

# Hydroxynitrile lyase-catalyzed Synthesis of Enantiopure Cyanohydrins in Biocatalytic Active Static Emulsions (BASE)

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## Introduction

Hydroxynitrile lyases (HNLs) catalyze selectively the C-C-coupling of hydrogen cyanide to carbonyl compounds yielding enantiopure cyanohydrins.<sup>[1-3]</sup> Due to the formation of two new functional groups (one hydroxyl and one nitrile group) these substances are considered as valuable intermediates for a variety of building blocks, including  $\alpha$ -hydroxy carboxylic acids and  $\alpha$ -aminonitriles.<sup>[4, 5]</sup> Unfortunately, the selective enzymatic reactions overlays with an undesired spontaneous reaction, which yields only a racemic product (Figure 1). Thus a high enantiomeric

excess can only be achieved if the spontaneous reaction is effectively suppressed.<sup>[1]</sup>

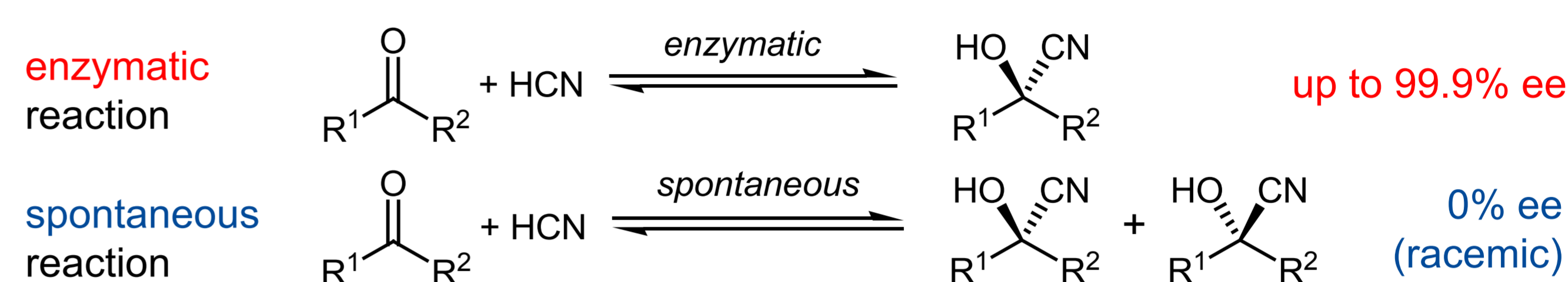


Figure 1. Enzymatic and spontaneous cyanohydrin synthesis

## Results

### Biocatalytic active static emulsions (BASE)

In this study we present the application of the hydroxynitrile lyase from *Manihot esculenta* (MeHNL) in biocatalytic active static emulsions (BASE) to suppress the spontaneous reaction. Biocatalytic active static emulsions consists of a hydrophobic matrix, in this study polydimethylsiloxane (PDMS), with dispersed domains of an aqueous phase (Figure 2).

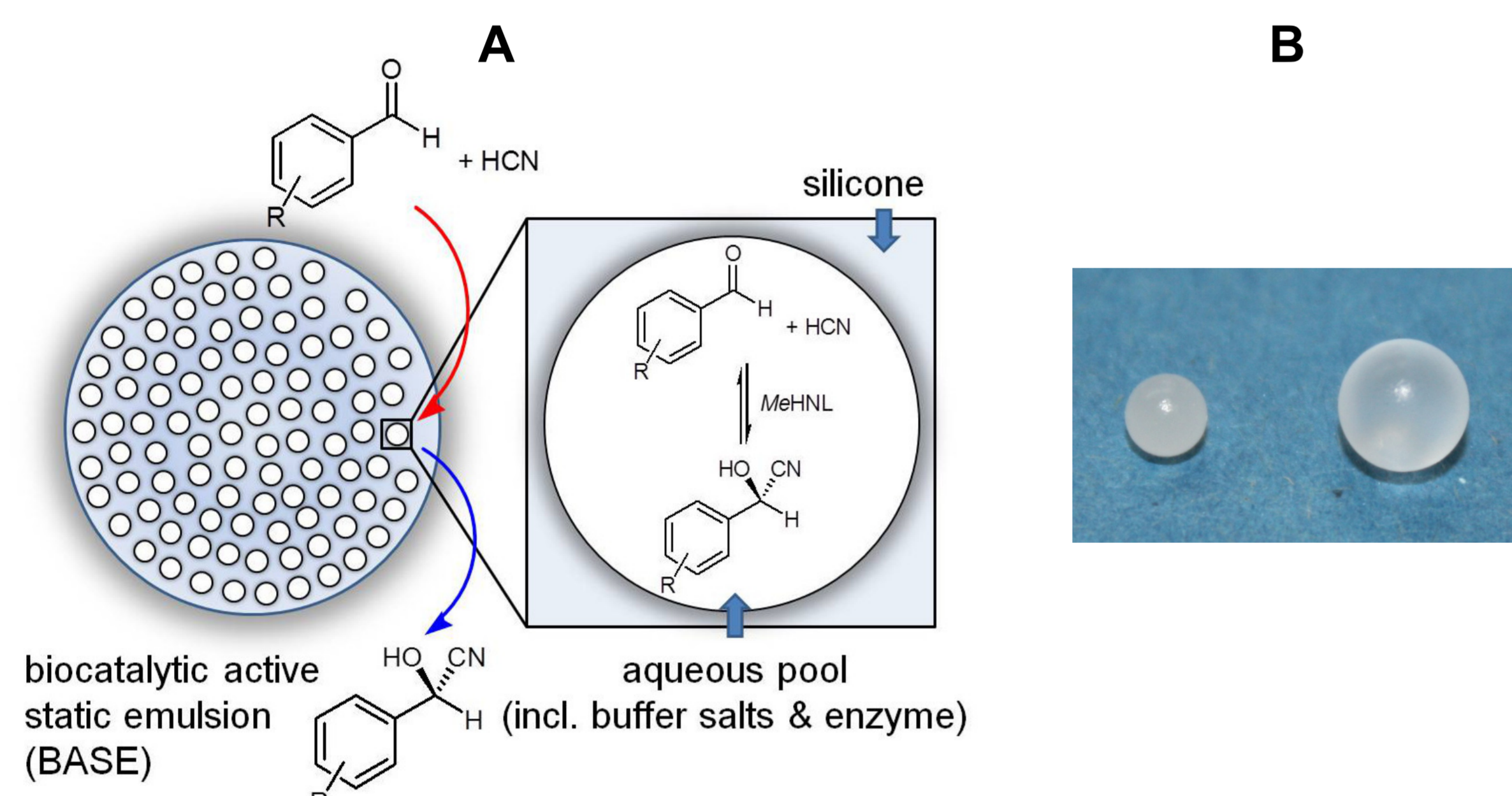


Figure 2. Schematic representation of the HNL-catalyzed synthesis of cyanohydrins in BASE within an organic solvent (A); swelling of BASE-MeHNL in diisopropylether (B).

The water domains represent small, independent water droplets (aqueous pools) that are scattered within the hydrophobic matrix and contain, besides water, the required buffer salts and MeHNL. During the time course of the reaction both substrates (carbonyl compound and hydrogen cyanide) diffuse through the hydrophobic matrix along the concentration gradient into the aqueous pools. The substrates are subsequently converted to the respective products, herein cyanohydrins, which diffuse in a similar manner out of the BASE spheres.

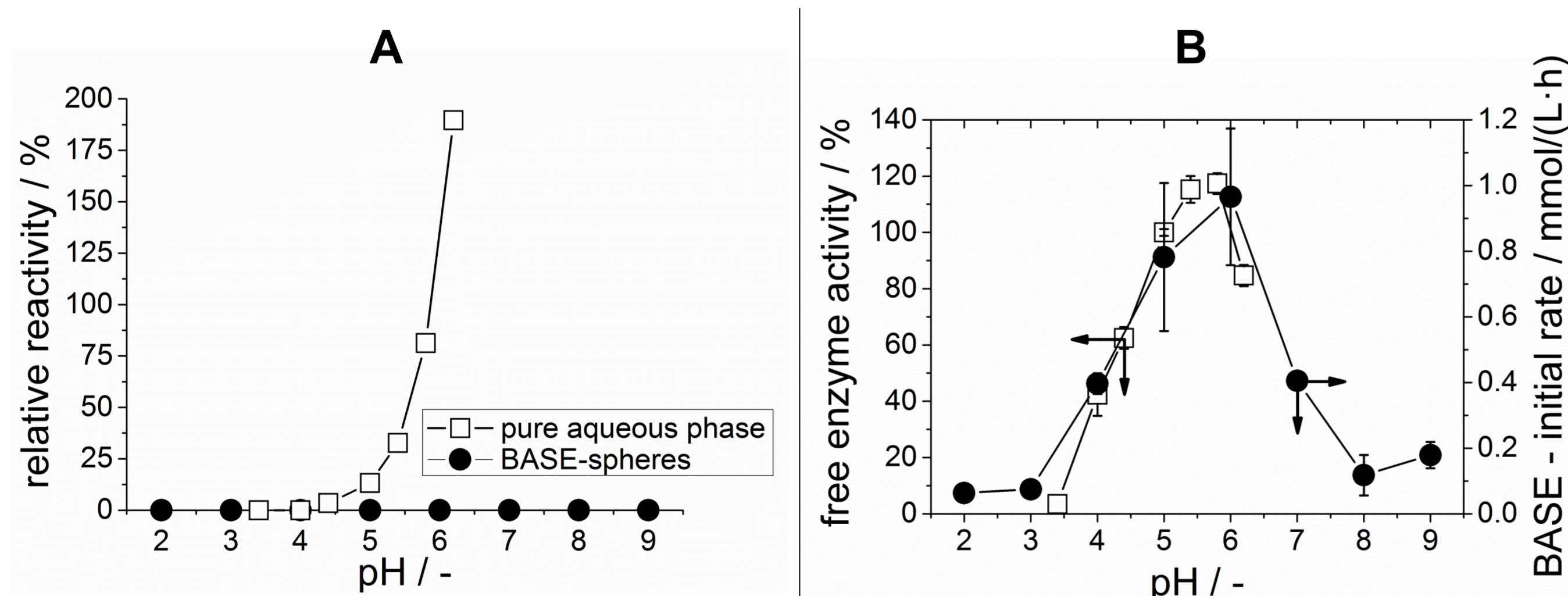


Figure 3. Comparison of the non-enzymatic reaction in pure aqueous phase and (unloaded) silicone spheres (A); Comparison of the pH-dependency of free (dissolved) MeHNL and immobilized) BASE-MeHNL (B); 100% represent enzyme activity at pH 5.<sup>[6]</sup>

As shown in Figure 3A, the application of BASE yields a total reduction of the undesired spontaneous reaction, even at a very unusual high pH. Consequently the entire pH range from 2 to 9 is now accessible for enantioselective catalysis, including its pH-optimum at pH 6 (Figure 3B).

### Synthesis of enantiopure cyanohydrins

The enantioselective synthesis of cyanohydrins was investigated at a series of different substrates, incl. benzaldehyde, halogen-substituted benzaldehyde derivatives and aliphatic methyl ketones. High yields with enantiomeric excess' of up to >99.5 % (S) were obtained (Table 1).

Table 1. BASE-MeHNL catalyzed synthesis of cyanohydrins

R <sup>1</sup>	R <sup>2</sup>	time / h	conversion / %	ee (S) / %
C <sub>6</sub> H <sub>5</sub>	H	6	> 99.5	> 99.5
2-F-C <sub>6</sub> H <sub>5</sub>	H	3	96	> 99.5
3-F-C <sub>6</sub> H <sub>5</sub>	H	6	93	99
4-F-C <sub>6</sub> H <sub>5</sub>	H	22	96	> 99.5
2-Cl-C <sub>6</sub> H <sub>5</sub>	H	22	96	> 99.5
3-Cl-C <sub>6</sub> H <sub>5</sub>	H	22	96	96
4-Cl-C <sub>6</sub> H <sub>5</sub>	H	22	91	93
2-Br-C <sub>6</sub> H <sub>5</sub>	H	22	97	> 99.5
3-Br-C <sub>6</sub> H <sub>5</sub>	H	22	96	96
4-Br-C <sub>6</sub> H <sub>5</sub>	H	22	90	> 99.5
<i>n</i> -C <sub>5</sub> H <sub>11</sub>	CH <sub>3</sub>	22	65	80
<i>n</i> -C <sub>6</sub> H <sub>13</sub>	CH <sub>3</sub>	22	43	83
<i>n</i> -C <sub>7</sub> H <sub>15</sub>	CH <sub>3</sub>	22	13	84
<i>n</i> -C <sub>8</sub> H <sub>17</sub>	CH <sub>3</sub>	22	9	84

250 mg BASE-MeHNL spheres (50 mM citrate phosphate buffer pH 6), 10 °C, 50 mM substrate, 250 mM hydrogen cyanide, 5 mL total volume.

BASE-immobilized MeHNL was also tested at larger scale (2 to 50 g) to show its synthetic practicability (results not shown here). Selected enantiomerically enriched cyanohydrins were hydrolyzed to the corresponding synthetically valuable mandelic acid derivatives and crystallized to improve the ee to an enantiopurity of >99 % e.e.(S).

## Summary

- The hydroxynitrile lyase from *Manihot esculenta* was immobilized in biocatalytic active static emulsions (BASE), which effectively suppresses the spontaneous reaction.
- High yields and ee were obtained with benzaldehyde, benzaldehyde derivatives and selected aliphatic ketones.
- Detailed information:  
**J. von Langermann and S. Wapenhensch**  
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