

Tools to evaluate processes for oxidase-based biocatalysis

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Tools to evaluate processes for oxidase-based biocatalysis

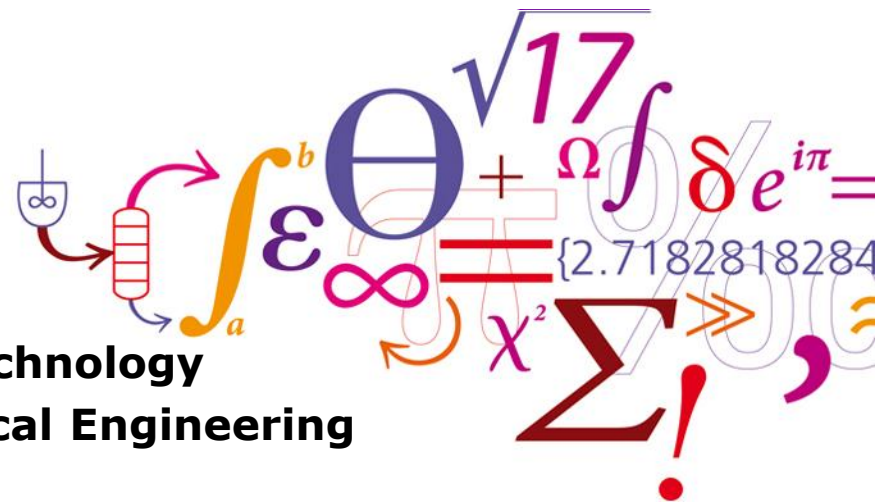
A stability perspective

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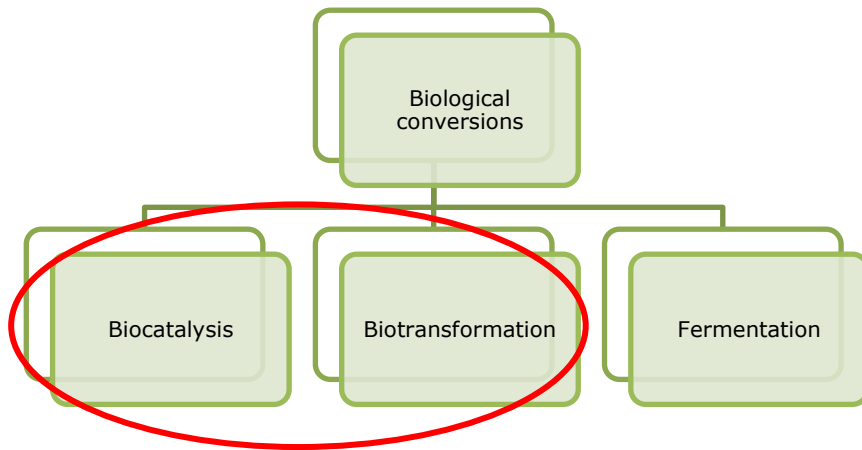
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Technical University of Denmark**



Agenda

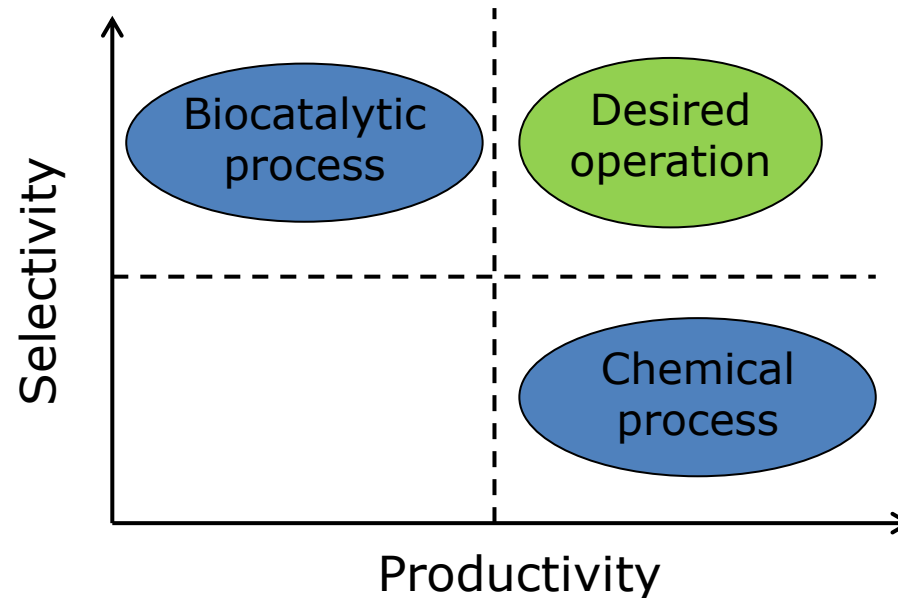
- Introduction
- Challenges involved in oxidase catalysed reactions
- Evaluation of stability issue in a process perspective
- Process tools employed in D-AAO as case study
- Take-home messages

Biocatalysis and Biotransformation



- Selective
- Water based reactions
- Mild conditions
- Catalyst properties can be altered

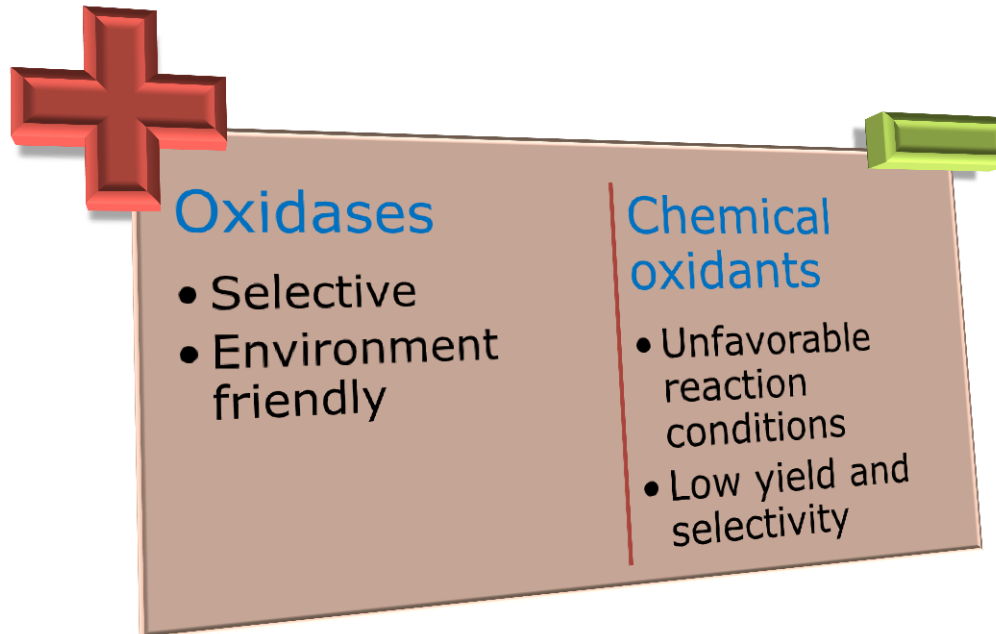
Chemical catalysis and Biocatalysis



Biocatalysis is very different to conventional chemical catalysis –
Different game, Different rules!

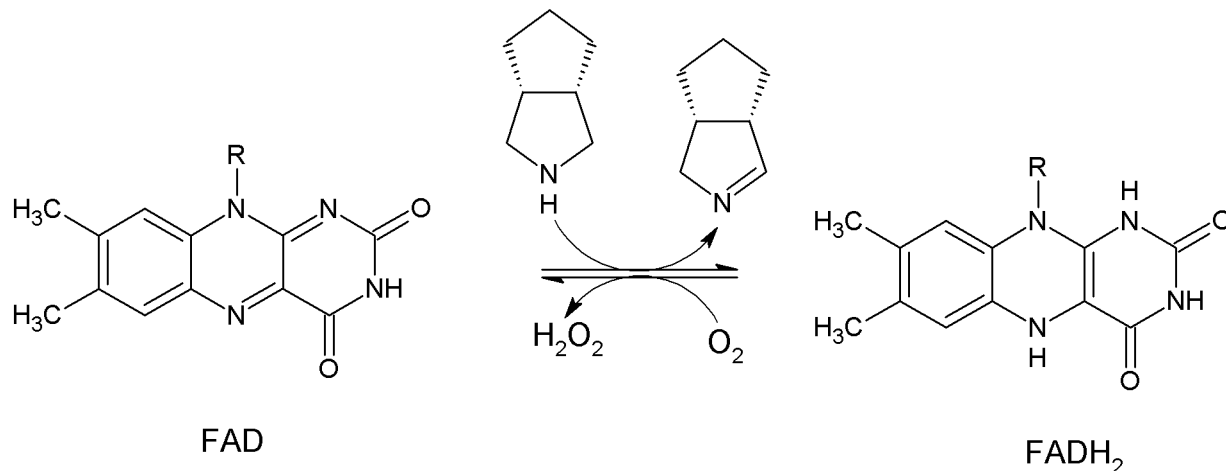
Biocatalysis for oxidation

- Oxidation reactions are key platforms for the development of building blocks for industrially relevant products.



Key reaction scheme of oxidases

- Two important oxidation reactions
 - C-O bond oxidation (alcohol and carbohydrate oxidases)
 - C-N bond oxidation (amine oxidases)
- Test system – A class of amine oxidase called MAO (EC 1.4.3.4)
- Reaction scheme of test system



Challenges

- The reaction scheme for MAO has indicated two key challenges that are associated
 - Supply of oxygen while maintaining stability
 - Hydrogen peroxide inactivation

The challenges are similar to that present in other oxidases, which will be explained further with the help of an example of D-AAO.

Importance of stability

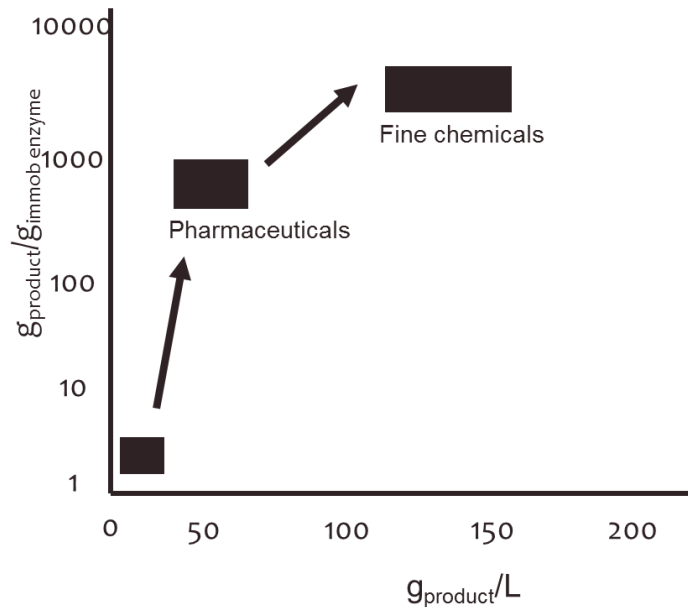


Fig: Productivity targets for different industrial sectors
(Woodley, personal communication)

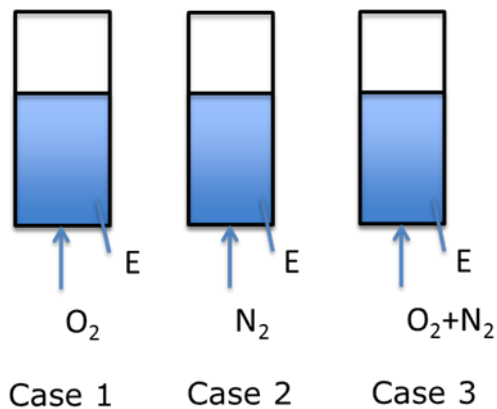
- Depending on the targeted chemical, the required catalyst productivity is depicted in the figure.
- It is inevitable that the biocatalyst be re-used a number of times to reach the productivity whilst keeping the cost down.

Measurement of Stability

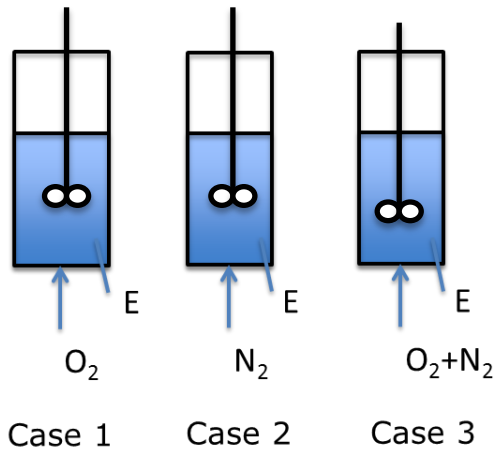
- Typically the stability of a biocatalyst is measured by the number of times it can be re-used in a particular reaction without loss in conversion.
 - Referred to as **operational stability**
- Operational stability is greatly affected by
 - Oxygen
 - Hydrogen peroxide
- In order to study the effect of oxygen on the stability, it is desirable to separate it from contributing effects.

Understanding deactivation by Oxygen

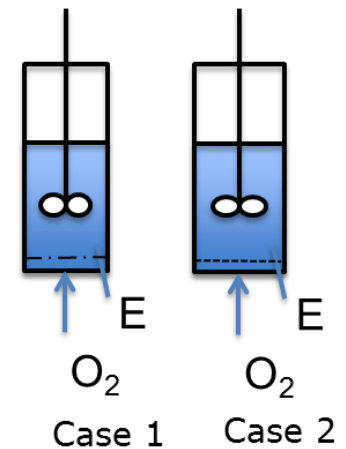
Oxygen concentration



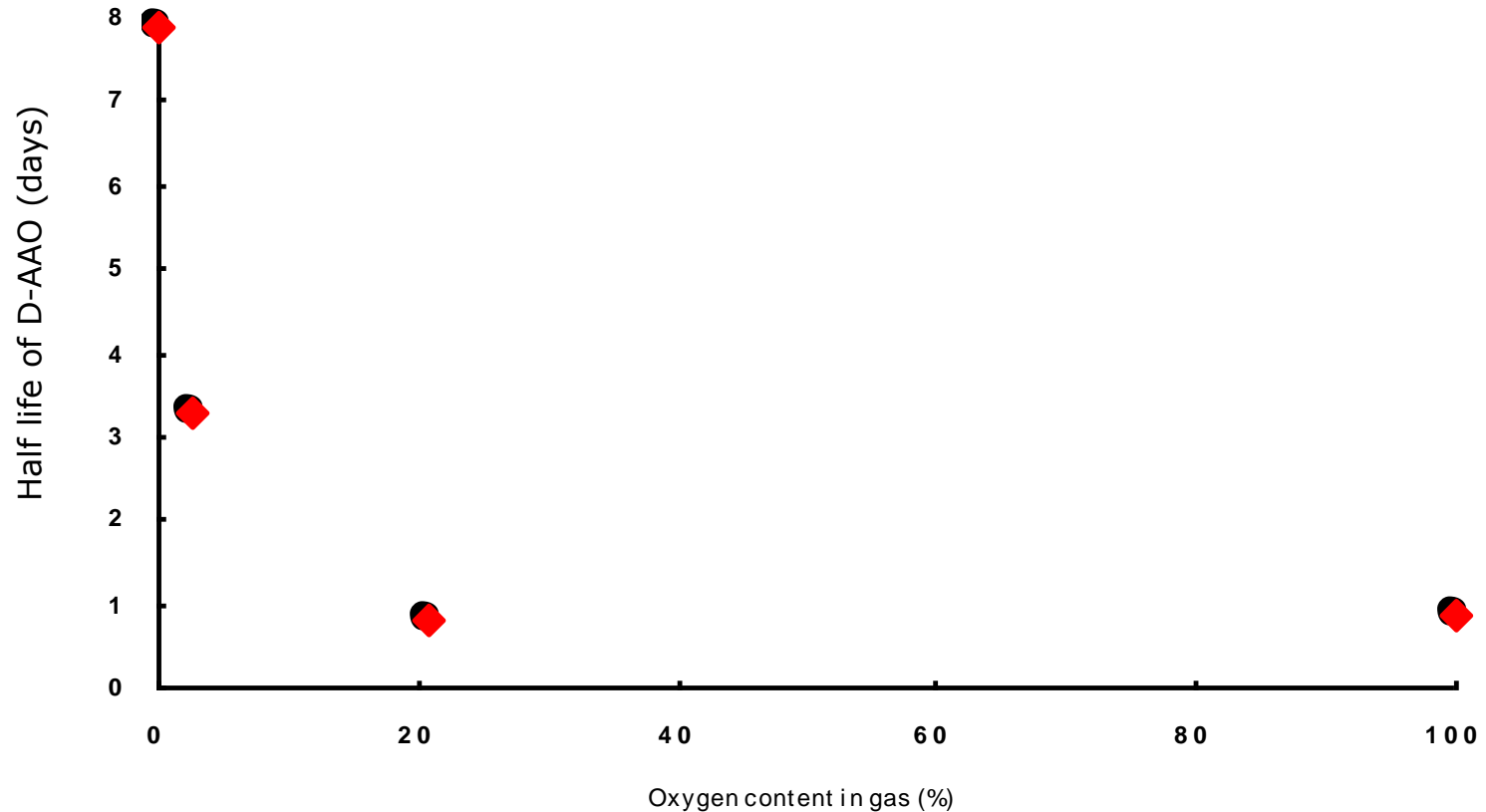
Aeration & agitation



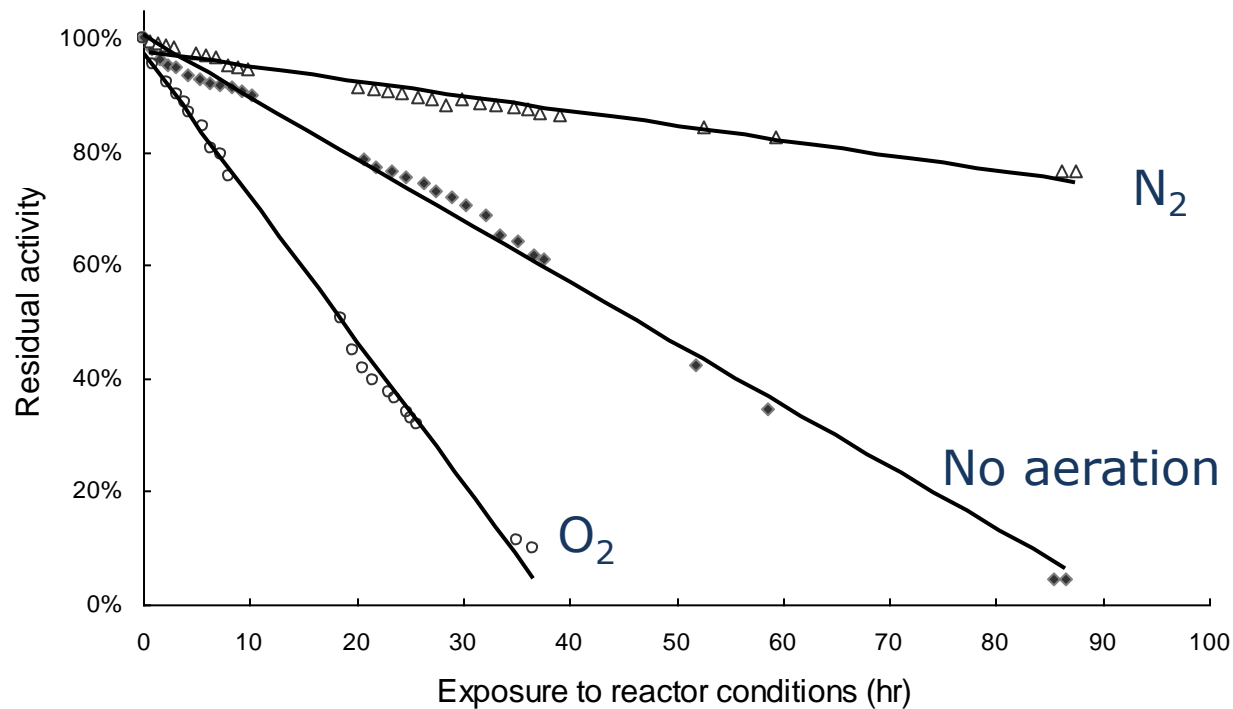
Bubble size



Oxygen concentration on stability

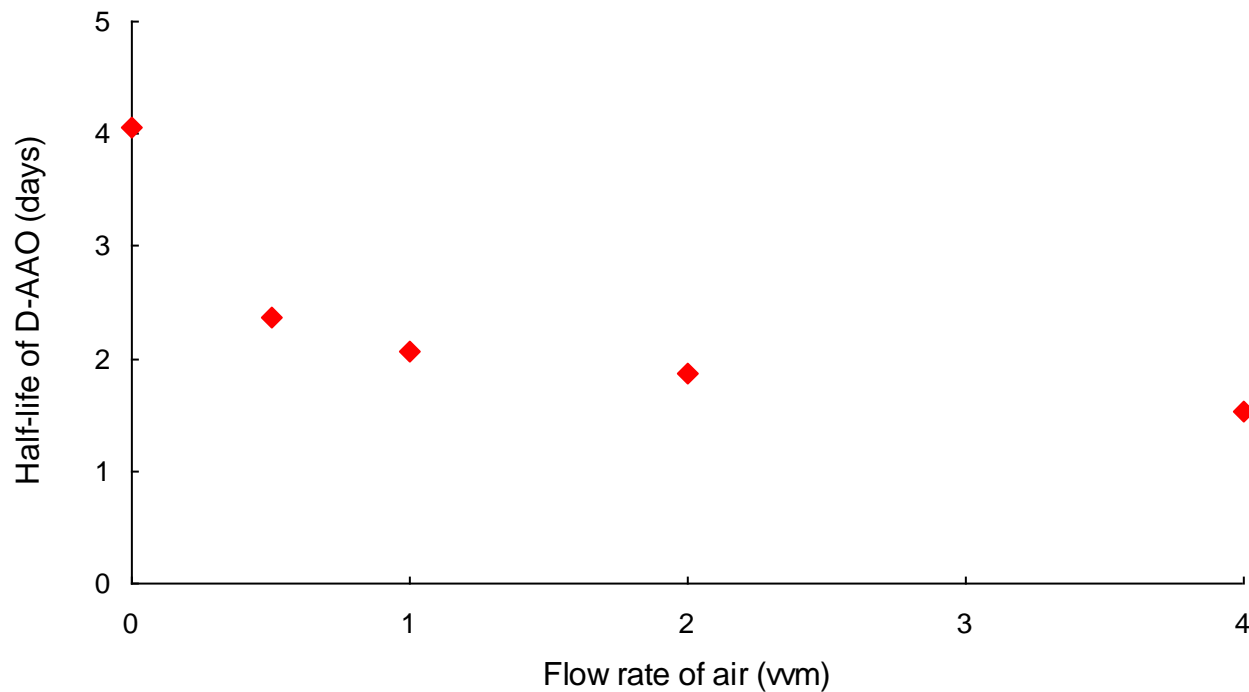


Gas effect on D-AAO stability

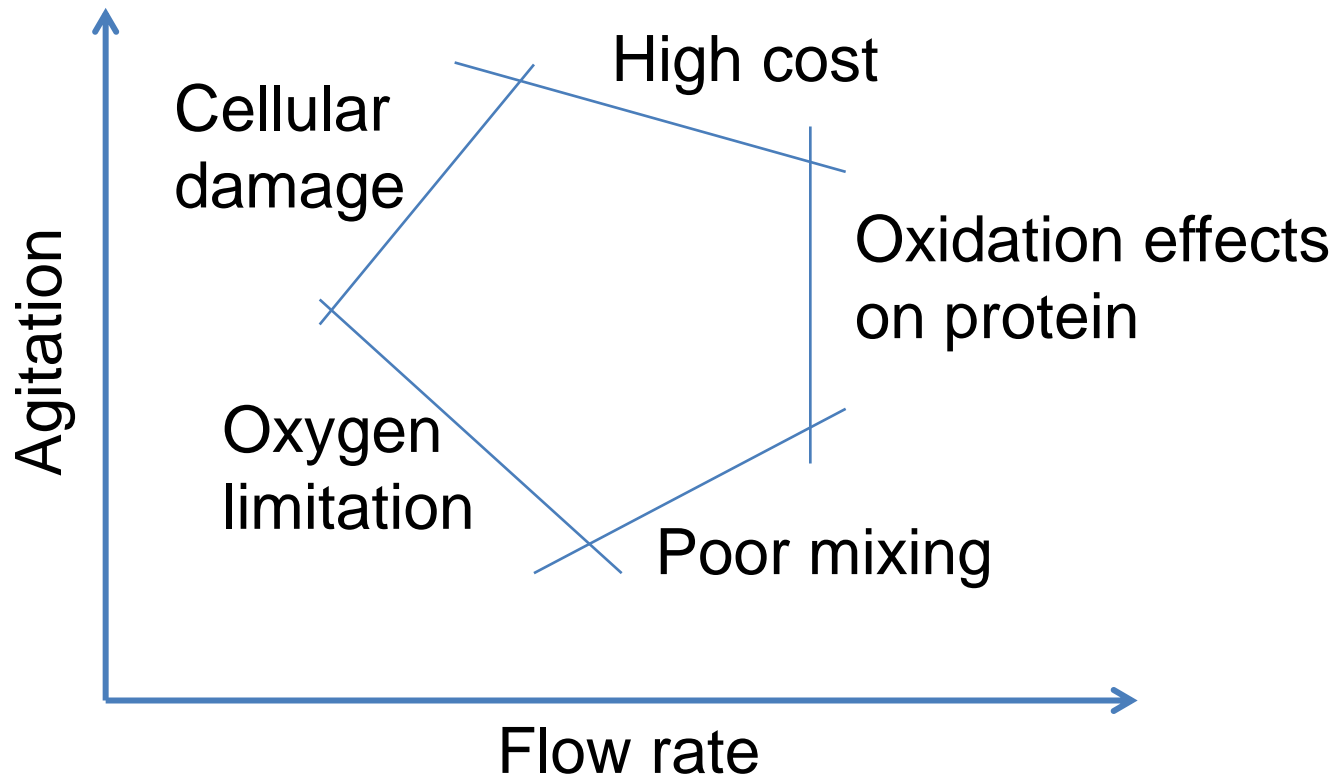


Tindall et. al., 2011

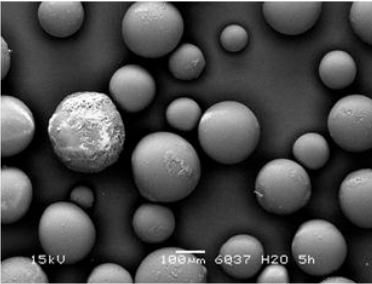
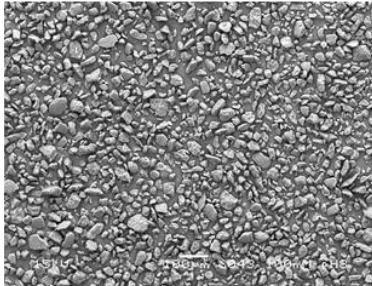
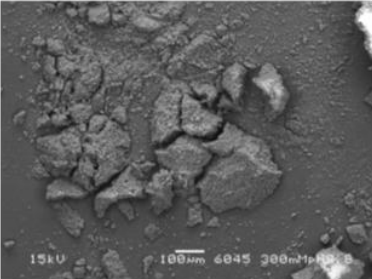
D-AAO stability with flow rates



Operation window

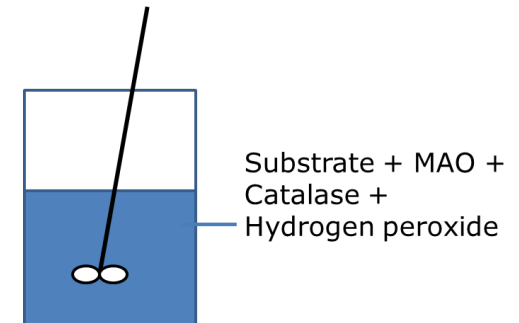
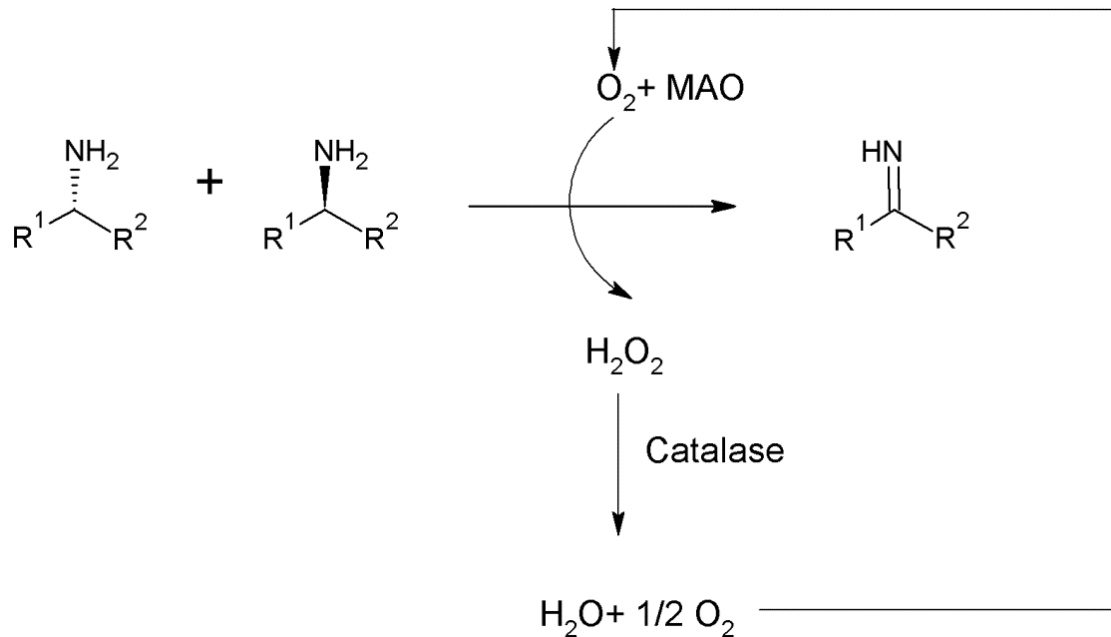


Effect of H₂O₂

<i>[H₂O₂] (mM)</i>	<i>Mean particle diameter (μm)</i>	<i>SEM</i>
0	175-125	
100	37-12	
300	< 5	

Dealing with Hydrogen peroxide

- Hydrogen peroxide is a known inhibitor of oxidases.

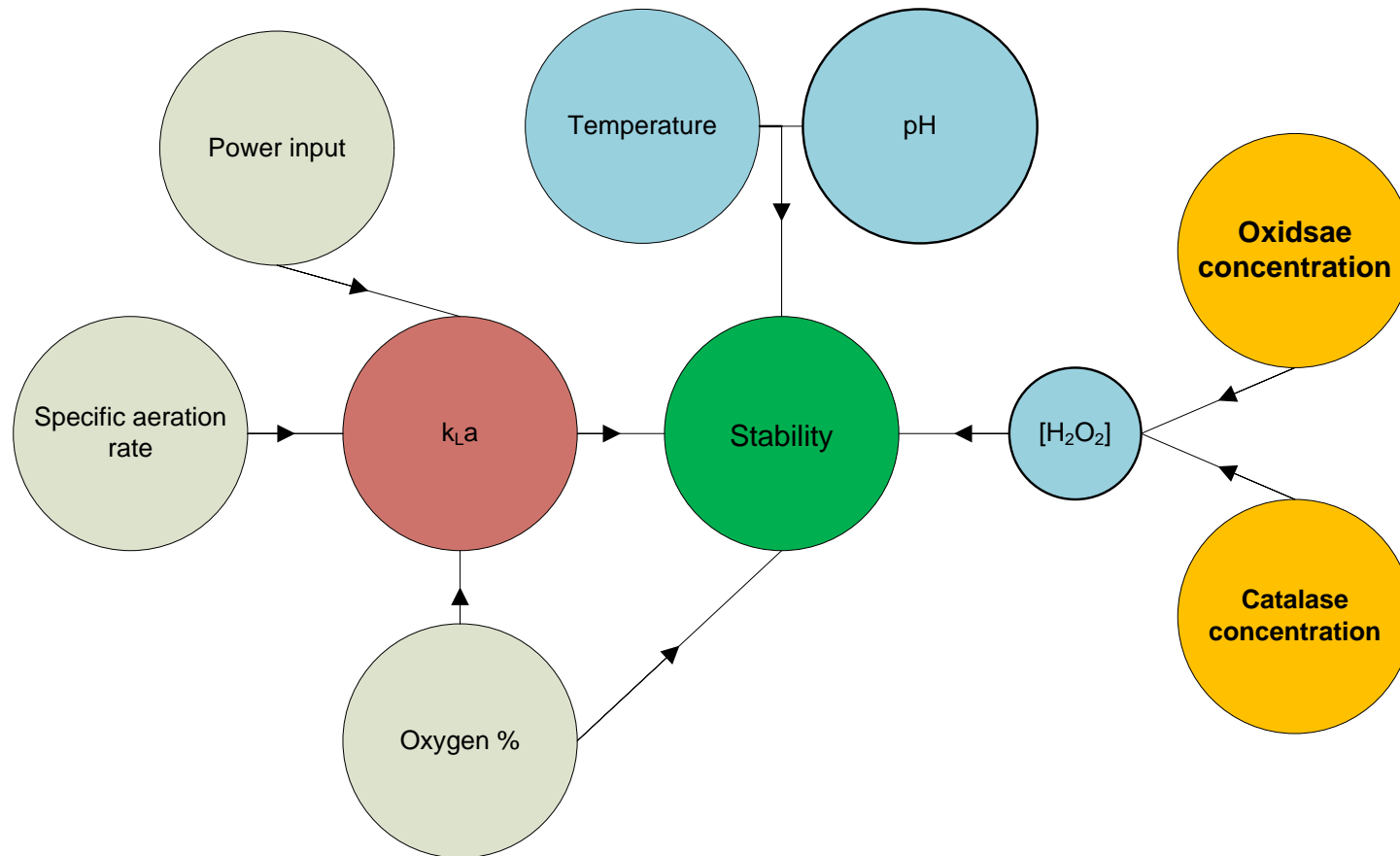


Strategy 1: Degradation of H₂O₂ by co-catalyst

Use of know-how in determining the deactivation mechanism

- Operational stability at operating conditions
- While re-use of immobilized catalyst can be made, stability of soluble catalyst can be evaluated by operating a continuous reactor and monitoring the conversion.

Effects contributing to stability of oxidases



Take home messages

- Oxygen effects on biocatalyst must be evaluated independently to understand the direct effects.
- Operational stability is of key importance for the success of a biocatalytic process and stability studies must be incorporated fairly early and go hand-in hand with process development.
- It is critical that the catalyst is evaluated under process conditions using scale-down strategy.
- The understanding gained through the studies can be used to develop operation windows which will aid as tools for process development.

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Thank you!

