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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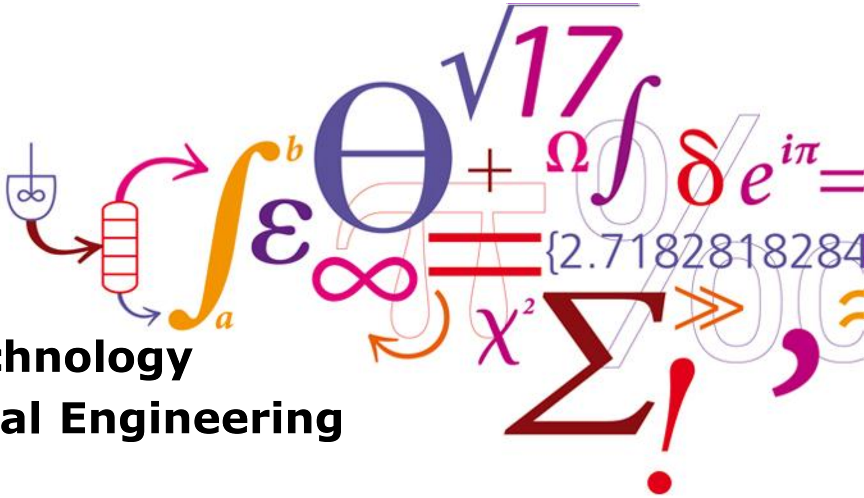

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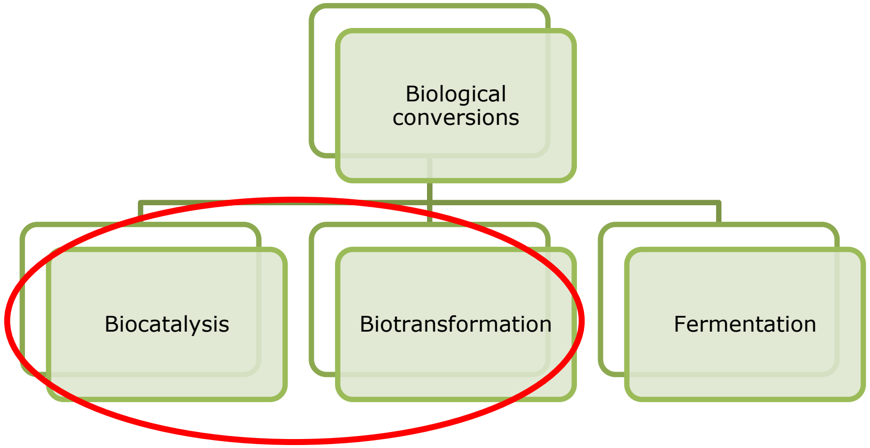
Agenda

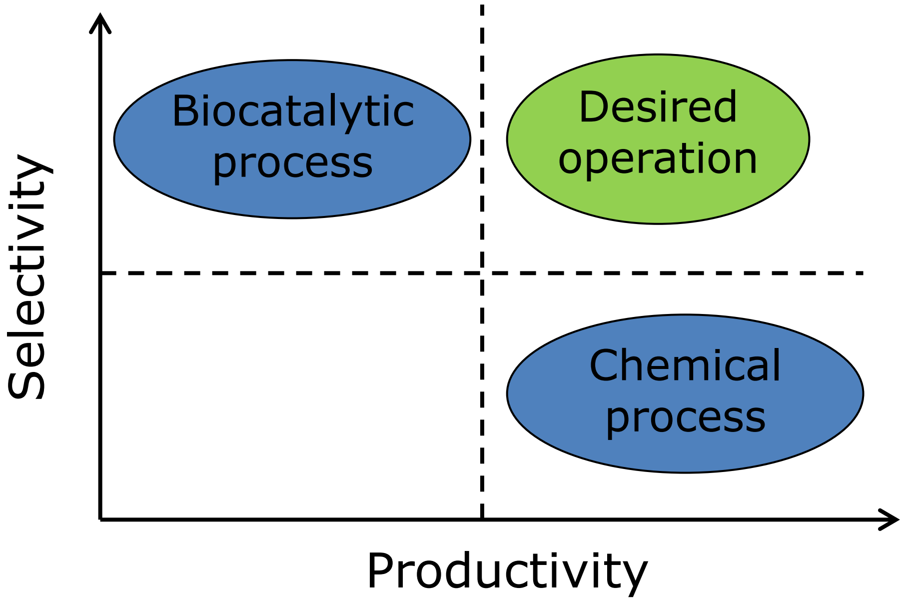
- Introduction
- Challenges involved in oxidase catalysed reactions
- Evaluation of stability issue in a process perspective
- Process tools employed in D-AAO as case study
- Take-home messages

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.

Oxidases

- Selective
- Environment friendly

Chemical oxidants

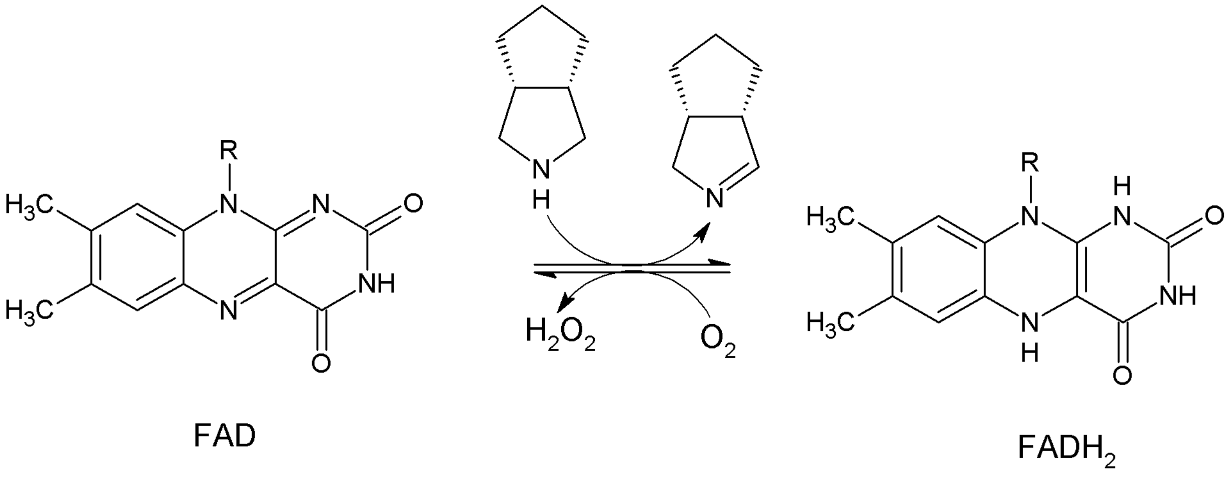
- Unfavorable reaction conditions
- Low yield and selectivity

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 oxidsae 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

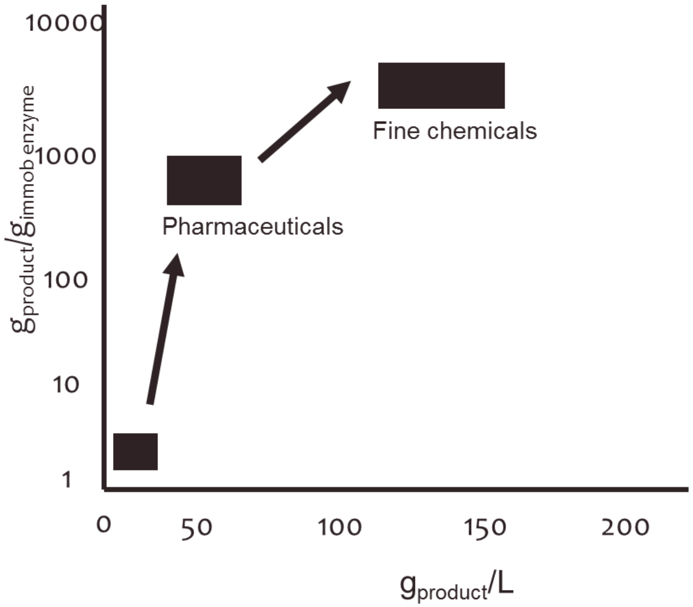


Fig: Productivity targets for different industrial sectors (Woodley, personal communication)

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

Measurement of Stability

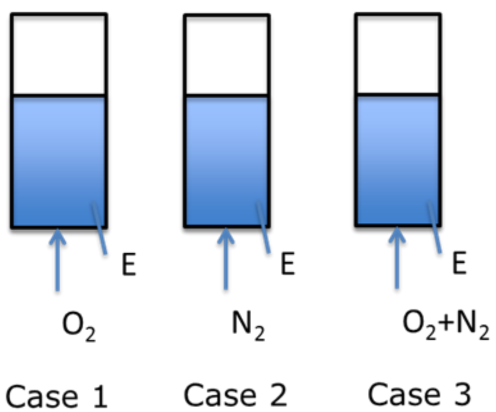
- Typically the stability of a biocatalyst is measured by the number of times it can be re-used in a particular reaction without loss in conversion.
  - Refered to as **operational stability**

- Operational stability is greatly affected by
  - Oxygen
  - Hydrogen peroxide

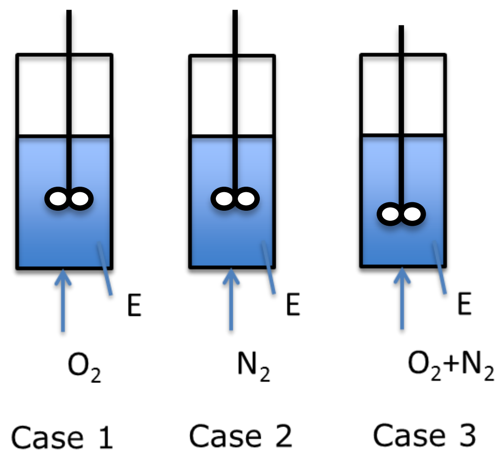
- In order to study the effect of oxygen on the stability, it is desireable to separate it from contributing effects.

Understanding deactivation by Oxygen

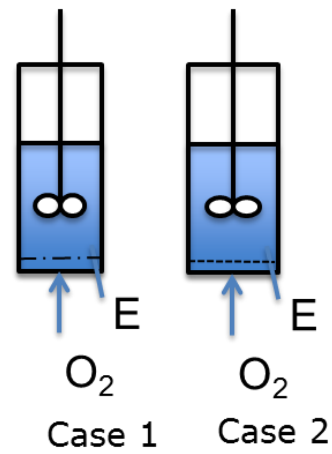
Oxygen concentration



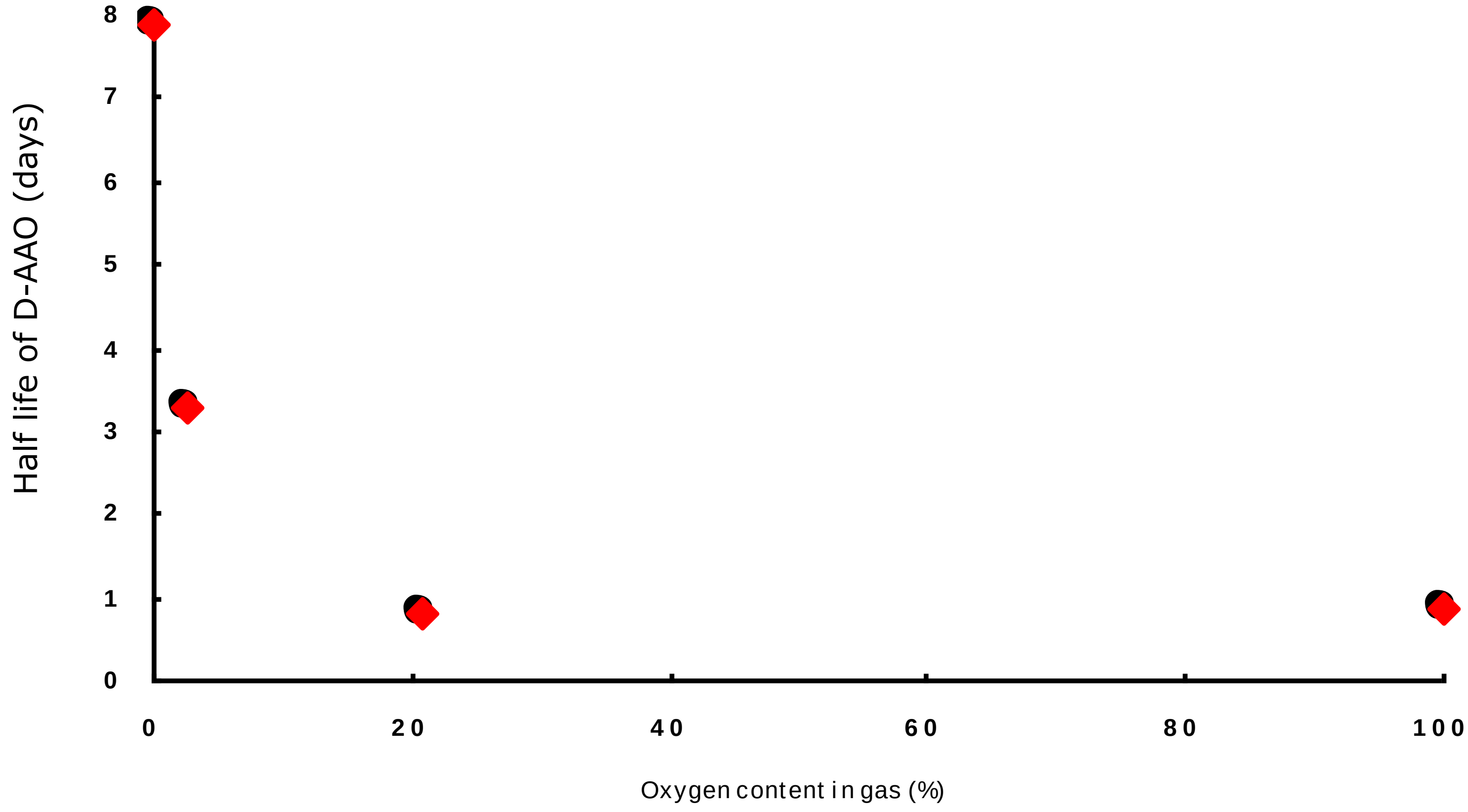
Aeration & agitation



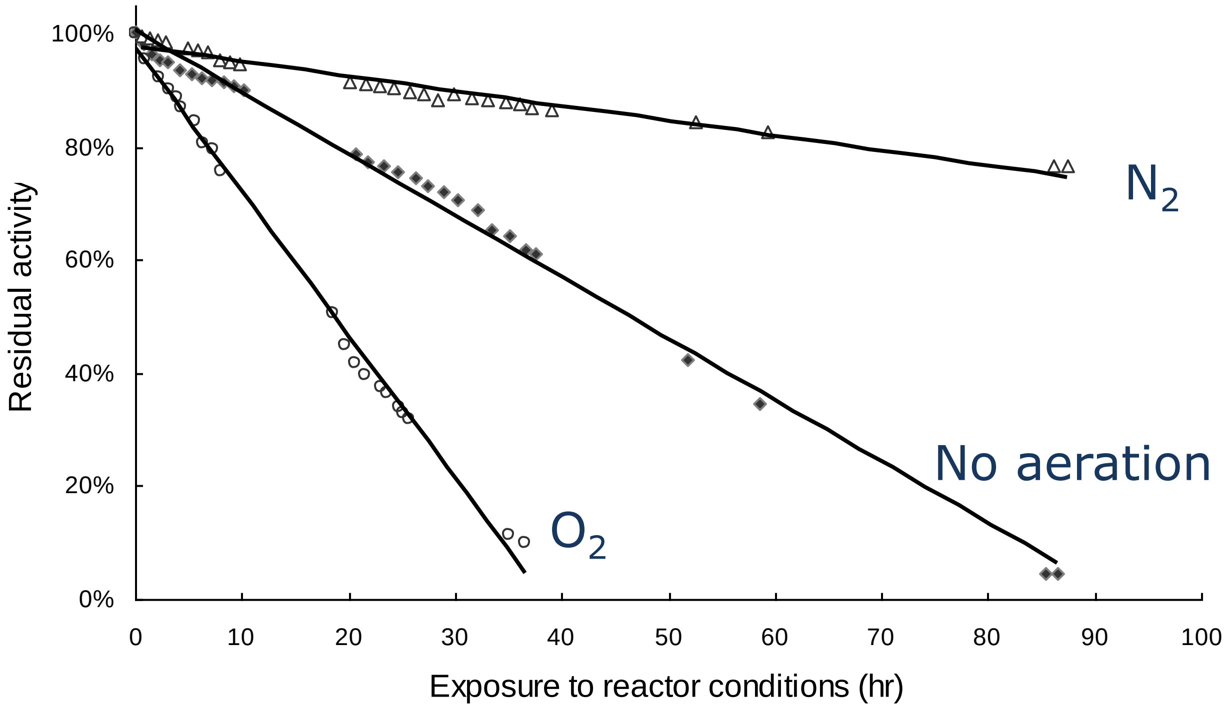
Bubble size



Oxygen concentration on stability

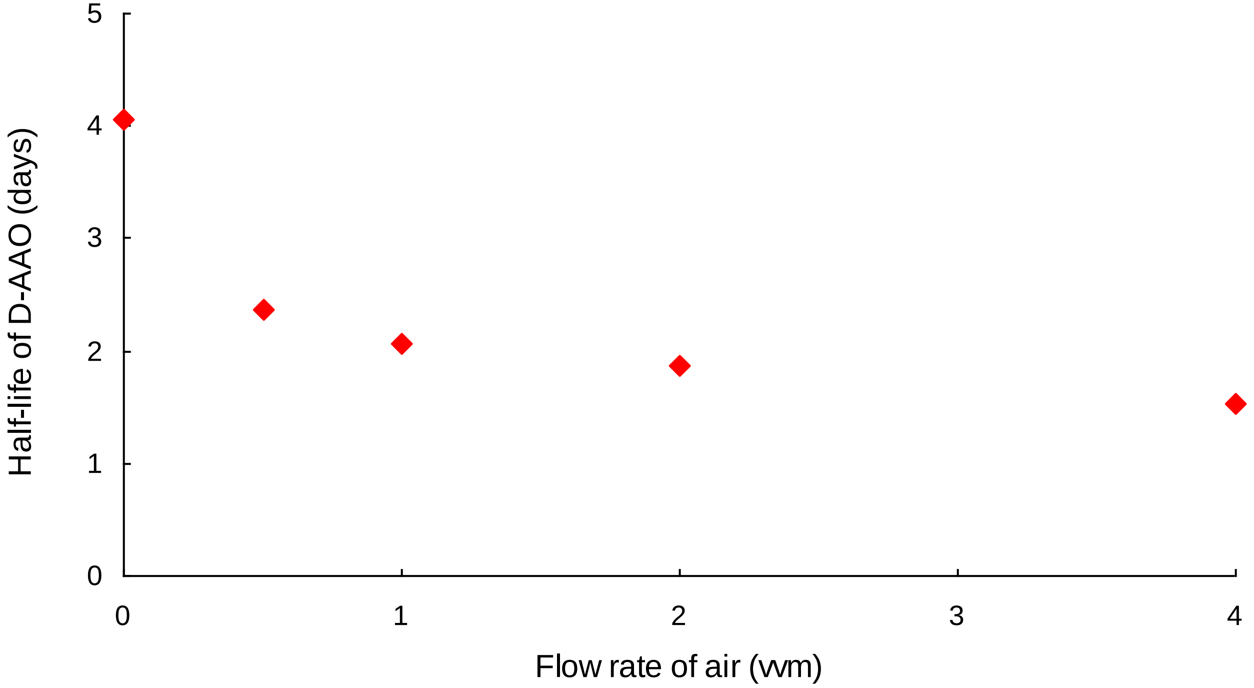


Gas effect on D-AAO stability

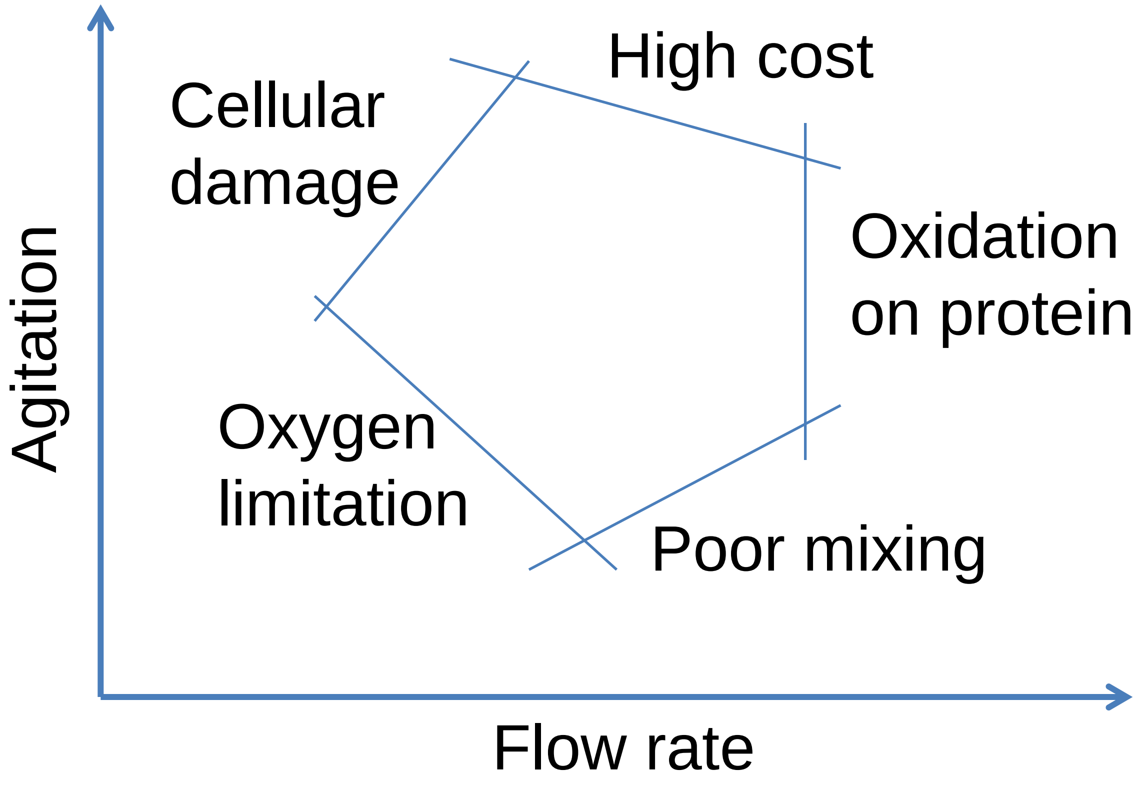


Tindall et. al., 2011

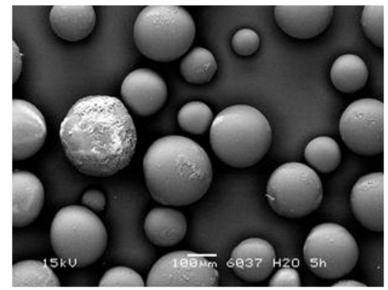
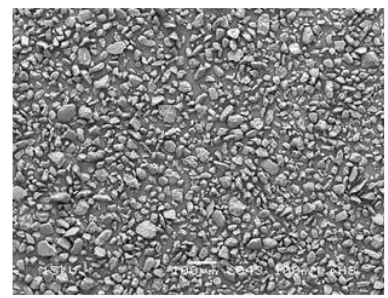
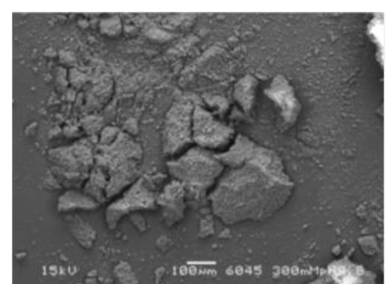
D-AAO stability with flow rates



Operation window

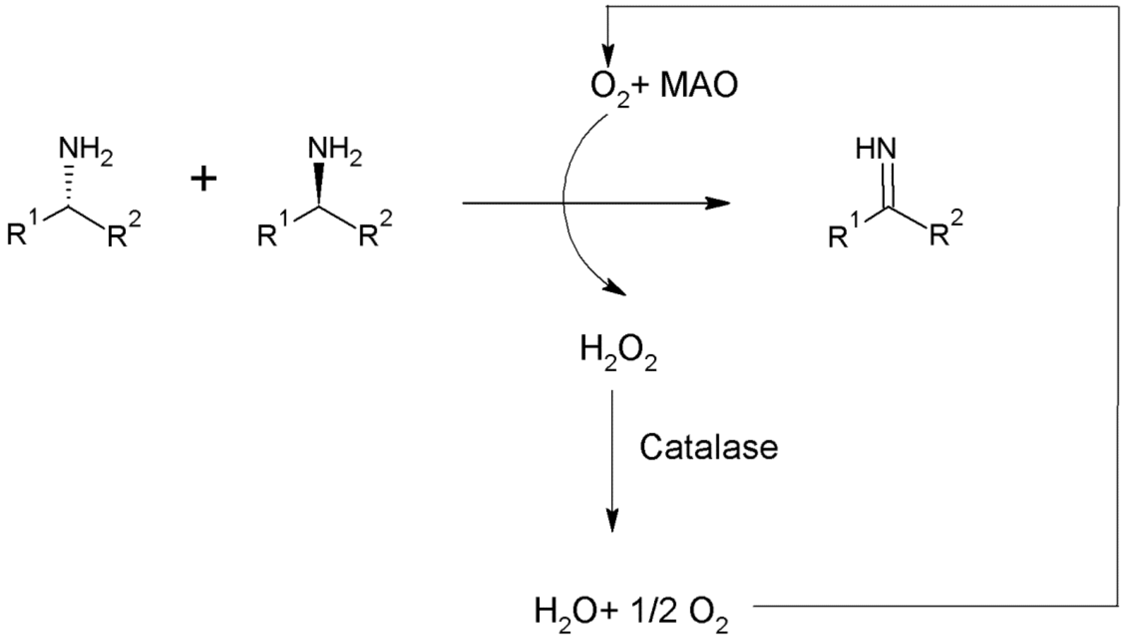


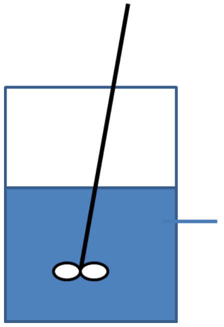
Effect of H_2O_2

$[H_2O_2]$ (mM)	Mean particle diameter (μm)	SEM
0	*175-125*	
100	*37-12*	
300	*< 5*	

Dealing with Hydrogen peroxide

- Hydrogen peroxide is a known inhibitor of oxidases.



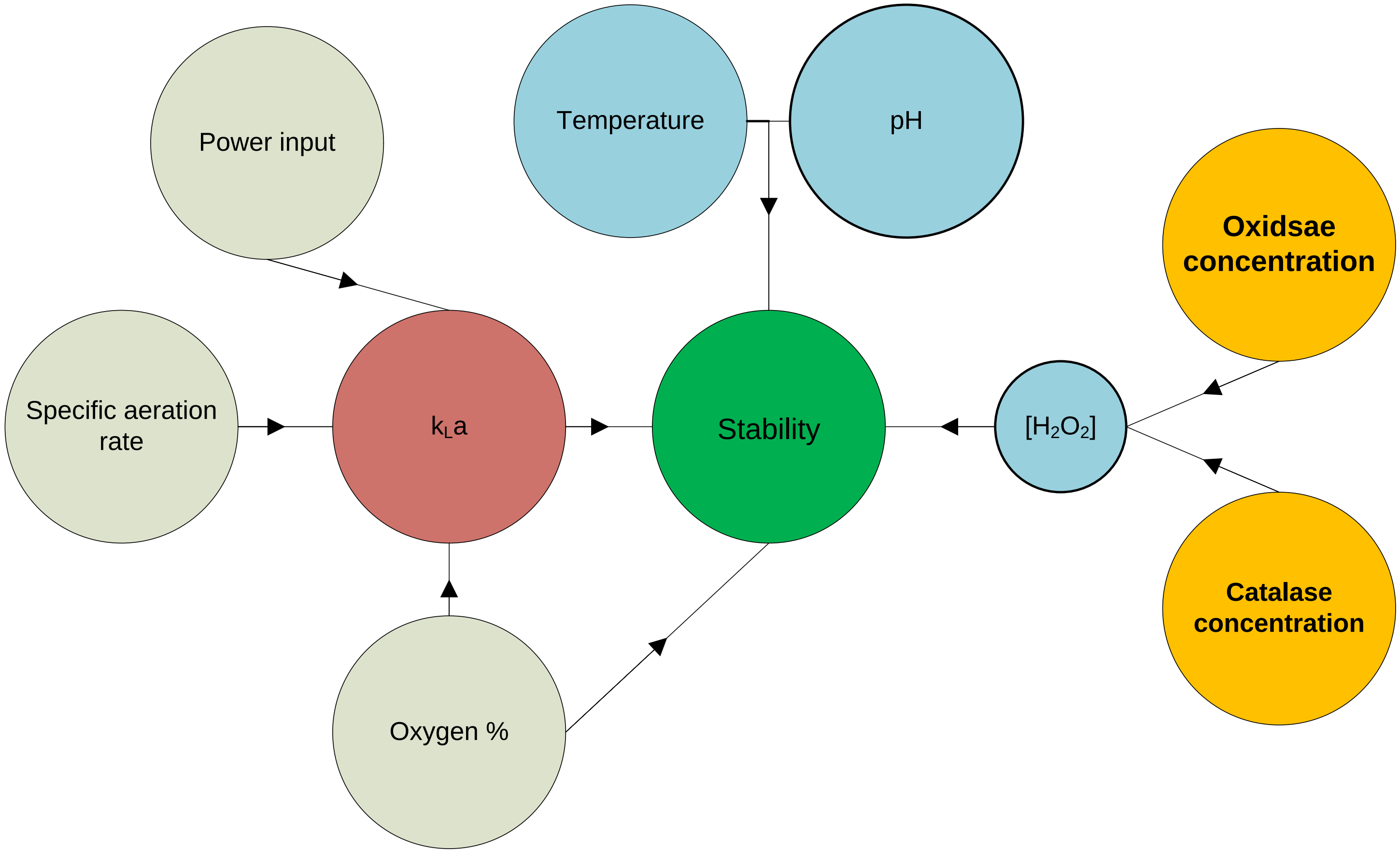


Strategy 1: Degradation of H_2O_2 by co-catalyst

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 soluable 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!