Biotransformation von in Wasser schwer löslichen Verbindungen mit ionischen Flüssigkeiten

Im Rahmen des 27. Osnabrücker Umweltgesprächs:

Ionische Flüssigkeiten – Fortschritte bei der Anwendung

Christina Kohlmann

24.06.2008

Biocatalysis

Advantages

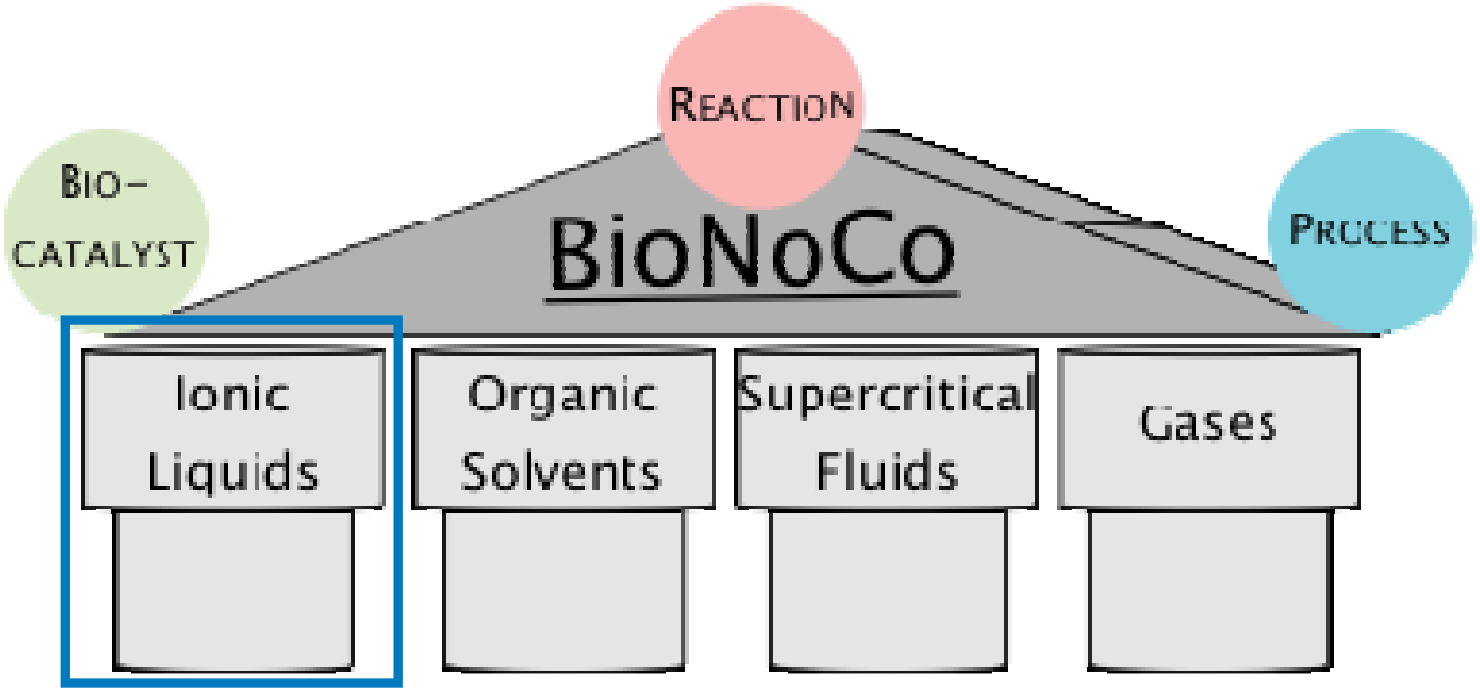
- Versatile catalysts
- High stereo-, regio- or enantioselectivity
- Mild reaction conditions
- Water as reaction medium

Disadvantages

- Separation of water
- Inhibition effects
- Limited to water soluble substrates

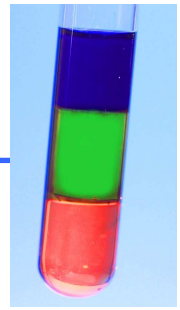
Biocatalysis using Non-Conventional Media

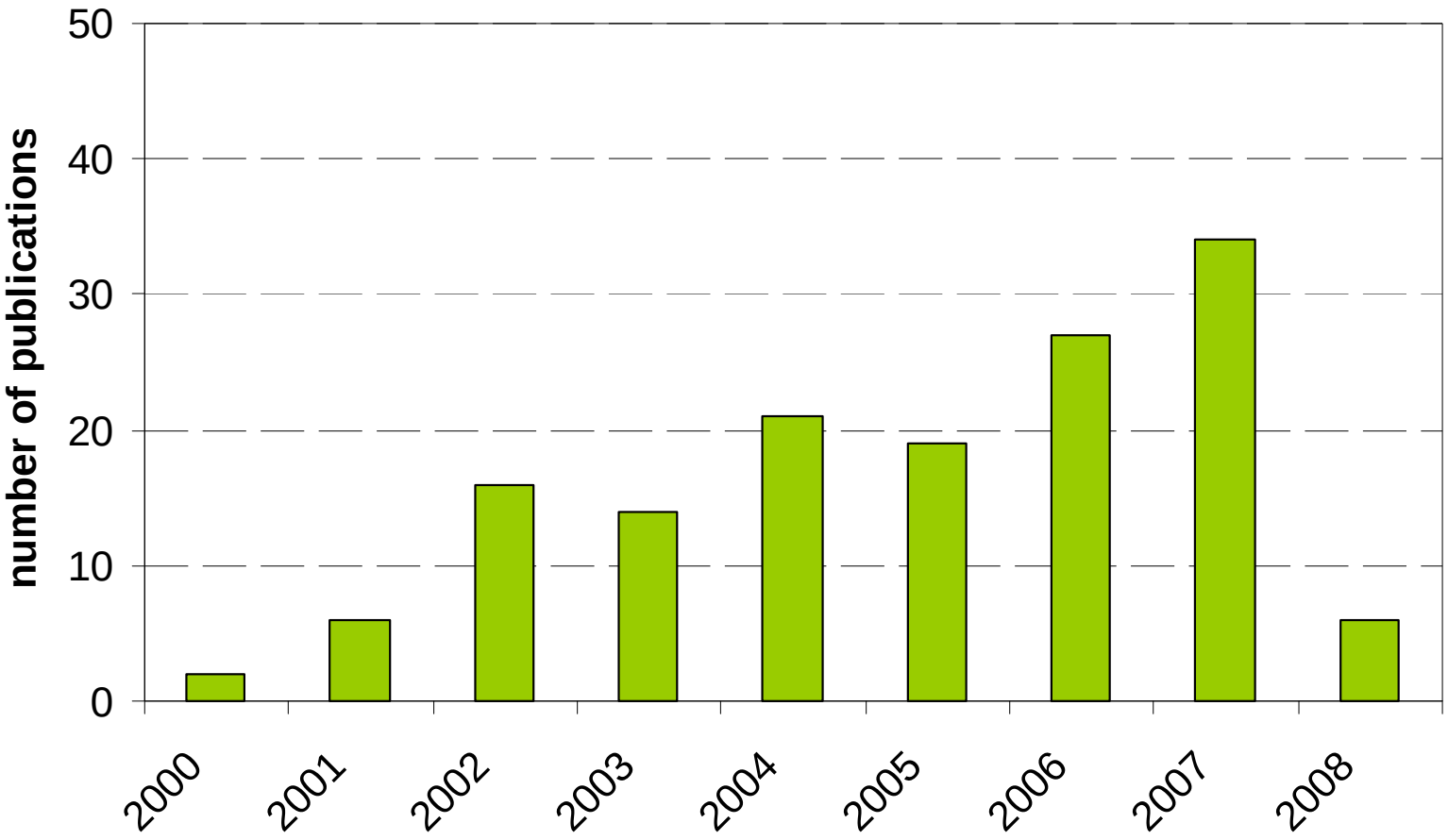
DFG research training group 1166 at RWTH Aachen University



http://www.bionoco.rwth-aachen.de/

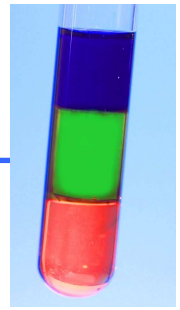
Biocatalysis in the presence of ILs



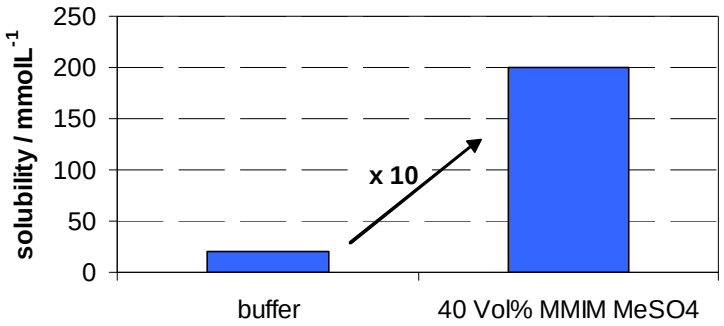


SciFinder search regarding „ionic liquids“ and „biocatalysis“ June 2008

Biocatalysis in the presence of ILs



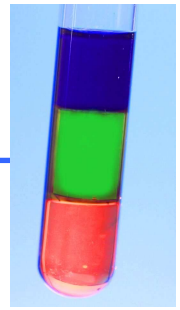
- **Increased substrate solubility**
  - Solubility of acetophenone[1]



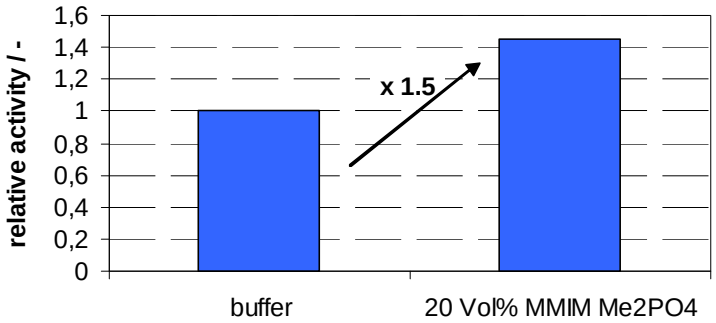
- **Higher activity**
- **Higher stability**
- **Increased selectivity**

[1]U. Kragl, M. Eckstein, N. Kaftzik, *Curr. Opin. Biotechnol.* 2002, *13*, 565

Biocatalysis in the presence of ILs

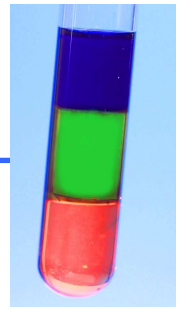


- **Increased substrate solubility**
- **Higher activity**
  - Activity of amino acid oxidase[2]
- **Higher stability**
- **Increased selectivity**

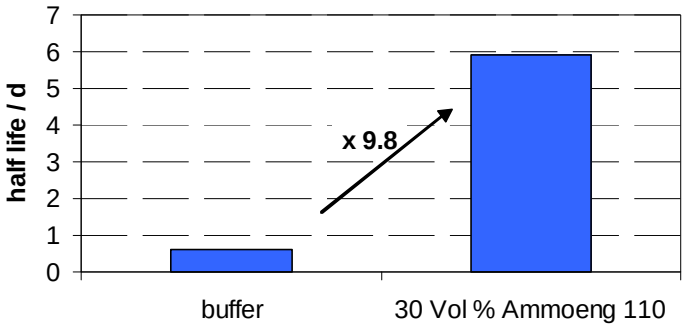


[2]S. Lutz-Wahl, E. M. Trost, B. Wagner, A. Manns, L. Fischer, *Journal of Biotechnology* **2006**, *124*, 163.

Biocatalysis in the presence of ILs

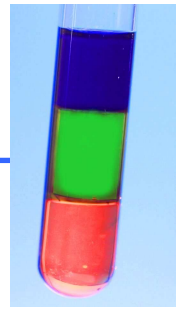


- **Increased substrate solubility**
- **Higher activity**
- **Higher stability**
  - Half life of an alcohol dehydrogenase[3]
- **Increased selectivity**



[3]S. Dreyer, U. Kragl, *Biotechnology and Bioengineering* **2008**, *99*.

Biocatalysis in the presence of ILs

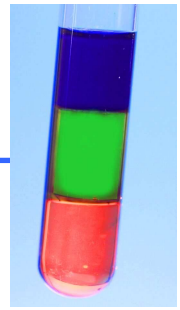


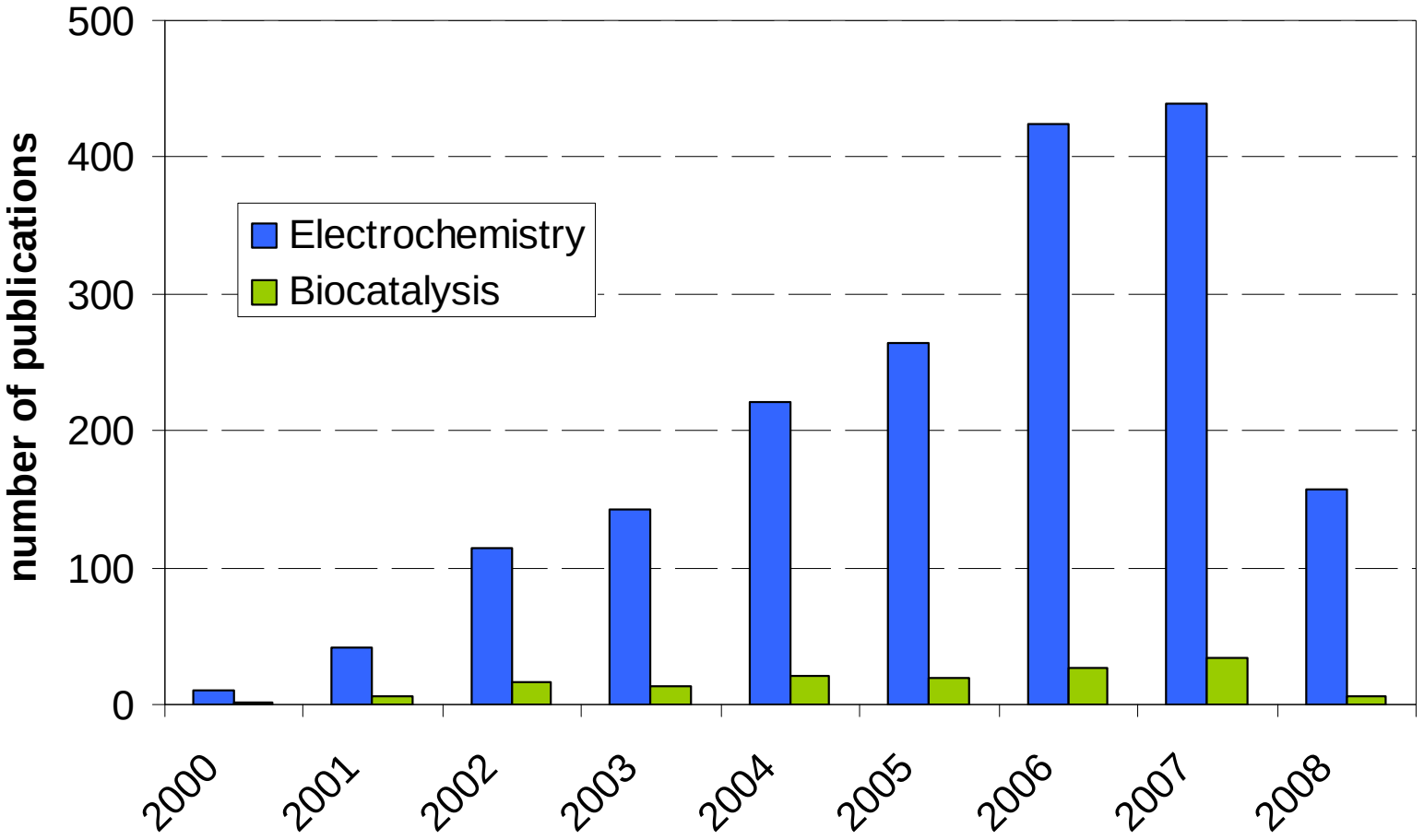
- **Increased substrate solubility**
- **Higher activity**
- **Higher stability**
- **Increased selectivity**
  - Stability of an Lipases from *Alcaligenes* sp.:[4]

    ee in MTBE = 0 *ee* in HMIM BF_4 und OMIM BF_4 > 98%
  - Reaction: kinetic resolution of *rac*-1-phenylethanol by transesterification with vinyl acetate

[4]S. H. Schöfer, N. Kaftzik, P. Wasserscheid, U. Kragl, *Chemical Communications* **2001**, 425.

Biocatalysis in the presence of ILs





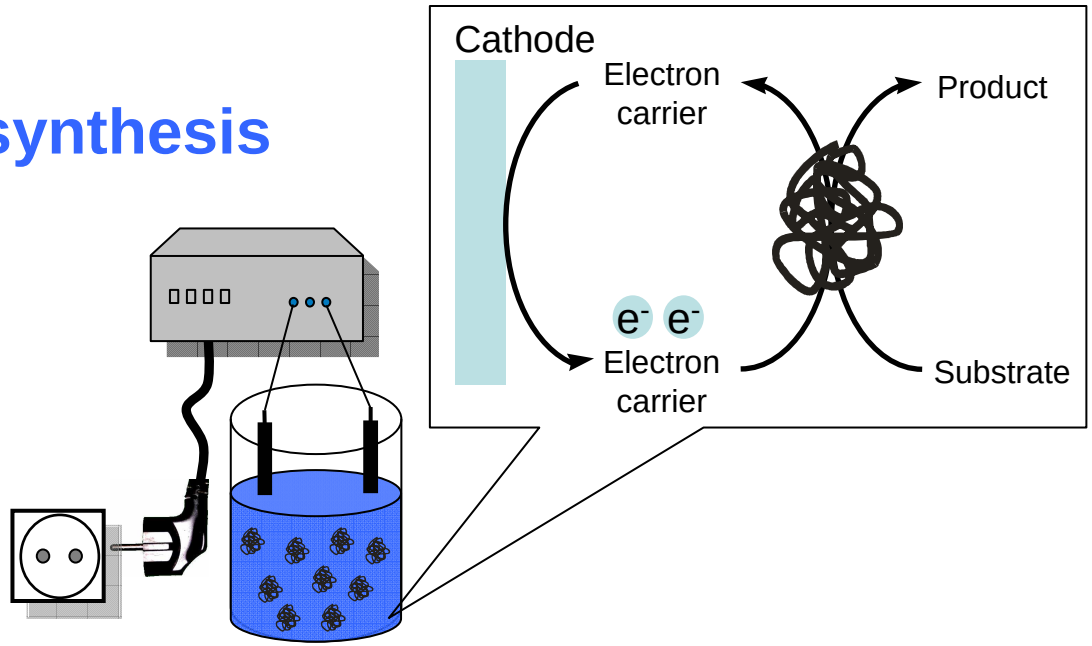
SciFinder search regarding „ionic liquids“ and „biocatalysis“ or “electrochemistry” June 2008

Electrochemistry with biocatalysts

Oxidoreductases = enzymatic redox catalysts

- Require source or sink for the involved electrons
- Electrons are usually generated or accepted by a further biocatalytic reaction or stoichiometric applied chemicals
- Anodes/Cathodes can also be used to take up or deliver the electrons

⇨ **Electroenzymatic synthesis**



Electroenzymatic synthesis

Advantages

- Electrons are among the cheapest redox equivalents available
- Reagent free
- Applied potential can be used to control reaction progress

Disadvantages

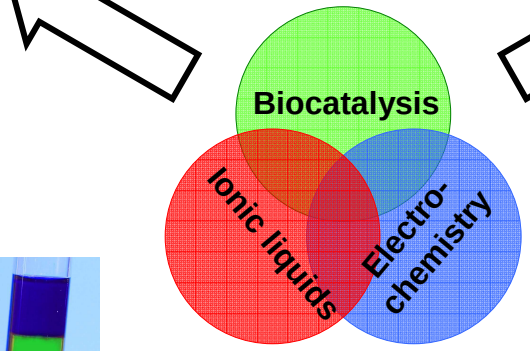
- By now relatively low productivities
- Low conductivity of the reaction medium
- Stablility of the biocatalyst

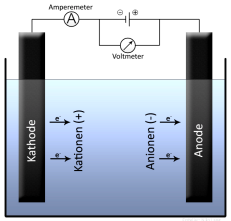
C. Kohlmann, W. Markle, S. Lütz, *Journal of Molecular Catalysis B: Enzymatic* **2008**, *51*, 57.

Electroenzymatic synthesis with ionic liquids

- Increased substrate solubility
- Higher stability of the enzyme

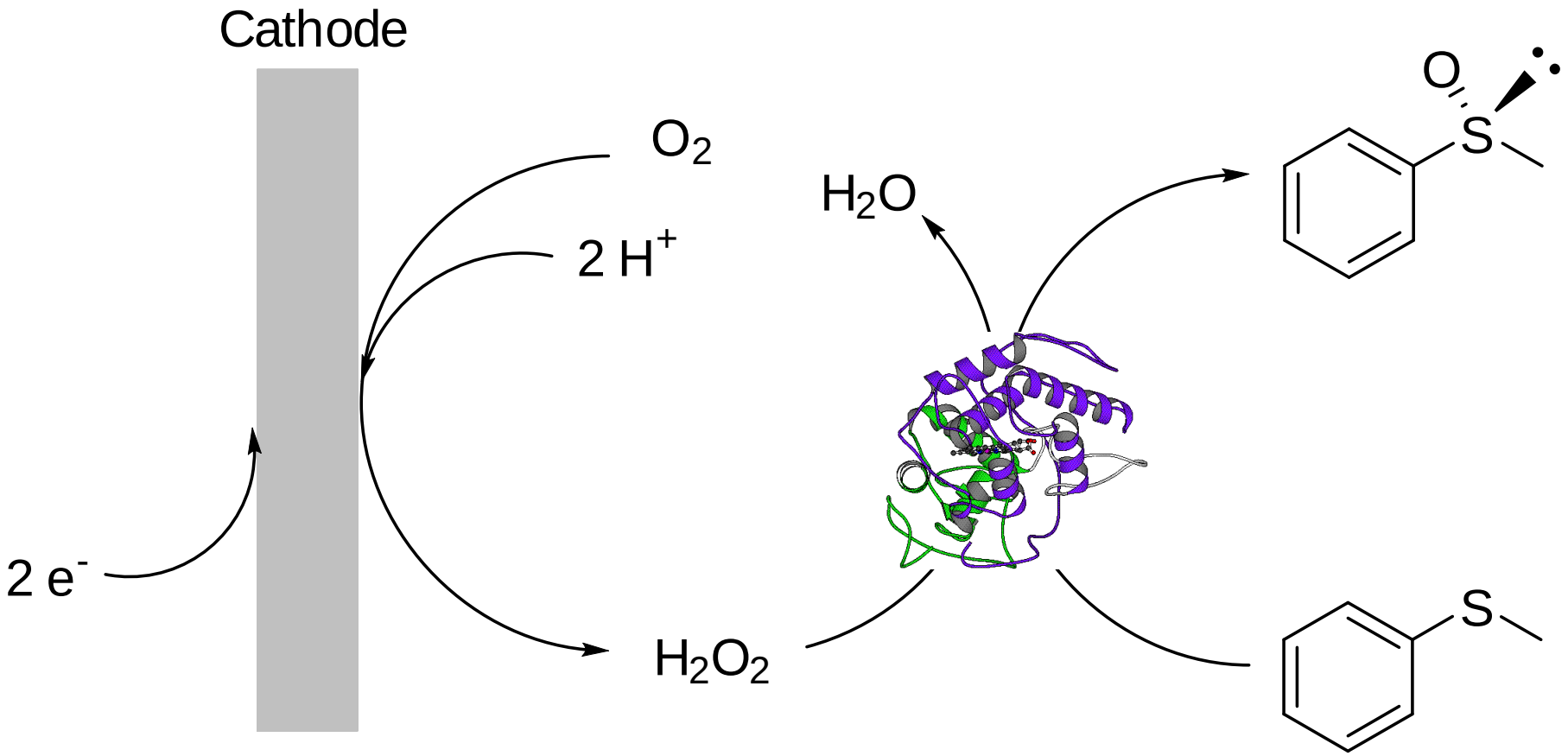
- Cheap redox equivalents
- Reagent free
- Control reaction progress by potential





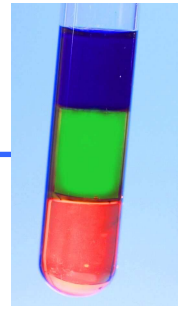
- Higher conductivity
- Higher productivity

Chloroperoxidase (CPO) catalysed oxidation of sulfides

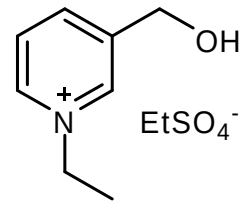


Lütz, S.; Steckhan, E.; Liese, A. *Electrochemistry Communications* **2004**, *6*, 583-587.

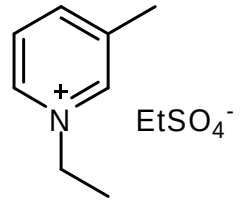
Ionic Liquids



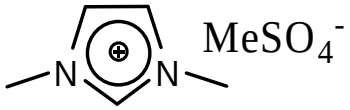
- 10 ILs under investigation
- Commercially available ILs
- Water-miscible ILs



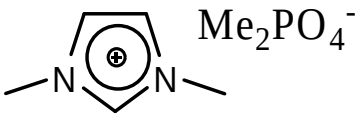
EM(OH)Py $EtSO_4$



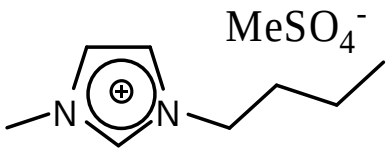
EMPy $EtSO_4$



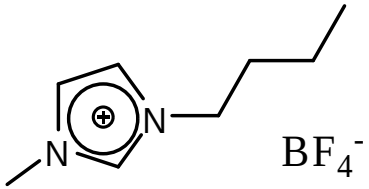
MMIM $MeSO_4$



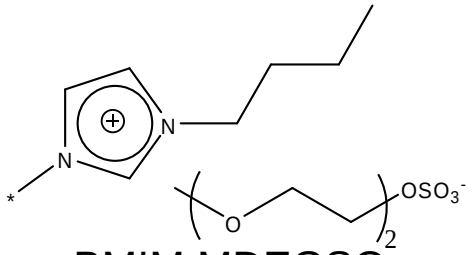
MMIM Me_2PO_4



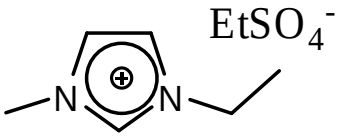
BMIM $MeSO_4$



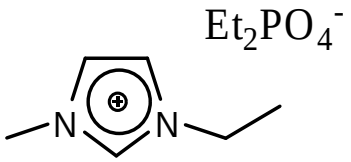
BMIM BF_4



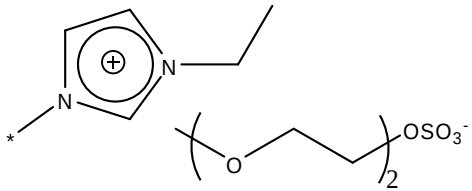
BMIM $MDEGSO_4$



EMIM $EtSO_4$

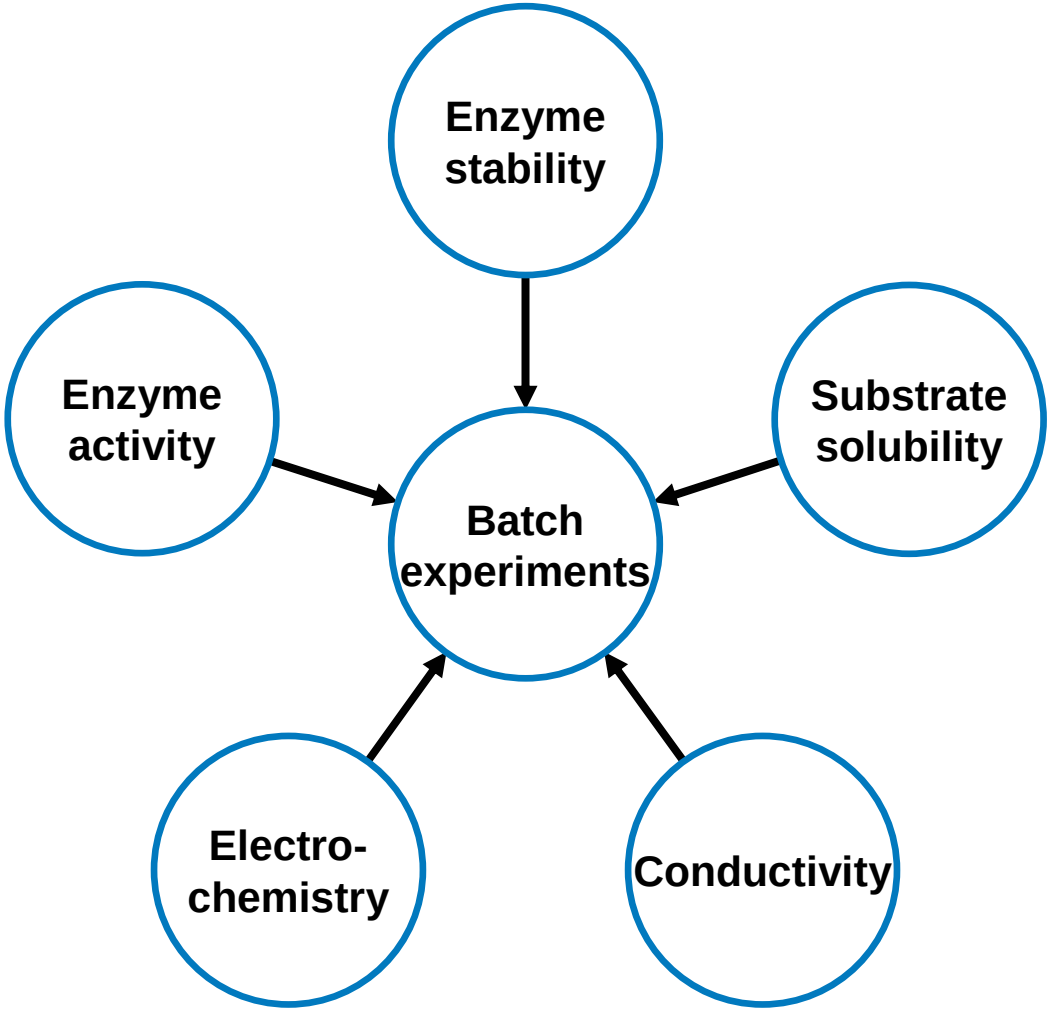


EMIM Me_2PO_4

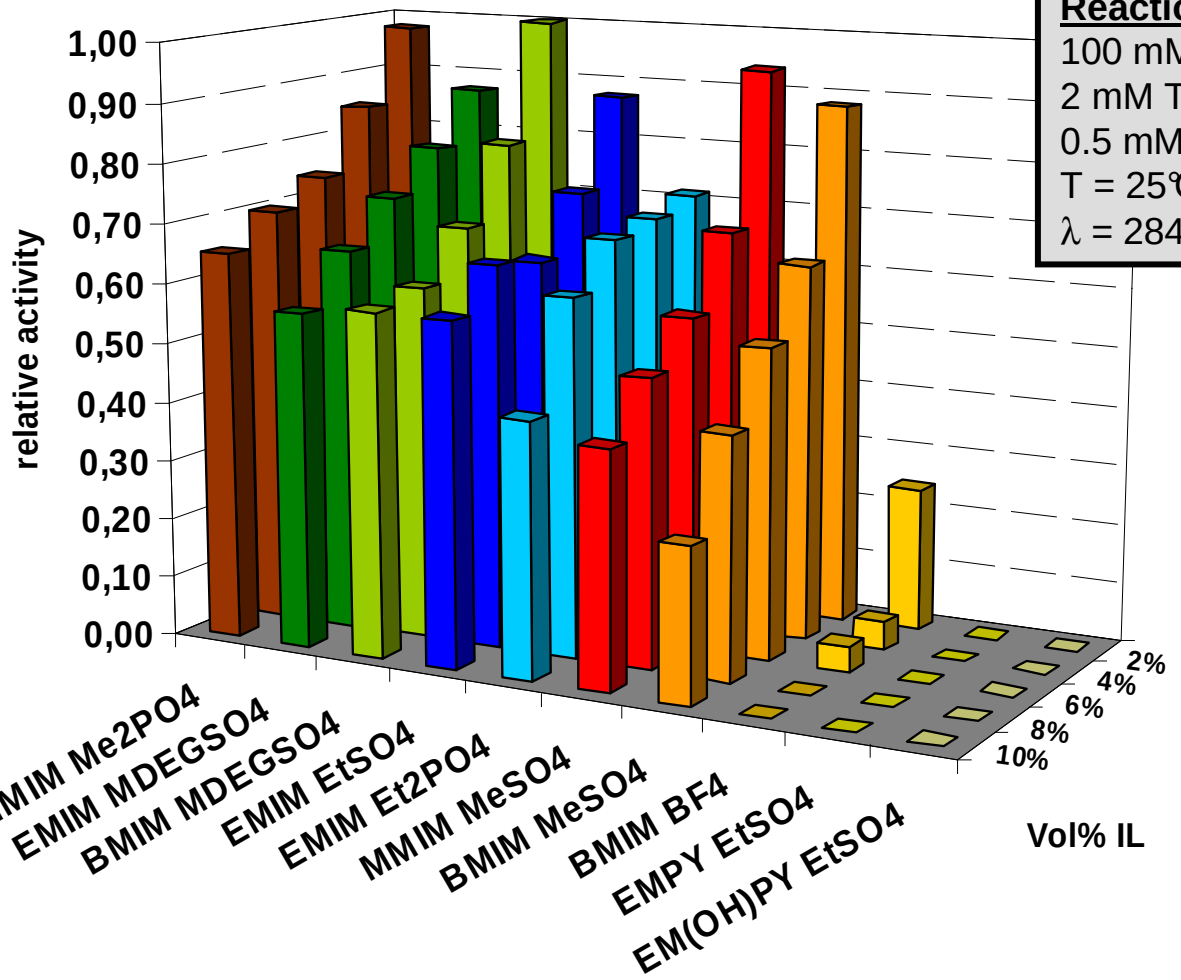


EMIM $MDEGSO_4$

Reaction systems

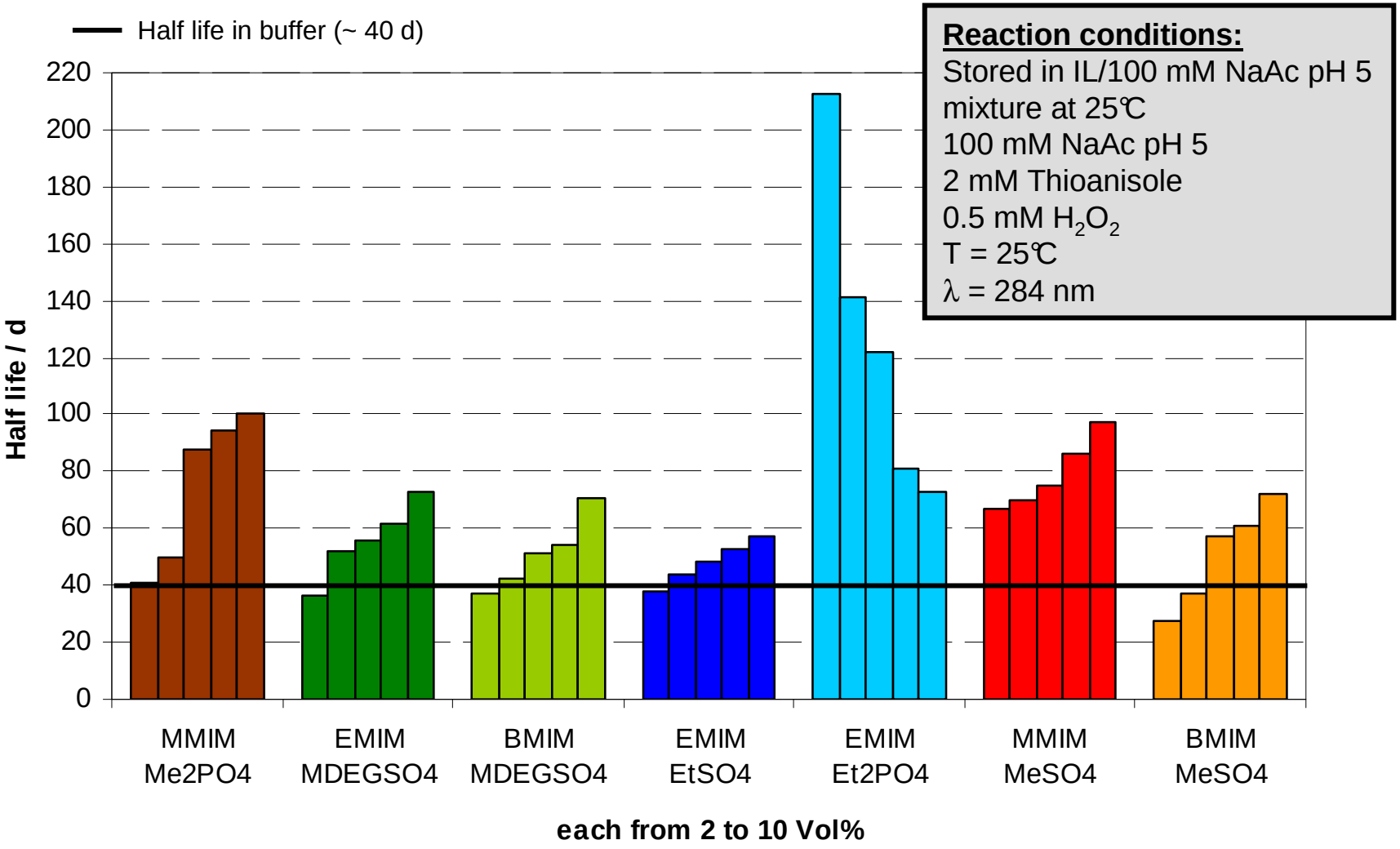


Activity of CPO in ILs

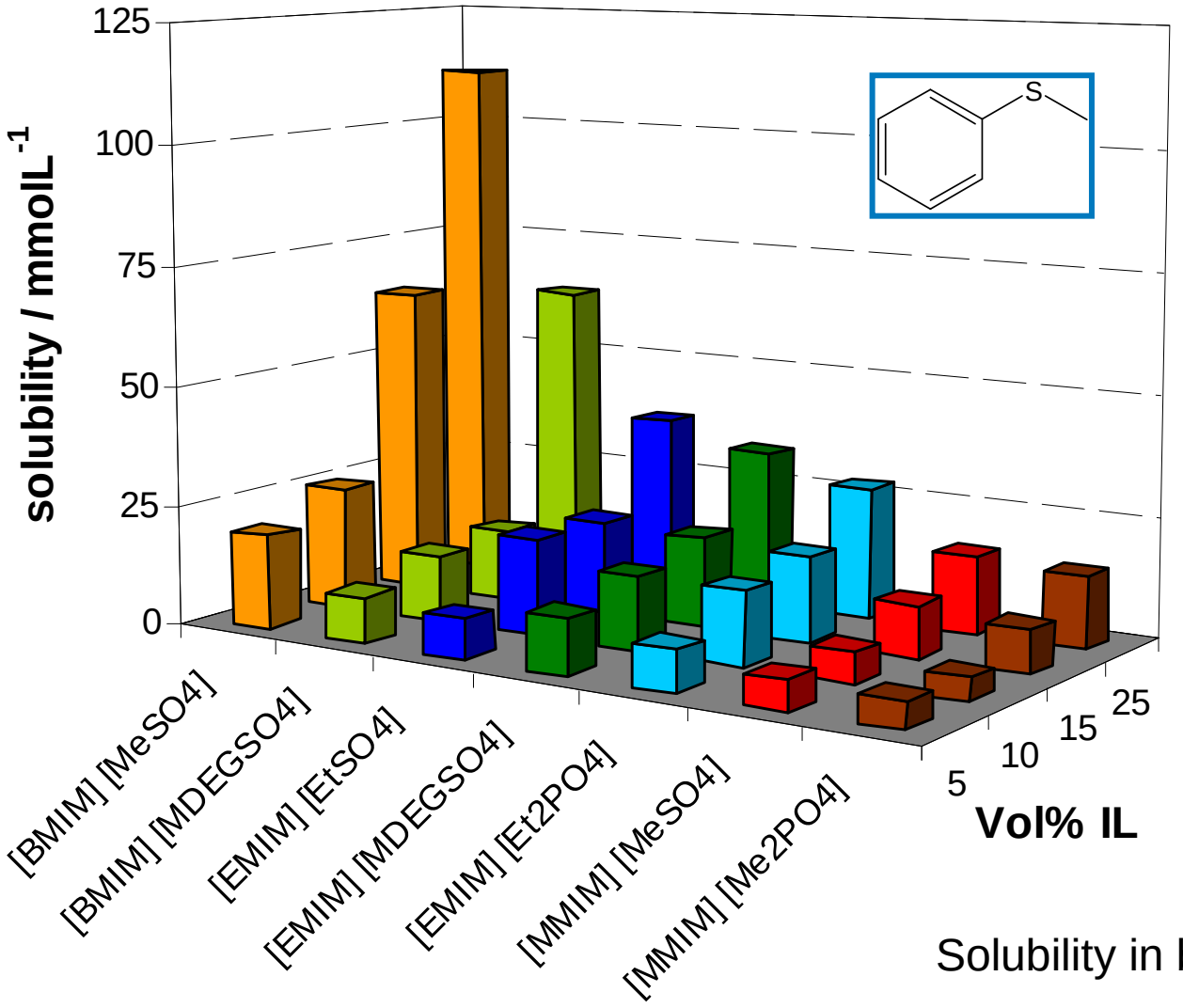


Reaction conditions:
100 mM NaAc pH 5
2 mM Thioanisole
0.5 mM H_2O_2
T = 25℃
λ = 284 nm

Stability of CPO in ILs



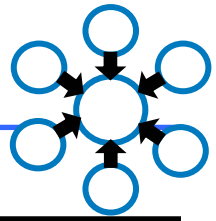
Substrate solubility with ILs

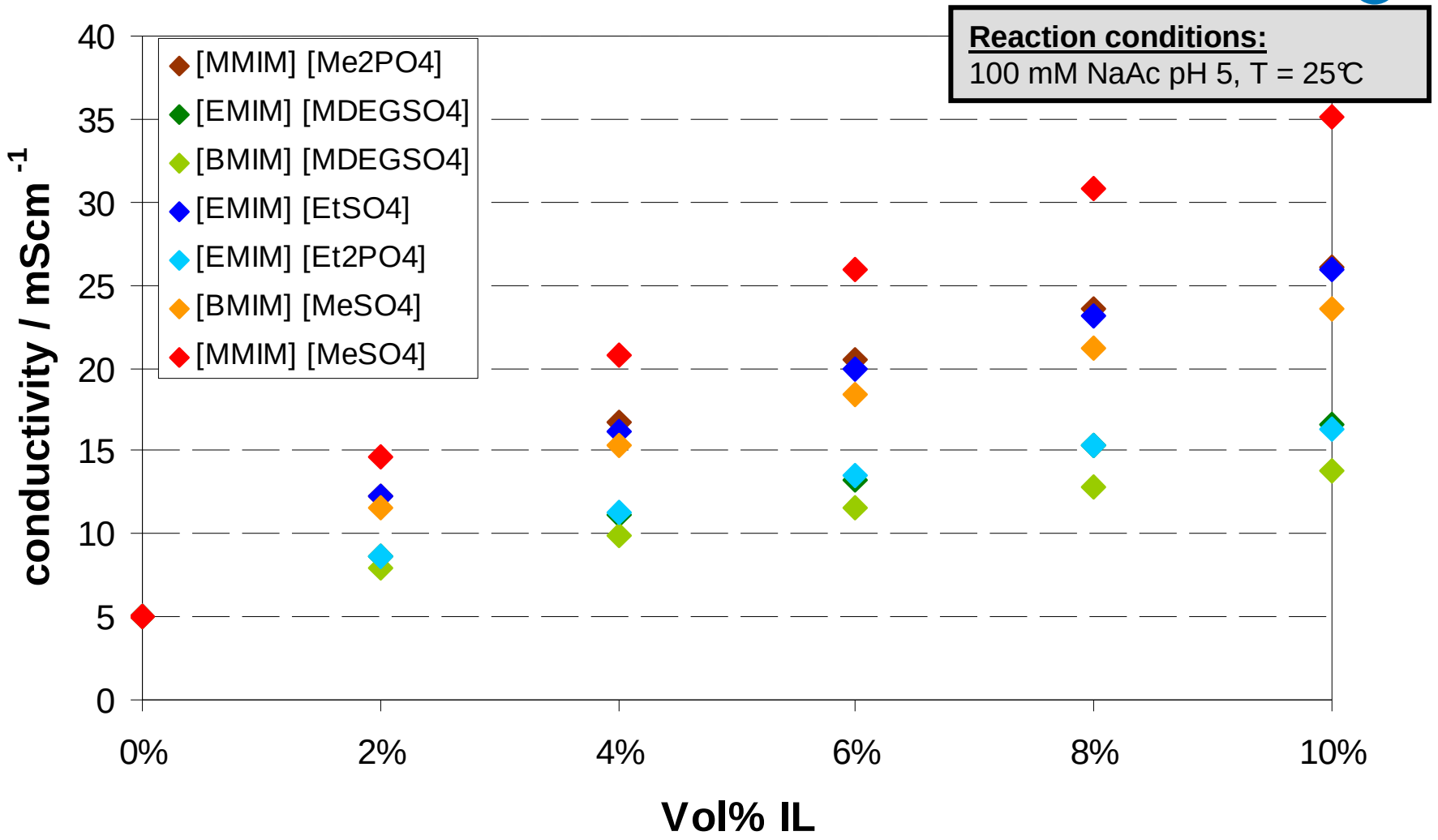


Reaction conditions:
100 mM NaAc pH 5
T = 25℃
Measured by GC

Solubility in buffer ~ 3.8 $mmolL^{-1}$

Conductivity with ILs





Electrochemical investigations

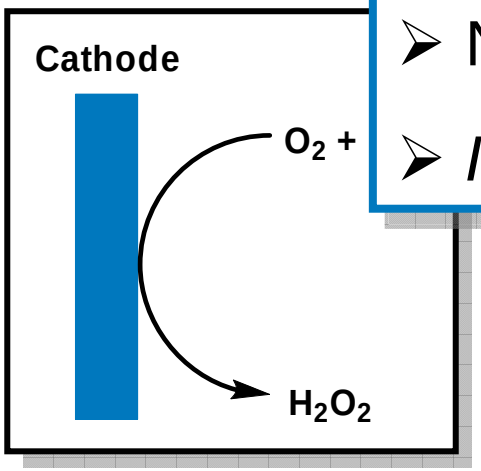
No current ⇨ Substrate, product & enzyme are suitable for the reaction!

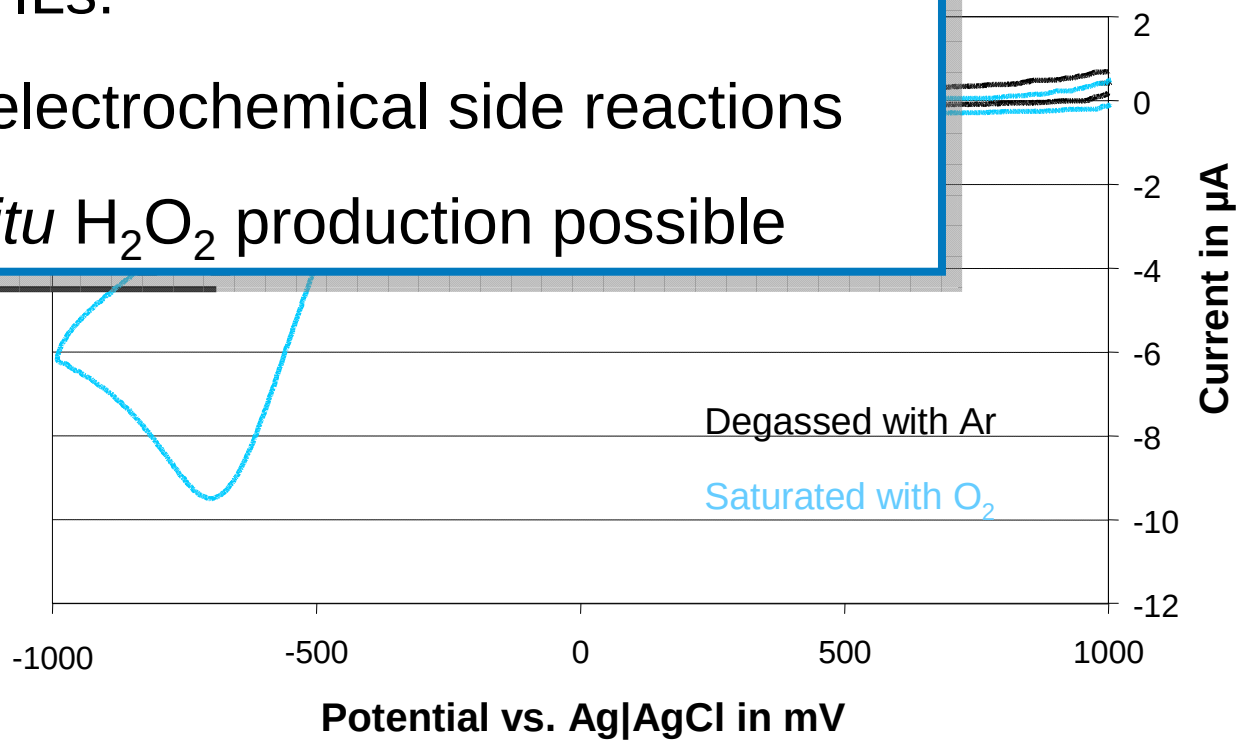
Reaction Conditions:
- 20 mL buffer pH 5
  90 V% 100 mM NaAc 50 mM $NaSO_4$
  10 V% *tert*-Butanol
- 0.4 mmol substrate or product
  or 20 µL enzyme

For all ILs:

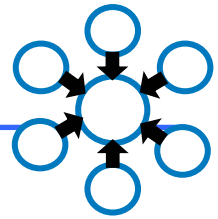
➢ No electrochemical side reactions

➢ *In situ* H_2O_2 production possible





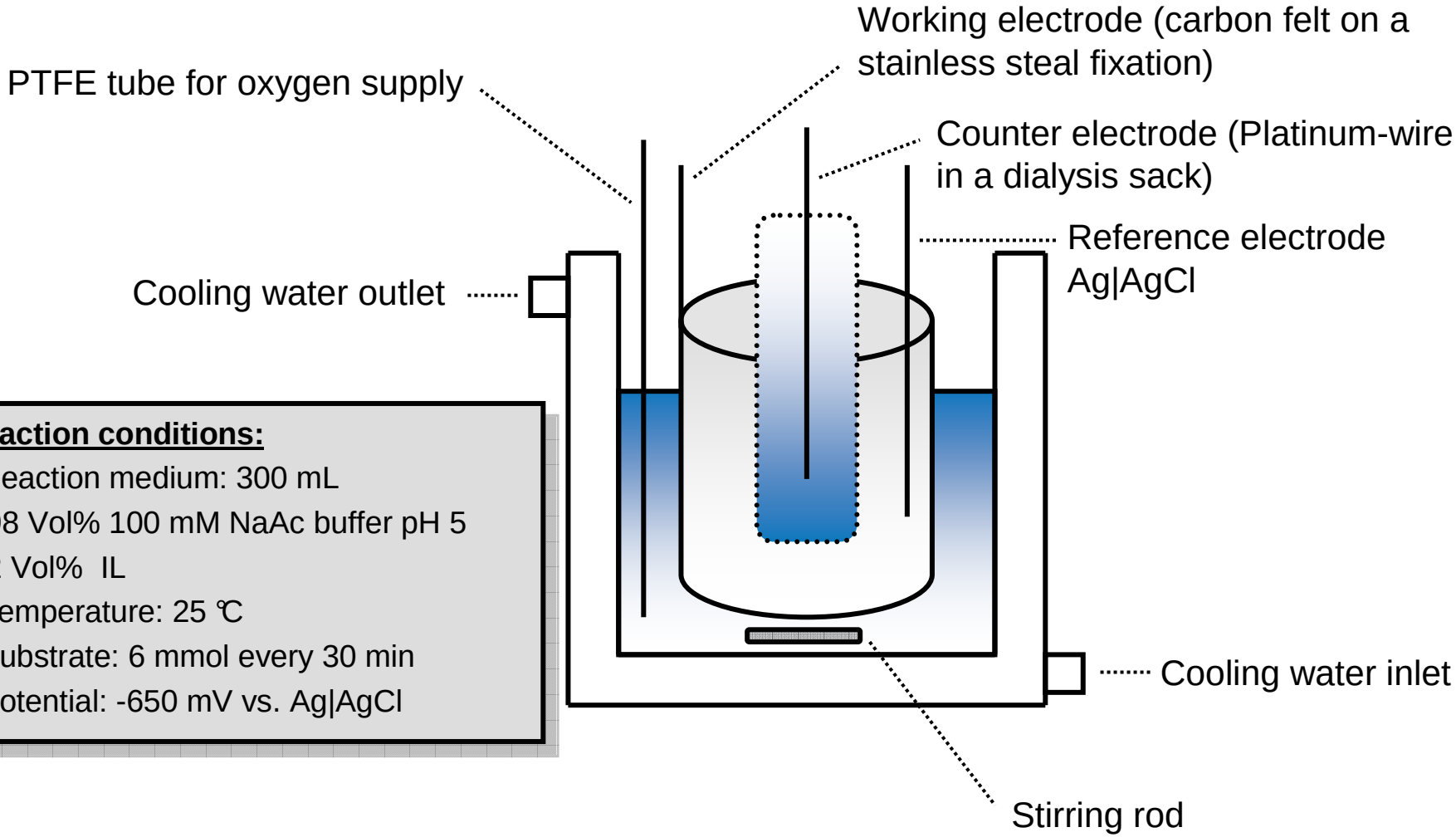
Summary of the preinvestigations



- CPO activity – fast decrease with higher amounts of IL
- CPO stability – in most cases increased
- Solubility increases
- Conductivity increases
- *In situ* H_2O_2 production is possible

- **Synthesis with very small amounts of ILs (⇨ 2 Vol%)**

Batch experiments

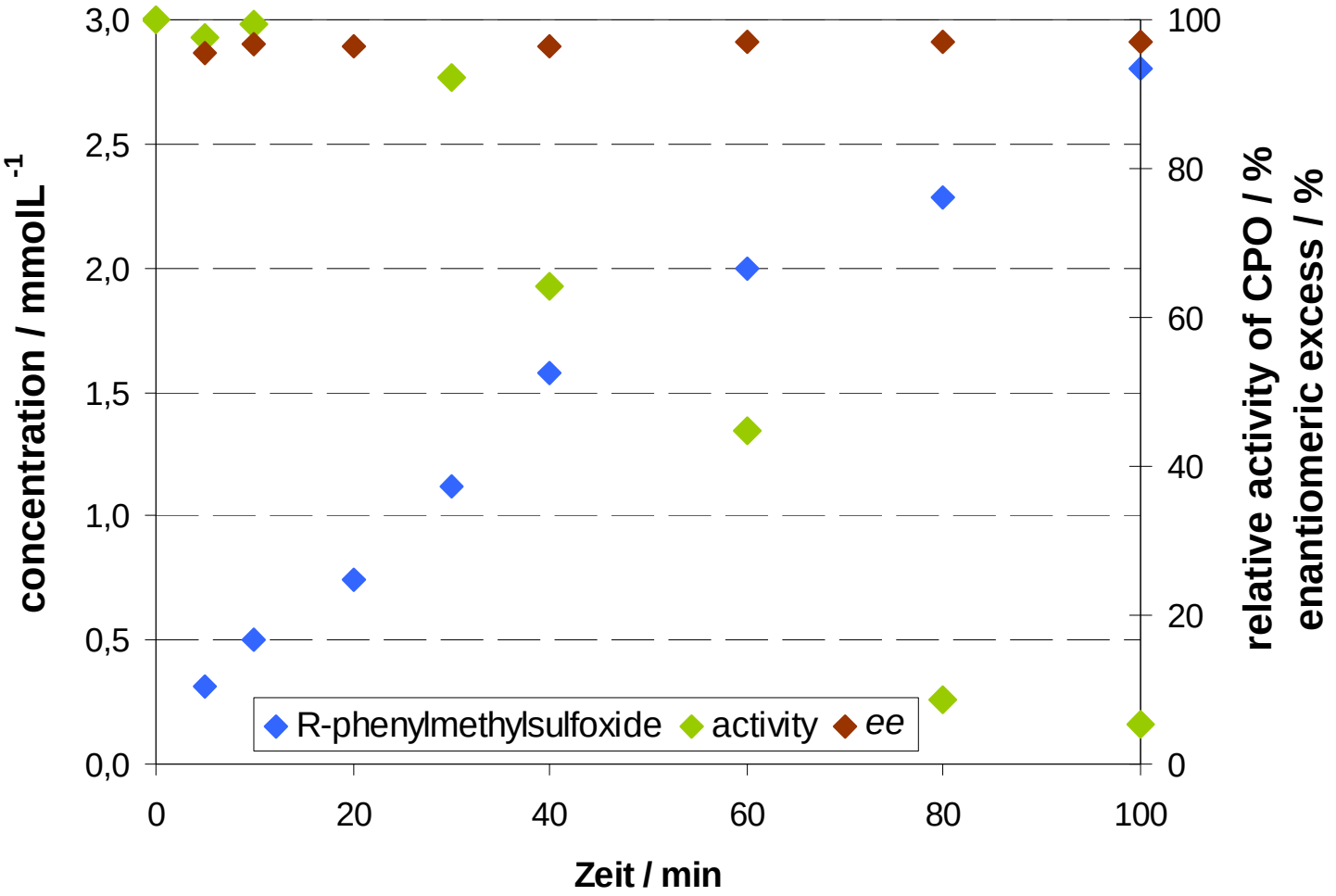


Reaction conditions:

- Reaction medium: 300 mL
  98 Vol% 100 mM NaAc buffer pH 5
  2 Vol% IL
- Temperature: 25 ℃
- Substrate: 6 mmol every 30 min
- Potential: -650 mV vs. Ag|AgCl

Batch experiments

Typical reaction progress:



Batch experiments

IL	Vol%	STY [$gL^{-1}d^{-1}$]	*ee* [%]	$ttn = \frac{n_{product}}{n_{catalyst}}$
In buffer	-	17.9	97	33000
S. Lütz, E. Steckhan, et al. (2004), Electrochemistry Communications **6**(6): 583-587.	30 Vol% *tert*-Butanol + Na_2SO_4	30.0	98.5	95000
MMIM Me_2PO_4	2	34.9	98	122000
BMIM $MDEGSO_4$	2	41.5	97	78000
MMIM $MeSO_4$	2	45.5	96	106000
BMIM $MeSO_4$	2	67.5	98	**138000**
EMIM $EtSO_4$	2	**75.4**	95	123000

STY = space time yield **_ee_ = enantiomeric excess** **_ttn_ = total turnover number**

Biocatalysis in the presence of ILs

Challenges

- Structure-properties-relationship

- IL requirements
  - Synthesise ILs with high purity
  - Produce less expensive ILs

- Develop effective methods for
  - Product recovery
  - Catalyst recycling
  - IL recycling

Biocatalysis in the presence of ILs

Perspective

- Improved biocatalyst performance
- Broadened substrate spectrum
- Novel reactor concepts
  - 2-phase or even 3-phase-systems
  - *In situ* product recovery by scF
- Less effort for safety installations

Not one single IL for all problems!
IL must be selected depending on the problem to be solved.

Acknowledgement

- Research Centre Jülich
  - Prof. Dr. Wandrey
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Thanks for your attention!!!