-Supporting Information-

Probing batch and continuous flow reactions in dry solvents: *Granulicella tundricula* Hydroxynitrile lyase (*Gt*HNL)

José Coloma,[a,b] Yann Guiavarc'h,[a,c] Peter-Leon Hagedoorn,[a] and Ulf Hanefeld[a]

[a]Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

[b] Universidad Laica Eloy Alfaro de Manabí, Avenida Circunvalación s/n, P.O. Box 13-05-2732, Manta, Ecuador

[c] Laboratory Reactions and Process Engineering, University of Lorraine, CNRS, LRGP, F-54000 Nancy, France

***Corresponding Author:** Ulf Hanefeld
Tel: +31(0)15-2789304; Fax: +31(0)15-2781415; Email: U.Hanefeld@tudelft.nl.
Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, Van der Maasweg 9, 2629 HZ Delft, The Netherlands

Table of contents

A. Gene and amino-acid sequences

GtHNL wild type – Gene sequence

5’CCATGGAGATTAAACGTGTTGGTTCTCAGGCTTCTGGTAAAGGTCCGGCTGATT
GGTTCACTGGTACTGTTCGTATCGATCCGCTGTTTCAGGCTCCGGATCCGGCATTA
GTAGCTGGTGCTTCTGTTACCTTTGAACCGGGTGCTCGTACTGCTTGGCATACTCA
TCCGTTAGGTCAGACTCTGATTGTAACTGCTGGTTGTGGTTGGGCTCAGCGTGAA
GGTGGTGCTGTTGAAGAAATTCATCCGGGTGATGTTGTATGGTTCTCTCCAGGTG
AAAAACACTGGCATGGTGCTGCACCAACTACCGCTATGACCCACCTGGCTATCCA
GGAACGTCTGGATGGTAAAGCTGTTGATTGGATGGAACACGTTACTGATGAACAG
TACCGTCGTTAAGCTT -3’

GtHNL wild type – Aminoacid sequence

M E I K R V G S Q A S G K G P A D W F T G T V R I D P L F Q A P D P A L V A G A S
V T F E P G A R T A W H T H P L G Q T L I V T A G C G W A Q R E G G A V E E I H P
G D V V W F S P G E K H W H G A A P T T A M T H L A I Q E R L D G K A V D W M
E H V T D E Q Y R R A

***Gt*HNL-A40H/V42T/Q110H**

5’CCATGGAGATTAAACGTGTTGGTTCTCAGGCTTCTGGTAAAGGTCCGGCTGATT
GGTTCACTGGTACTGTTCGTATCGATCCGCTGTTTCAGGCTCCGGATCCGGCATTA
GTAGCTGGTCACTCTACTACCTTTGAACCGGGTGCTCGTACTGCTTGGCATACTCA
TCCGTTAGGTCAGACTCTGATTGTAACTGCTGGTTGTGGTTGGGCTCAGCGTGAA
GGTGGTGCTGTTGAAGAAATTCATCCGGGTGATGTTGTATGGTTCTCTCCAGGTG
AAAAACACTGGCATGGTGCTGCACCAACTACCGCTATGACCCACCTGGCTATCCA
CGAACGTCTGGATGGTAAAGCTGTTGATTGGATGGAACACGTTACTGATGAACAG
TACCGTCGTTAAGCTT -3’

***Gt*HNL-A40H/V42T/Q110H – Aminoacid sequence**

M E I K R V G S Q A S G K G P A D W F T G T V R I D P L F Q A P D P A L V A G H S
T T F E P G A R T A W H T H P L G Q T L I V T A G C G W A Q R E G G A V E E I H P
G D V V W F S P G E K H W H G A A P T T A M T H L A I H E R L D G K A V D W M E
H V T D E Q Y R R A

Aminoacids in red show the mutations at positions 40, 42 and 110.

B. SDS-PAGE purified *Gt*HNL-A40H/V42T/Q110H

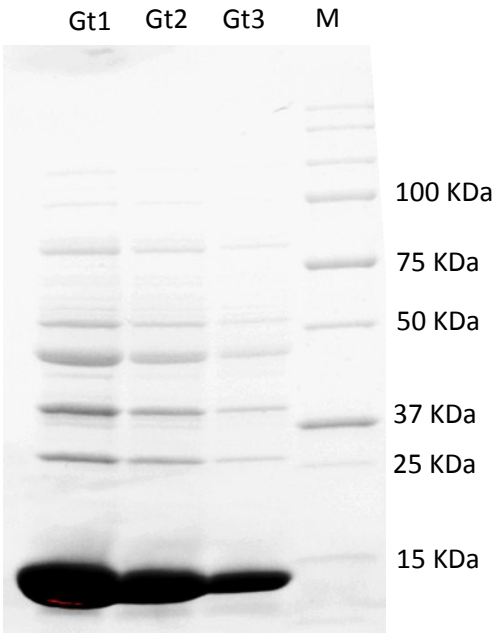

Figure S1. SDS PAGE of purified GtHNLA40H/V42T/Q110H. Sample M is the marker and G1 to Gt3 are different dilutions of purified *Gt*HNLA40H/V42T/Q110H

C. Calculation of reaction volume in continuous flow

Interbead volume (Non porous glass beads)

A commonly used inter-beads volume for non-porous beads of similar or close diameters even for well packed columns is between 30% and 40% of the total volume. Since 150 mg of glass beads correspond to 0.5 mL bulk volume, the inter-beads volume is about:

0.5 mL x 35% = 0.175 mL

Pore volume (Celite R-633)

According to El-Sayed[1], Celite R-633 has a total pore volume of 1.46 mL g^{-1}.

0.150 g Celite R-633 x 1.46 mL g^{-1} = 0.219 mL

Reaction volume = 0.219 mL + 0.175 mL = 0.394 mL

D. Progress of the synthesis of (*R*)-mandelonitrile with non-immobilized *Gt*HNL-TV

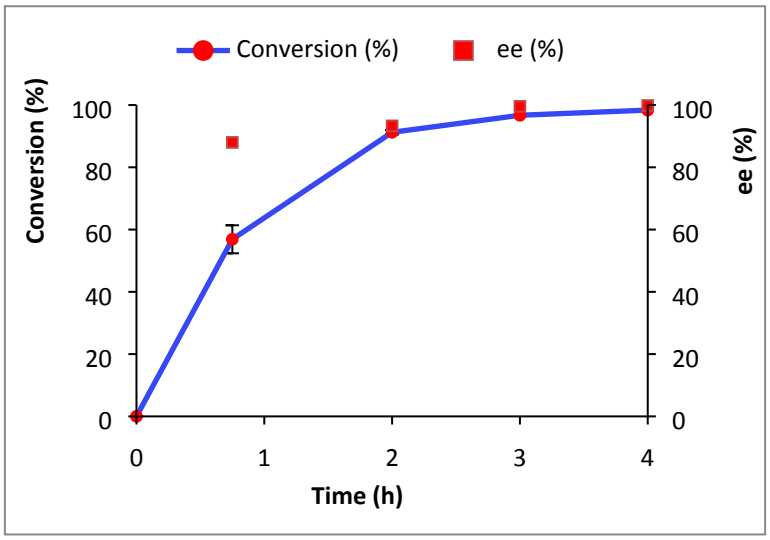

Figure S2: Kinetic trace for the synthesis of (*R*)-mandelonitrile with non-immobilized *Gt*HNL-TV. Conditions: Ratio benzaldehyde : HCN in acetate buffered MTBE, pH 4, 1:4, *Gt*HNL-TV (11 mg, 50 U). The reaction was stirred at 1000 rpm at 5 °C.

E. Benzaldehyde and HCN kinetics

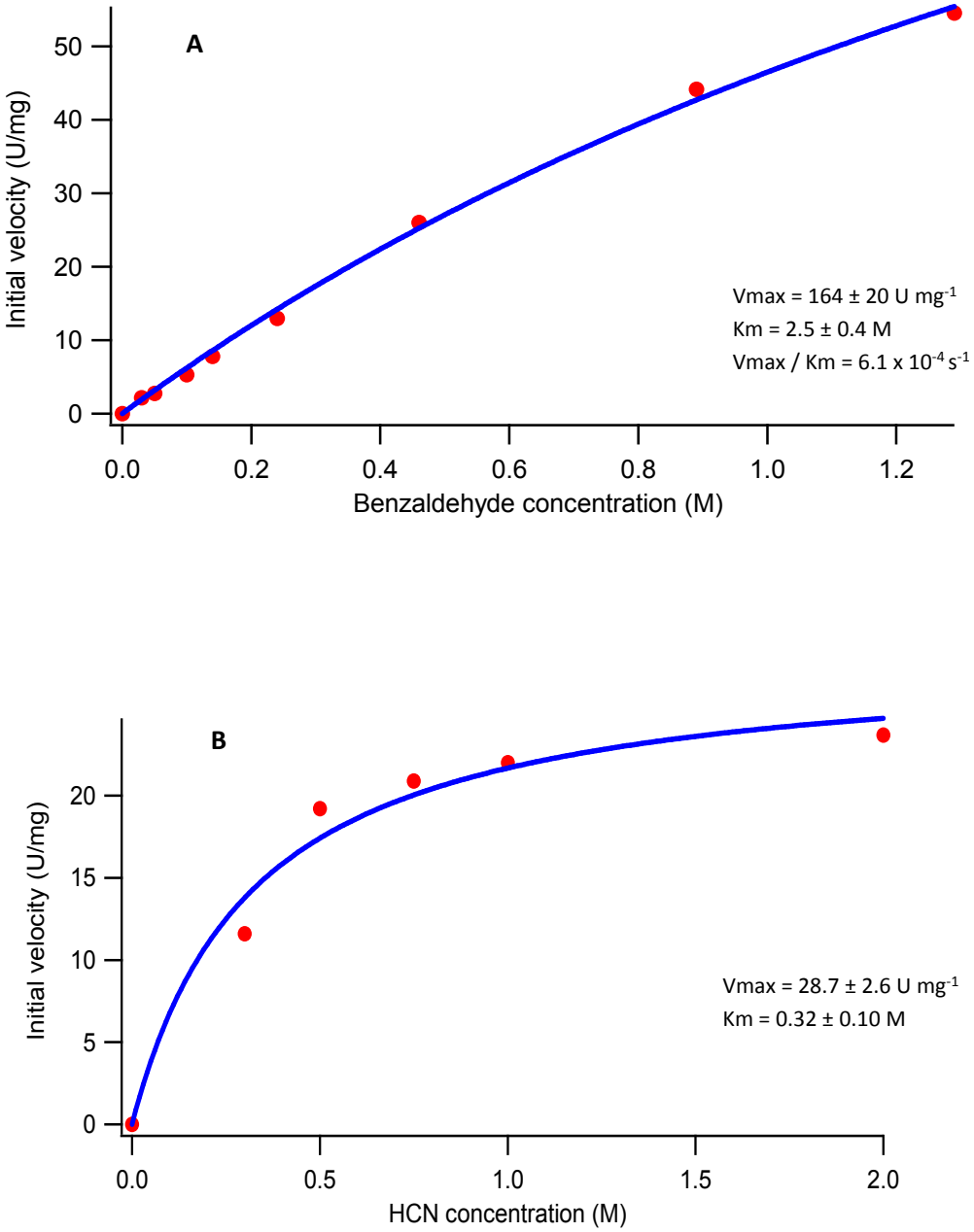

Figure S3. Kinetic traces for the synthesis of (*R*)-mandelonitrile at different benzaldehyde (S3-A) and HCN (S3-B) concentrations. Conditions: Ratio benzaldehyde : HCN in acetate buffered MTBE, pH 4, 1:4, 2 mL HCN in acetate buffered MTBE (1.5–2 M) pH 4, *Gt*HNL-TM immobilized on 50 mg Celite R-633 (50 U). The reaction was stirred at 700 rpm, 5 °C. Single measurement points were fitted by using Michaelis-Menten equation. Standard deviation was calculated with Igor Pro 5.0.5 software

F. Substrate incubation for evaluation of background reaction during 8 hours

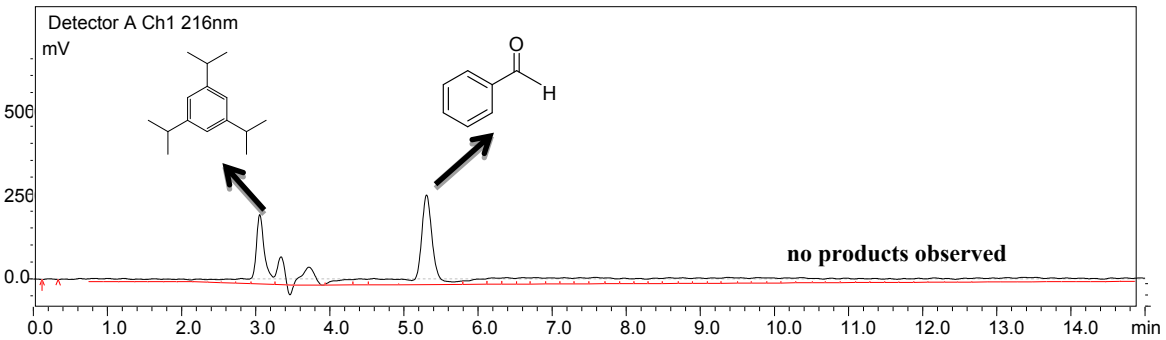

Figure S4. HPLC detection of benzaldehyde and 1,3,5 tri-isopropylbenzene during 8 hours of incubation: Conditions: Ratio benzaldehyde : HCN in buffered MTBE, pH 4, 1:4, 100 µL benzaldehyde (1 mmol), 2 mL HCN in acetate buffered MTBE, pH 4**.** The reaction was stirred at 1000 rpm at 5 °C.

G. Identification of substrates and products during the synthesis of (*R*)-mandelonitrile

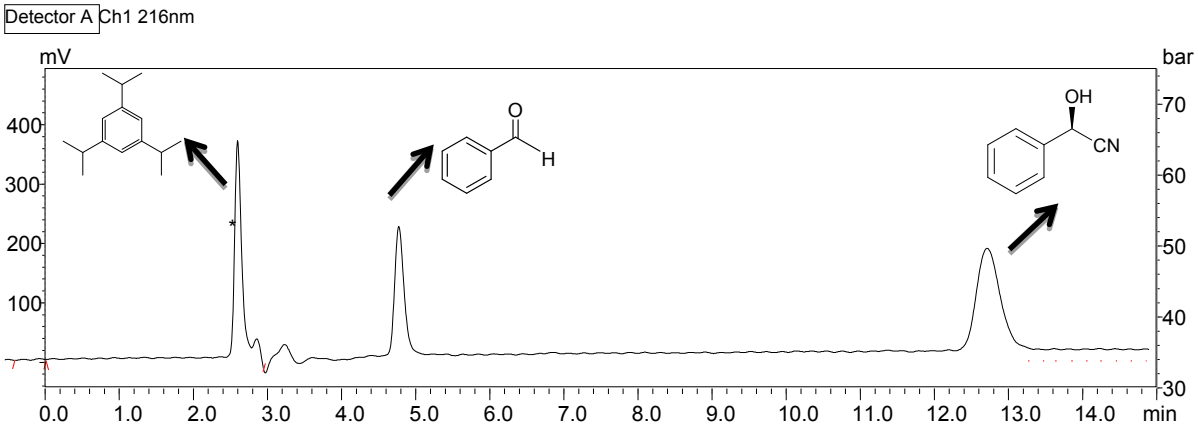

Figure S5. HPLC detection of benzaldehyde, 1,3,5 tri-isopropylbenzene and (*R*)-mandelonitrile. Conditions: Ratio benzaldehyde : HCN in buffered MTBE, pH 4, 1:4, a CFR with *Gt*HNL-TV immobilised on 150 mg Celite R-633 (150 U). Reactions was performed at room temperature.

H. Progress of the synthesis of (*R*)-mandelonitrile with immobilized *Gt*HNL-TV in nylon and regular paper tea bag.

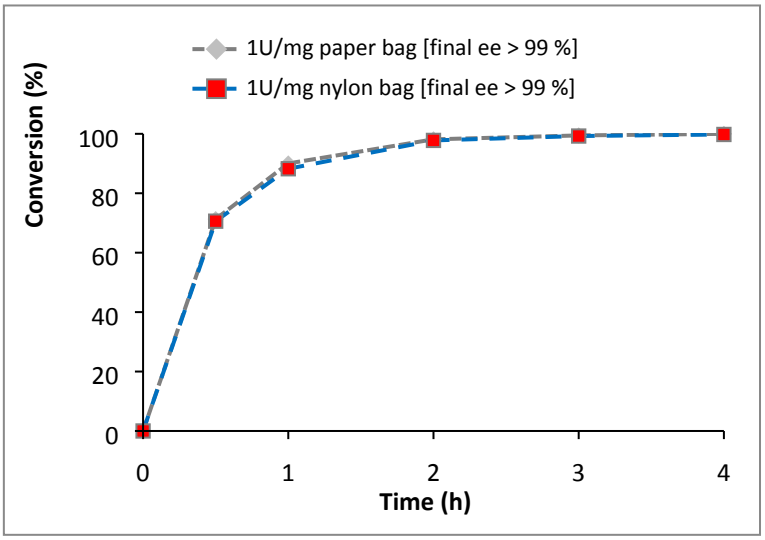

Figure S6: Synthesis of (*R*)-mandelonitrile with immobilized *Gt*HNL-TV in nylon and regular paper tea bags. Paper tea bags are commonly nylon enforced. Conditions: Ratio benzaldehyde : HCN in acetate buffered MTBE, pH 4, 1:4, *Gt*HNL-TV (11 mg, 50 U). The reaction was stirred at 1000 rpm at 5 °C.

I. Size of tea bags for BR and RBR reactions

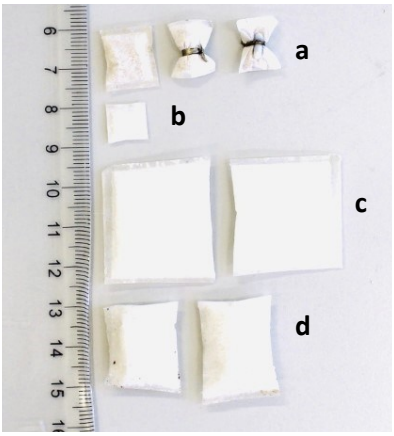

Figure S7: Tea bags with different sizes for tightly or loosely packed *Gt*HNL-TV on Celite R-633. The ruler shows the size in cm. a) tightly packed Celite immobilised *Gt*HNL-TV (50 mg) for BR, b) tightly packed Celite immobilised *Gt*HNL-TV (18 mg) for BR, c) loosely packed Celite immobilised *Gt*HNL-TV (773 mg) for RBR and d) tightly packed Celite immobilised *Gt*HNL-TV (773 mg) for RBR.

Table S1: Size of tea bags for tightly or loosely packed *Gt*HNL-TV on Celite R-633 for BR and RBR reactions.

Reactor	Type of packing	Size	Amount of enzyme -carrier
BR	Tight	1.3 x 1.6 cm	50 mg
BR	Tight	0.7 x 0.8	18 mg
RBR[1]	Loose	2.5 x 2.6 cm	773 mg
RBR[1]	Tight	1.8 x 2.2 cm	773 mg

[1] 2 bags with equal amount of GtHNL-TV on Celite.

SI References

[1] A.H.M.M. El-Sayed, W.M. Mahmoud and R.W. Coughlin, *Biotechnol. Bioeng*., **1990**, *36*, 83-91.