Key References:

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Technical University of Denmark

Kinetic Evaluation of Oxygen-Dependent Enzymes Converting Poorly Water-Soluble Substrates

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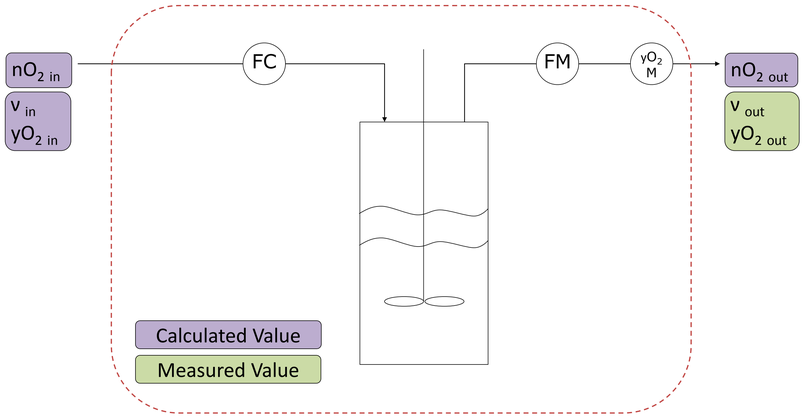
Reaction kinetics: a generic approach

- Accurate analytics are key at all scales.
- *Online, non-intrusive* methods are preferred to **overcome sampling challenges** brought about by **heterogeneous reaction mixtures**.
- Oxygen mass balance for kinetic estimations applied in the fields of respirometry (wastewater treatment) & solid state fermentations.



Theory and model system

Glucose oxidase (GOx) used as a model system for validation

$$\frac{1}{2}O_2 + \text{Glucose} \xrightarrow[\text{Catalase}]{\text{GOx}} \text{Gluconic acid} + H_2O$$

n_{O_2} [mmol.min^{-1}] in gas outlet stream found by ideal gas law:

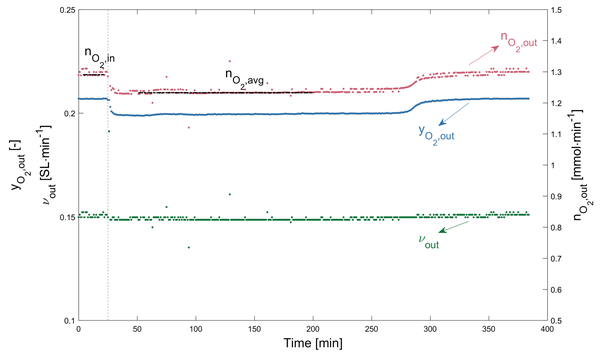
$$n_{O_2,out} = \frac{P.v_{out}}{RT} \times y_{O_2,out}$$

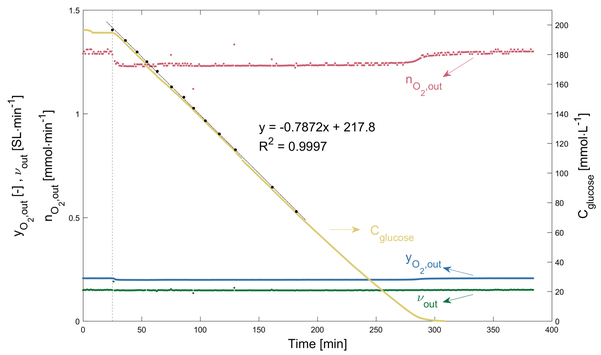
Initially when no reaction is taking place (at steady state):

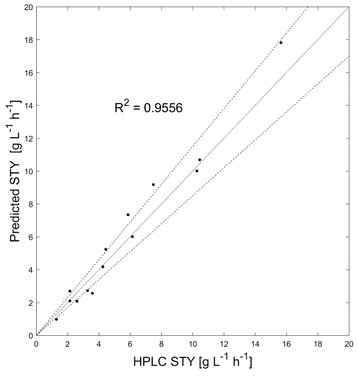
$$n_{O_2,in} = n_{O_2,out}$$

R_{O_2} (= ½$R_{glucose}$) [mmol.L^{-1}.min^{-1}]:

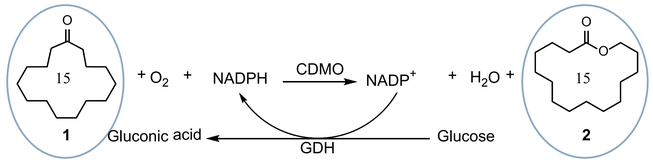
$$R_{O_2} = \frac{dC_{O_2}}{dt} = \frac{(n_{O_2,in} - n_{O_2,out})}{V}$$







Figures 1 & 2 (above): Measured variables, $y_{O_2,out}$ (blue) and v_{out} (green), and calculated $n_{O_2,out}$ (red) as a function of time at 500 rpm and 1.0 vvm. Grey dashed line indicates the experiment start by addition of 200 mg.L^{-1} GOx. Predicted $C_{glucose}$ (yellow) overlaid with HPLC data.
Figure 3 (left): Parity plot of space-time yields (STYs) measured by HPLC vs. those predicted by oxygen mass balance. Dashed lines indicate 85% similarity; dotted line indicates unity.



Scheme 1: BVMO-catalysed oxidation of **poorly water-soluble** cyclopentadecanone (**1**, circled) to cyclopentadecanolide (**2**, circled) by cyclododecanone monooxygenase (CDMO). NADPH cofactor was recycled using glucose dehydrogenase (GDH).

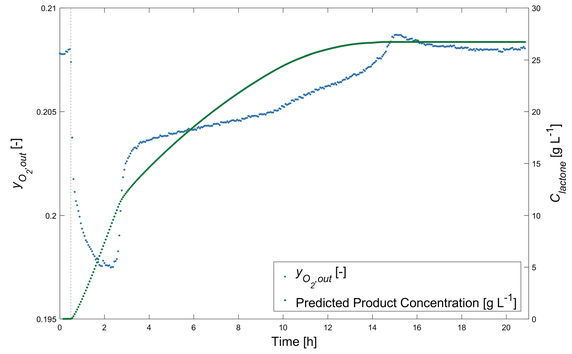


Figure 4: Reaction kinetics of the oxidation shown in *Scheme 1* predicted by oxygen mass balance. Grey line indicates the experiment start by addition of 5% (v/v) CDMO cell-free extract.

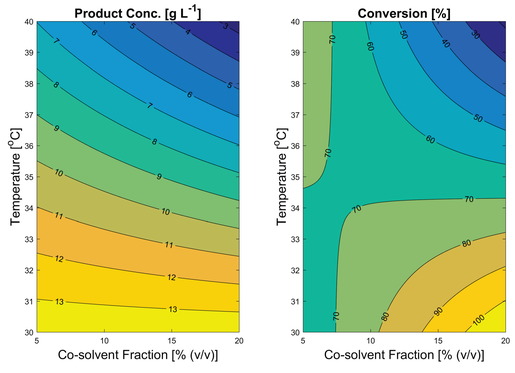


Figure 5: Full factorial Design of Experiments (DoE) output for product concentration and conversion as a function of varied environmental parameters: temperature and co-solvent (methanol) fraction. Conversions of **1** to **2** estimated using gas-phase oxygen mass balances.

Conclusion

Accurate kinetic predictions by off-gas oxygen mass balance hold potential for *any* oxygen-dependent reactions where representative sampling is challenging (e.g. heterogeneous reactions).

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