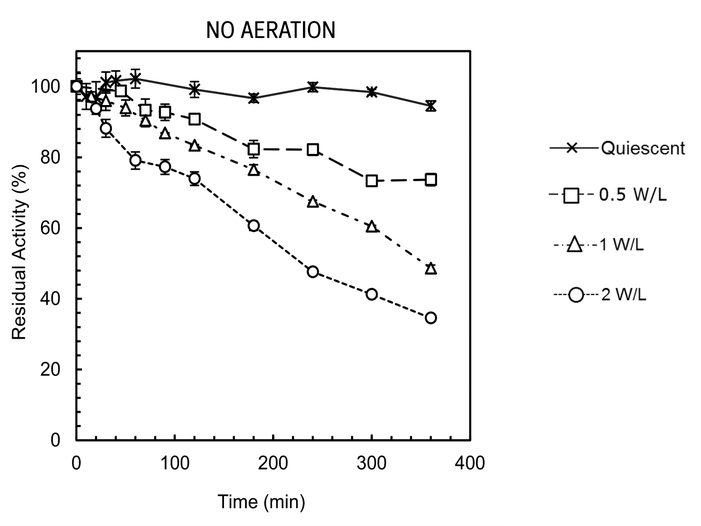
$$Specific\ interfacial\ area\ (a) = \frac{A}{V_l} = \frac{6 \cdot \varepsilon}{(1-\varepsilon) \cdot d_{32}}$$

NOX stability correlates with the increase of interfacial area.



$$Half - life = \frac{\ln 2}{k_d}$$

NOX stability in a stirred tank under non aerated conditions.



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[1] Pedersen *et al* (2015) *Org. Process Res. Dev.* **19**, 1580
[2] Bommarius *et al* (2005) *Biotechnol Prog* **21**, 1663
[3] Colombié *et al* (2001) *Enzyme Microb Technol* **28**, 820
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