DECHEMA FORSCHUNGSINSTITUT

Chemical Technology

Photocatalytic *in situ* hydrogen peroxide production for biocatalysis based on peroxidases

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Motivation

The use of enzymes such as oxidoreductases in organic synthesis offers highly selective, efficient and therefore sustainable chemical processes. However, industrial utilization of oxidoreductases is often limited by their requirement of expensive cofactors.
Peroxidases have the advantage that their cofactor, hydrogen peroxide, is economically priced and can be easily synthesized. The challenge for utilization of peroxidases is their poor stability against their own cofactor, hydrogen peroxide.
This problem can be overcome by employing *in situ* generation techniques that guarantee a constant but low hydrogen peroxide concentration. However, the state of the art methods for achieving this all have certain disadvantages of their own, a satisfactory solution has yet to be found. Heterogeneous photocatalysis will be studied here as a possible alternative to previous methods that could overcome their drawbacks.

Previous H_2O_2 supply methods for peroxidases

method	additional needs	max. TTN	disadvantage
stoichiometrically [1]	-	4,9k	high H_2O_2 concentration
stepwise / continuous dosing [2]	pump system	41k / 108k	H_2O_2 concentration (hot spots)
sensor controlled dosing [2]	on-line analytics, pump system	148k	H_2O_2 concentration (hot spots)
enzymatically (GOD) [3]	2nd enzyme + cofactor, electron donor	250k	side product
electrochemically [4]	Electrodes, current	1150k	modified reactor system
photocatalitically (homogeneneous) [1]	light, electron donor	22,4k	cosubstrate for regeneration and difficult seperation of catalyst

Aim

The aim of the project is to provide the peroxidase with ideal and therefore mild concentration of hydrogen peroxide to raise the stability of the enzyme and thereby TTN (total turnover number) of the reaction. For this purpose, the photocatalytic generation of hydrogen peroxide by titanium dioxide and other photocatalysts (e.g. Fe_2O_3, ZnO) will be explored and optimized under different conditions. Afterwards, this process will be combined with the peroxidase to form an integrated photobiocatalytic process (*cf.* Figure 1).

Advantages of this system are the homogeneous allocation of hydrogen peroxide within a set concentration regime and avoidance of detrimental concentration peaks. Also, no additional reagents are needed and separation of the catalyst by filtration is trivial. The only energy source needed is light, which is provided contactless from outside the reactor.

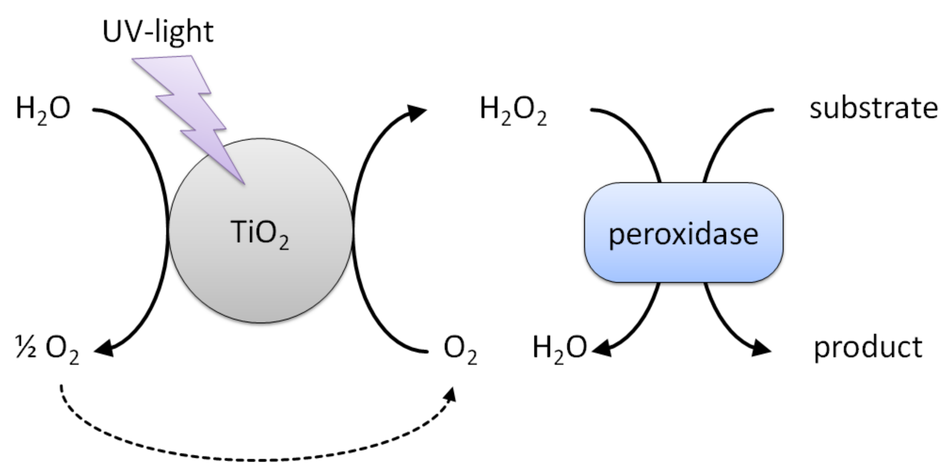

Figure 1: Reaction scheme for the photobiocatalytic synthesis of various products via *in situ* generation of H_2O_2 followed by peroxidase-catalysis.

H_2O_2-build-up with TiO_2 photocatalysis

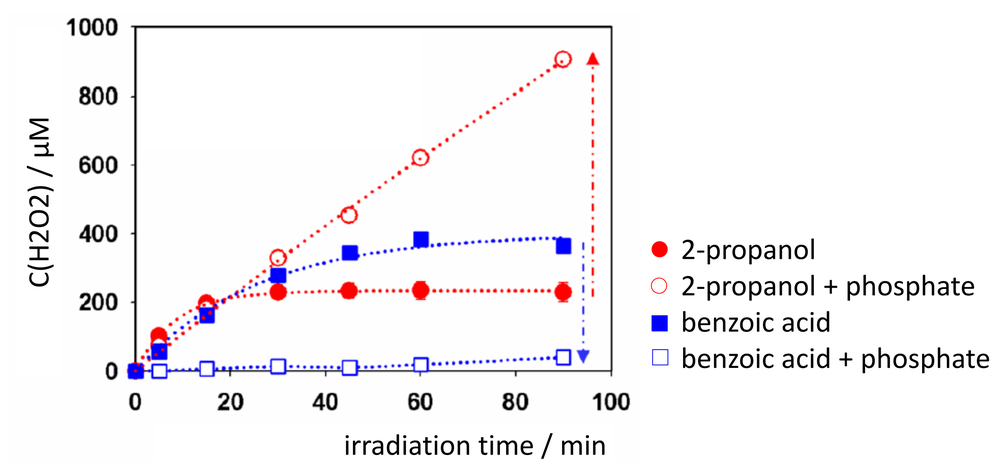

Figure 2: Photocatalytic H_2O_2 production depending on the presence of phosphate and electron donors. (λ≥320 nm, O_2-equilibrated, 0.5 g/l TiO_2, pH = 3.0, 0.1 M phosphate, 5 vol% 2-propanol or 0.5 mM benzoic acid) [5]

Preliminary results / proof of principle

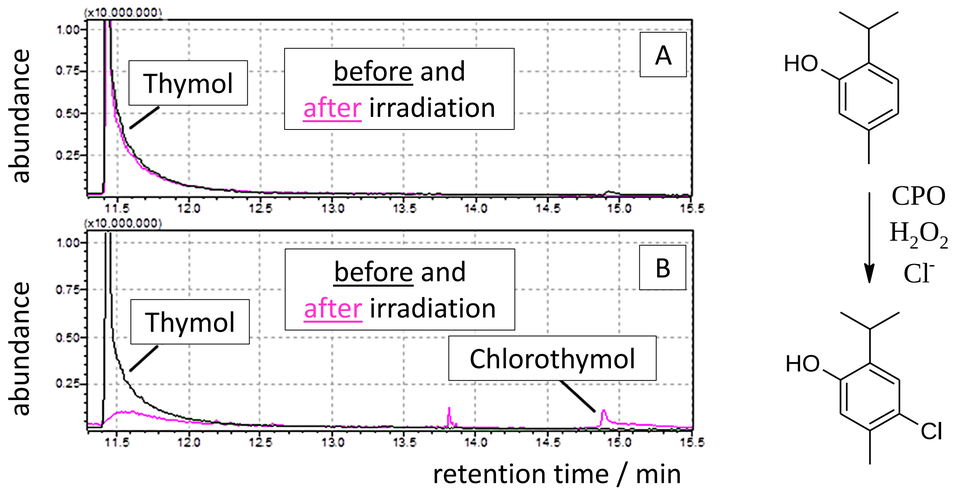

Figure 3: GC-MS-data of the reaction from Thymol to Chlorothymol without (A) and with (B) titanium dioxide. (4 U/ml chloroperoxidase, 100 mM acetate-buffer pH = 3.7, 50 mM phosphate, 25 mM , 0,5 g/l TiO_2 (only B), 300 min UVA-irradiation: 105W lamp with λ_{peak} = 365 nm)

Outlook

- Study of the photocatalytic H_2O_2-build-up under different conditions (catalyst, pH, T, light intensity, solvent)
- Optimizing the *in situ* H_2O_2 generation for the reaction of thioanisole to *(R)*-methylphenylsulfoxide catalyzed by CPO in a batch system
- Getting from a batch system to a continuous process

References

[1] D. I. Perez, M. M. Grau, I. W. C. E. Arends, F. Hollmann, *Chem. Comm.*, **2009**, 6848-6850.
[2] J. D. Leak, R. A. Sheldon, J. M. Woodley, P. Adlercreutz, *Biocatal. Biotransformation*, **2009**, 1-26.
[3] F. Van de Velde, F. Van Rantwijk, R. A. Sheldon, *Trends Biotechnol.*, **2001**, 73-80.
[4] D. Holtmann, T. Krieg, L. Getrey, J. Schrader, *Catalysis Communications*, **2014**, 82-85.
[5] G. Moon, W. Kim, A. D. Bokare, N. Sung, W. Choi, Energy Environ. Sci., **2014**, 4023, SI.