A COMPARTMENTED BIOCATALYTIC CASCADE REACTION WITH CONTRARY REACTION CONDITIONS

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Introduction

Enzymes are very versatile catalysts that catalyze selectively a vast variety of reactions under typically mild reaction conditions.[1,2] Since enzymes often operate under similar reaction conditions their simultaneous use in multistep reactions is obvious.[3] Such reactions cascades provide several advantages[4], eg. intermediate products do not need to be isolated, toxic concentrations of intermediates are prevented and thermodynamically unfavored reactions with low equilibrium conversions can be pushed to the product side.

In this study we present a conceptional design for an orthogonal cascade reaction that consists of contrary reaction conditions. Two enzymes (alcohol dehydrogenase evo-1.1.270 and the hydroxynitrile lyase from *Manihot esculenta*, *Me*HNL) were localized in specifically chosen reaction compartments that contain optimal but different pH. These compartments were suspended in an external solvent, which allowed the reactants to diffuse relatively freely between the applied compartments to achieve optimal reactivity.

Results

Incompatibility of reaction conditions

Alcohol dehydrogenases and hydroxynitrile lyase are typically considered as incompatible due their very different reaction requirements (Figure 1). While hydroxynitrile lyases are used at low pH to prevent product decomposition or racemization[5], alcohol dehydrogenase-catalyzed reductions are usually performed at neutral pH and moderate reaction temperatures.

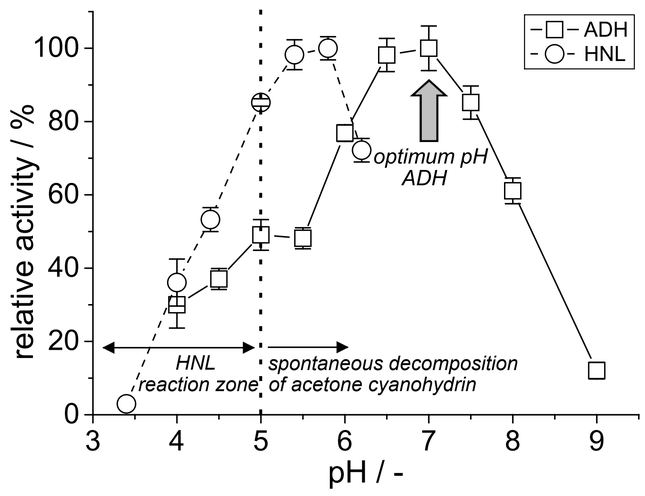

Figure 1: Alcohol dehydrogenase evo-1.1.270 and hydroxynitrile lyase *Me*HNL exhibit very different reaction requirements

Compartmentation of biocatalysts

Evo-1.1.270 and *Me*HNL were each entrapped as enzyme solution in aqueous domains in silicone or polyurethane (PUR) polymer matrices, which also include buffer salts and cofactor $NADP^+$/NADPH (in case of the alcohol dehydrogenase). (Figure 2; b, c).[6]

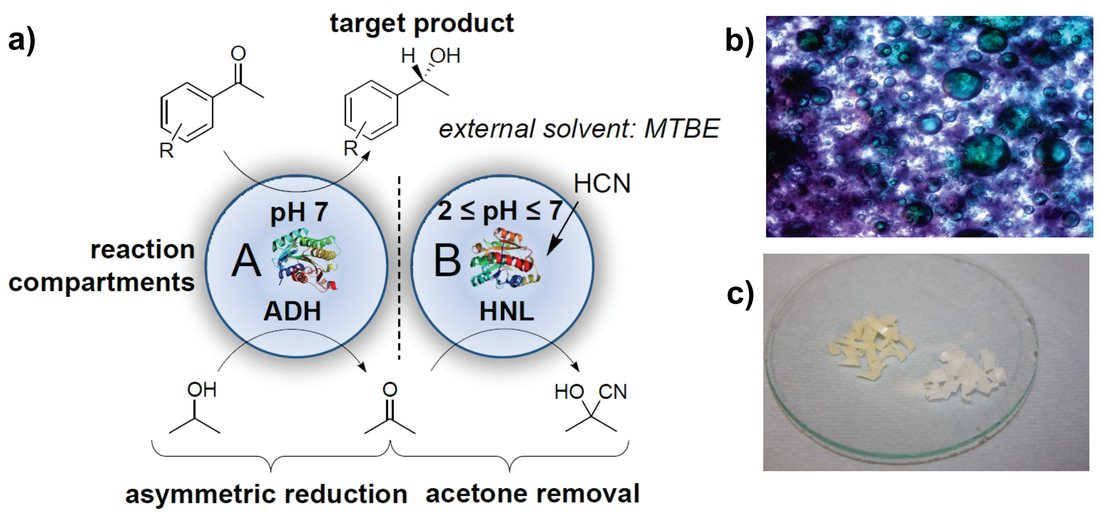

Figure 2: a) cascade reaction with two reaction zones; compartment A: alcohol dehydrogenase; compartment B: hydroxyntitrile lyase; **b)** microscopic image of compartments (purple-green) **c)** PUR based *Me*HNL (yellow) and evo-1.1.270-compartments (white), particle size: 1-2 mm

In reaction compartment A the prochiral ketone in converted to the corresponding (*R*)-alcohol. The oxidized cofactor $NADP^+$ is regenerated to NADPH + H^+ by a classical substrate-coupled approach with 2-propanol yielding acetone. Acetone is then further converted to acetone cyanohydrin by *Me*HNL in reaction compartment B (Figure 2; a). The reactants diffuse from the surrounding solvent through the polymer matrix into the compartments and in a similar way back into the external solvent. The shown three phase reaction represents a diffusion barrier.

Compartmented cascade reaction

As shown in Figure 2 the single compartmentation of the ADH-catalyzed reaction (pH 7) yields a conversion of only 56% (filled triangle). The addition of the second hydroxynitrile lyase reaction compartment (pH 3), which removes *in situ* byproduct acetone, facilitates an increase of overall conversion to 88%.

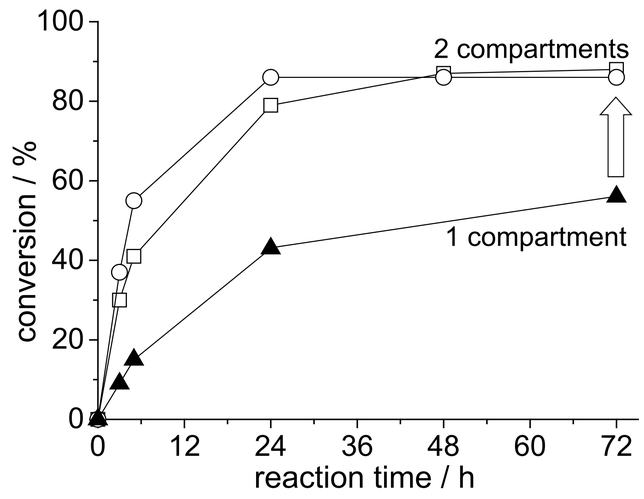

Figure 2: evo-1.1.270-*Me*HNL cascade reaction with one or two compartmented reaction zones; open circle: silicone-based compartments, open squares: polyurethane-based compartments

The ratio of prochiral ketone and 2-propanol affects the equilibrium conversion within the cascade reaction (Table 1). Higher 2-propanol concentrations facilitate an increase of conversion to (*R*)-1-phenylethanol. The highest conversion was found with an acetophenone : 2-propanol-ratio of 1 : 10.

Table 1: 5 ml MTBE, 10 mM acetophenone, [a]400 mg evo-1.1.270-PUR-preparation, 30 °C; [b]evo-1.1.270-*Me*HNL-PUR-preparation + 200 mM hydrogen cyanide, 21 °C, conversion after 3d

Ratio acetophenone: 2-propanol	conversion [%][a] only evo-1.1.270	conversion [%][b] evo-1.1.270-*Me*HNL-cascade
1:1	30	49
1:2	39	55
1:3	42	62
1:5	45	77
1:10	56	88
1:15	54	85

Summary

- Successful combination of two enzymes with contrary reaction requirements by an independent localization in polymer compartments.
- Increase of overall conversion by *in situ* byproduct removal (acetone cyanohydrin).
- **Detailed results:**

  D. Uhrich, J. C. Peinemann, S. Wapenhensch, J. von Langermann "Conceptual Design of a Compartmented Biocatalytic Cascade Reaction with Contrary Reaction Requirements", *submitted*

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