Key References:

Technical University of Denmark

Solid Phase Biocatalytic Baeyer-Villiger Oxidations

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Oxidative Biocatalysis: Renewed Industrial Interest

Oxygen functional groups are essential for intermediates and products within the polymers, fine and agro chemicals, vitamins, flavours and fragrances, and active pharmaceutical ingredients industries.

New ROBust OXidases are targetted to:
- Exploit the full potential of biocatalytic oxidations
- Match industrial standards of chemocatalytic oxidation

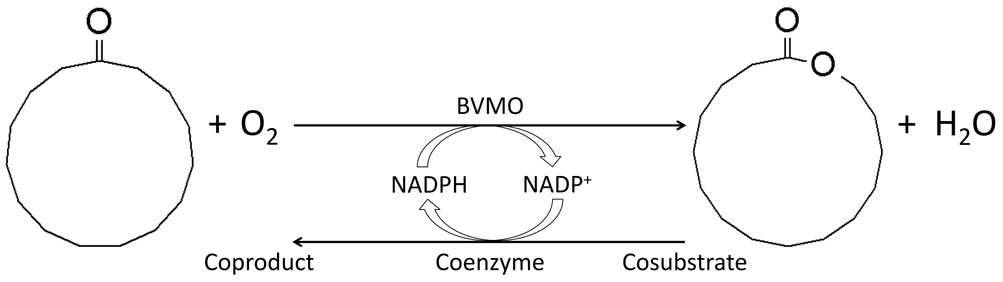
Pharma	• Hydroxylations and dealkylations for pharmaceutical metabolite synthesis • Selective oxidation of alcohols and lactols/deoxysugars for pharma
Nutrition	• Hydroxylations of aromatics for vitamin intermediate production • Hydroxylation of alkenes as intermediates for nutritional products • Selective oxidation of alcohols and lactols/deoxysugars for nutrition
Fine/specialty chemicals	• Hydroxylation of alkenes in flavours and fragrance production • Oxidation of solid surfaces in coating applications • Oxidation of macrocyclic molecules to musk ketones/lactones
Materials	• Oxidation of (macro)cyclic ketones for polyesters • Oxidation of (macro)cyclic ketones for performance polymers • Synthesis of monomers for biobased polymers (with novel functionalities)

Enzymatic Baeyer-Villiger Oxidation Advantages

- Broad substrate scope
- Substitution of conventional oxidants with molecular oxygen
- Superior regio-, chemo- and enantioselectivity

Biocatalytic process steps offer potentially more efficient processes and satisfy the fulfilment of 'green chemistry' legislation.

Target Reaction

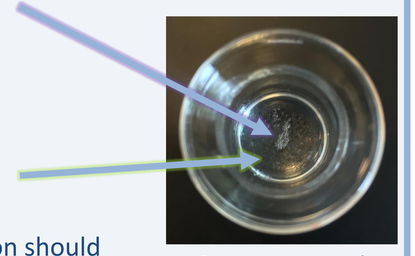


Solid Phase Properties

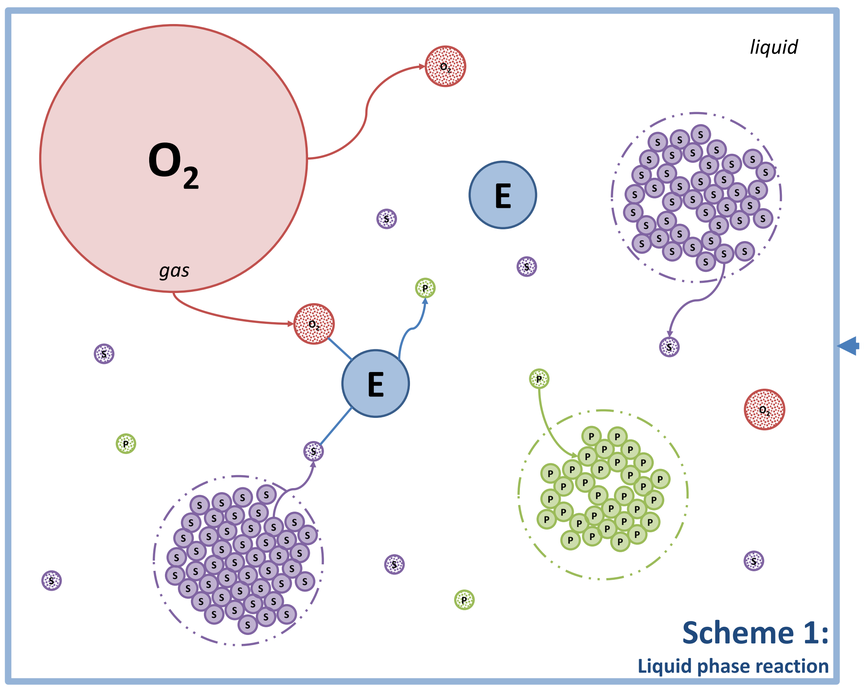
Substrate/product solubility in water @ 28 °C (mean ± SD, n = 3):

- Cyclopentadecanone (substrate)
  = 25.4 ± 4.7 mg.l^{-1}
  = 113 ± 21 µM
- Cyclopentadecanolide (product)
  = 24.2 ± 5.7 mg.l^{-1}
  = 101 ± 24 µM

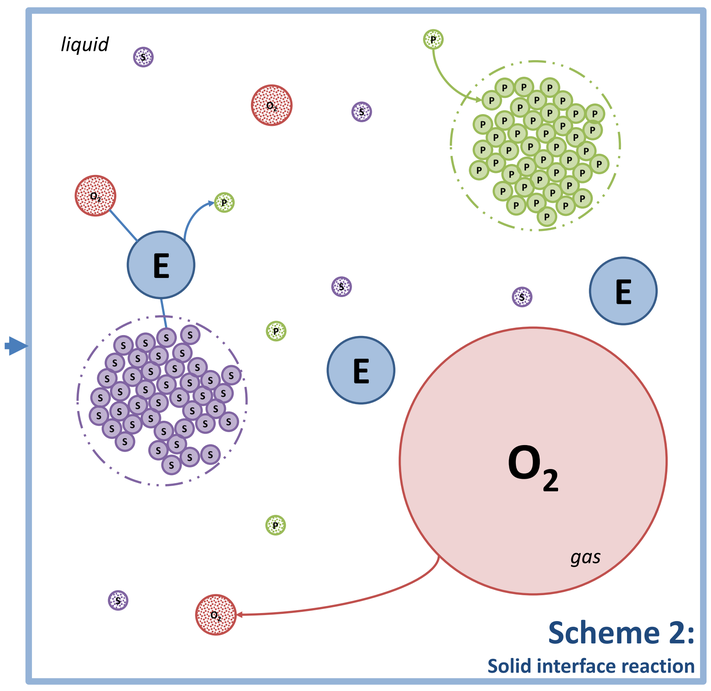
K_m of enzyme = 24 µM ∴ the reaction should proceed at V_{max} at substrate saturation.



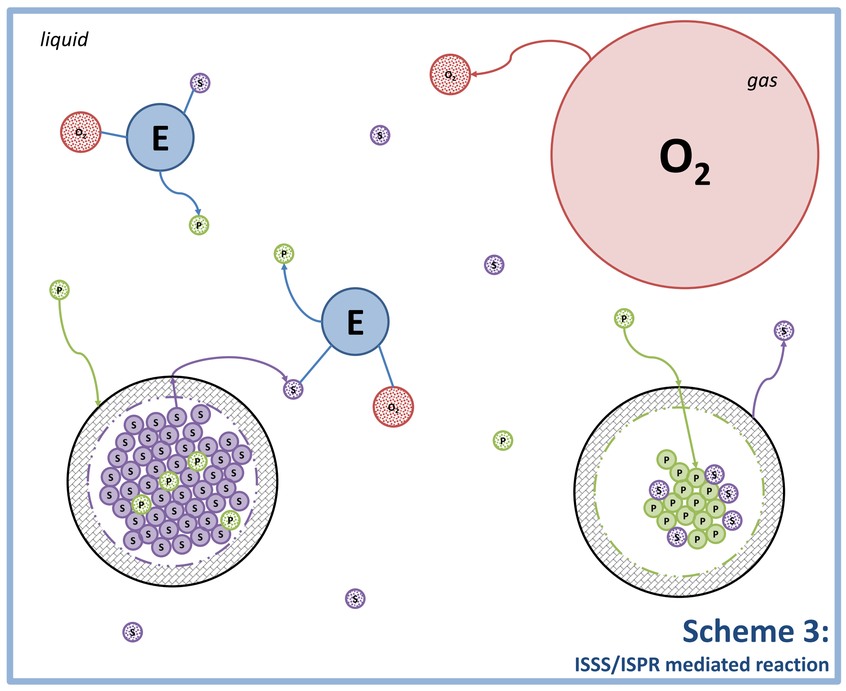
Separate crystal formation



Scheme 1:
Liquid phase reaction



Scheme 2:
Solid interface reaction



Scheme 3:
ISSS/ISPR mediated reaction

Process Challenges

- Representative sampling
- Analytical methods
- Enzyme characterisation assays

Solution

Sacrificial samples of 1 m*l* reaction vol. with total organic phase extraction for gas chromatography analysis.

Drawback: reaction conditions difficult to control (pH, oxygen supply)

Addressing Potential Surface Interaction Deactivation

In situ product removal (ISPR) and *in situ* substrate supply (ISSS) methods:
- Liquid-liquid extraction
- Absorption
- Adsorption

Solvents and/or solid sorbents must be biocompatible and assessed in terms of the potential introduction of mass transfer limitations.

Future work

Characterise reaction kinetics as a function of:
- Enzyme concentration
- Substrate loading
- Product loading
- Stability towards co-solvents
- Stability towards bubbling

Establish reaction location and rate limiting factor (dissolution or enzyme kinetics).

Cofactor regeneration mechanism using whole-cells (substrate/product mass transfer to be addressed).

Upscaling reactions to reactor environment for better process control.

Multiphase Reactor Options

- Stirred tank reactor
- Fluidised bed reactor
- Two-liquid phase biocatalytic reactor

Ultimate Objective

Minimise the water fraction of two-phase reactions to operate at high, industrially-relevant substrate loadings and reduce the cost of downstream processing.

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