Electronic Supplementary Information

Efficient hydration of 2-amino-2, 3-dimethylbutyronitrile to 2-amino-2, 3-dimethylbutyramide in a biphasic system via an easily prepared whole-cell biocatalyst

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1.1 Preparation of whole-cell biocatalyst

Genomic DNA of *Pseudonocardia thermophila* CGMCC 4.1303 was extracted using a Wizard® Genomic DNA Purification Kit (Promega, Beijing, China). NHase coding gene was amplified using polymerase chain reaction, and the primers were designed based on a conserved protein sequence with GenBank accession numbers: 24158676 and 24158677. (Forward primer: 5'-TATCATATGAACGGCGTGTACGACGTC-3' with Nde I site being underlined, and reverse primer: 5-CGCAAGCTTTCACGCGACCGCCTTCGCCGGT-3 with Hind III site being underlined). Then the PCR fragments were inserted into the simultaneously digested pET-28a (+) vector, and the isolated recombinant plasmids were transformed into *E. coli* BL21 (DE3) cells. A single colony was picked into 3 mL of LB medium supplemented with 50 ug/mL kanamycin, and shaken overnight at 37 ^{o}C, 200 rpm as seed. 0.5 mL of the seed was inoculated into 50 mL of optimized fermentation medium (16 g/L D-Sorbitol, 20 g/L peptone, 10 g/L angel yeast extract, and 2.5 g/L $Na_2HPO_4 \cdot 12H_2O$) supplemented with 50 ug/mL kanamycin in 250 mL shake flasks and cultured at 37^{o}C,200 rpm. When cell density of OD_{600} reached 0.8-1.0, 0.1 mM of IPTG and 0.3 mM of $CoCl_2$ were added to induce the NHase expression at 20 ^{o}C, 200 rpm for 24 h. During the induction process, 0.3 mM of $CoCl_2$ was added to medium in 4h, 8h and 12h, respectively. Cells were harvested by centrifugation at 8, 000 rpm for 10 min, then resuspended and washed by 100 mM sodium phosphate buffer (pH 7.0). The resulted recombinant *E. coli*whole cells containing NHase were used as the whole-cell biocatalyst in this work.

1.2 Effects of substrate, product, HFE-7100 and protein leakage on the enzyme activities of whole cells

The toxicities of ADBN to the enzyme activities of whole cells (30 U/mL of enzyme amount in reaction mixture) were assessed by adding 180 mM, 130 mM and 120 mM of ADBA in HFE-7100/H_2O (10 %, v/v) biphasic system, glycerol carbonate/H_2O (5 %, v/v) homogeneous system and single aqueous phase with 120 mM of ADBN in each reaction system, respectively, at 200 rpm and 20 ^{o}C for 8 h. Additionally, the toxicities of ADBA to the enzyme activities of whole cells (30 U/mL of enzyme amount in reaction mixture) in HFE-7100/H_2O (10 %, v/v) biphasic system, glycerol carbonate/H_2O (5 %, v/v) homogeneous system and single aqueous phase were also evaluated by adding 180 mM, 130 mM and 120 mM of ADBA in each reaction system, respectively, at 200 rpm and 20 ^{o}C for 8 h. The toxicity of HFE-7100/H_2O (10 %, v/v) biphasic system to the enzyme activities of whole cells (30 U/mL of enzyme amount in reaction mixture) were also tested at 200 rpm and 20 ^{o}C for 8 h, and the single aqueous phase with 30 U/mL of enzyme amount was employed as blank control to investigate the effect of protein leakage on the enzyme activity of whole cells during the continuous operation process. The whole cells were taken out at 1h, 2h, 3h, 4h, 5h, 6h, 7 h and 8h from the different reaction systems by centrifugation at 8000 rpm, and washed twice using 100 mM phosphate buffer (pH 7.0), then used for assay the residual enzyme activity of whole cells. The enzyme relative activity (%) used to evaluate the level of ADBN toxicity to the enzyme activity of whole cells was defined as the percentage of the residual enzyme activity to the initial enzyme activity of wet whole cells (109 U/g). The leaked protein content of supernatants in HFE-7100/H_2O (10 %, v/v) biphasic system and single aqueous phase was assayed by the method of Bradford.[1]

1.2.1 Effects of substrate and product on the enzyme activities of whole cells

The results shown in Fig.S4A indicated that the enzyme activities of whole cells decreased gradually when the incubation time was over 2 h in different reaction systems, which contained 120 mM ADBN with the threshold concentration of ADBA that resulted in the reaction be terminated, implying that the long time incubation of whole cells using 120 mM ADBN would cause the

deactivation of enzymes existed in whole cells. Subsequently, the toxicity of product to whole cells was excluded as shown in the Fig.S4B, which indicated that the reduction trend of enzyme activity of whole cells was exhibited similarly in the presence of 180 mM of ADBA in HFE-7100/H_2O (10 %, v/v) biphasic system and that of 120 mM in single aqueous phase, confirming the fact that substrate is toxicity to the enzyme activity of whole cells when the incubation time was over 2 h. However, as shown in the Fig.S2 in "Electronic Supplimentary Information", over 60 % of substrate was converted rapidly to product in 60 min, resulting in the reaction be terminated by product inhibition before the substrate toxicity deactivate the enzymes in whole cells, suggesting that the termination of reaction under different substrate concentrations is mainly attributed to the product inhibition instead of substrate toxicity.

1.2.2 Effects of HFE-7100 on the enzyme activities of whole cells

The toxicity of HFE-7100/H_2O (10 %, v/v) biphasic system to the enzyme activity of whole cells was also tested, and some intriguing results shown in Fig.2B indicated that enzyme activity of whole cells raised approximately 10 % compared with the initial value when 10 % (v/v) of HFE-7100 was used to construct the biphasic reaction system. Same phenomenon is also observed in the NHase catalyzed process for hydration of ADBN to ANBA using an organic solvent/H_2O biphasic system.[2] With 10 % (v/v) *n*-hexane in reaction system, 22.3 % of NHase activity of whole cells was improved in the biocatalytic hydration process, which was potentially attributed from the increase of the permeability of whole cells in the presence of organic solvent.

1.2.3 Effects of protein leakage on the enzyme activities of whole cells

The protein leaked into the supernatants of HFE-7100/H_2O (10 %, v/v) biphasic system and single aqueous phase were determined. The results shown in Fig. S4C indicated that protein leakage is the main reason leading to the enzyme activity decrease of whole cells. The protein release rate was calculated to be approximately 0.13 mg mL^{-1} h^{-1} and 0.172 mg mL^{-1} h^{-1}, respectively, in HFE-7100/H_2O (10 %, v/v) biphasic system and single aqueous phase, and the total leaked protein amount reached to approximately 7 % and 10 % of total protein amount of whole cells (18.5 mg mL^{-1}) after 8 batches, basically consistent with the reduction trend of enzyme activity of whole cells as shown in the Fig.S4B. Combined the results showed in the Fig.4 of the manuscript and SDS-PAGE analysis of protein leakage in Fig.S5, it could be undoubtedly concluded that the decrease of ADBA yield with a prolonged reaction time after seven batches reaction mainly resulted from the leakage of NHase from the whole-cell into aqueous phase during the long operating course.

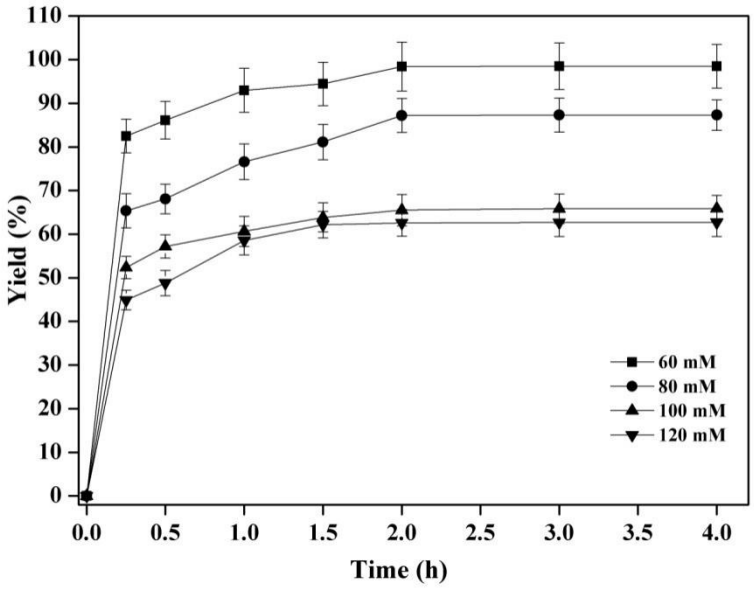


Figure S1. Effects of ADBN concentrations on ADBA yield in single aqueous phase. Reaction conditions: temperature 20 °C, pH 7.0, 30 U/mL, reaction volume (400 μL) and shaking speed (200 rpm).

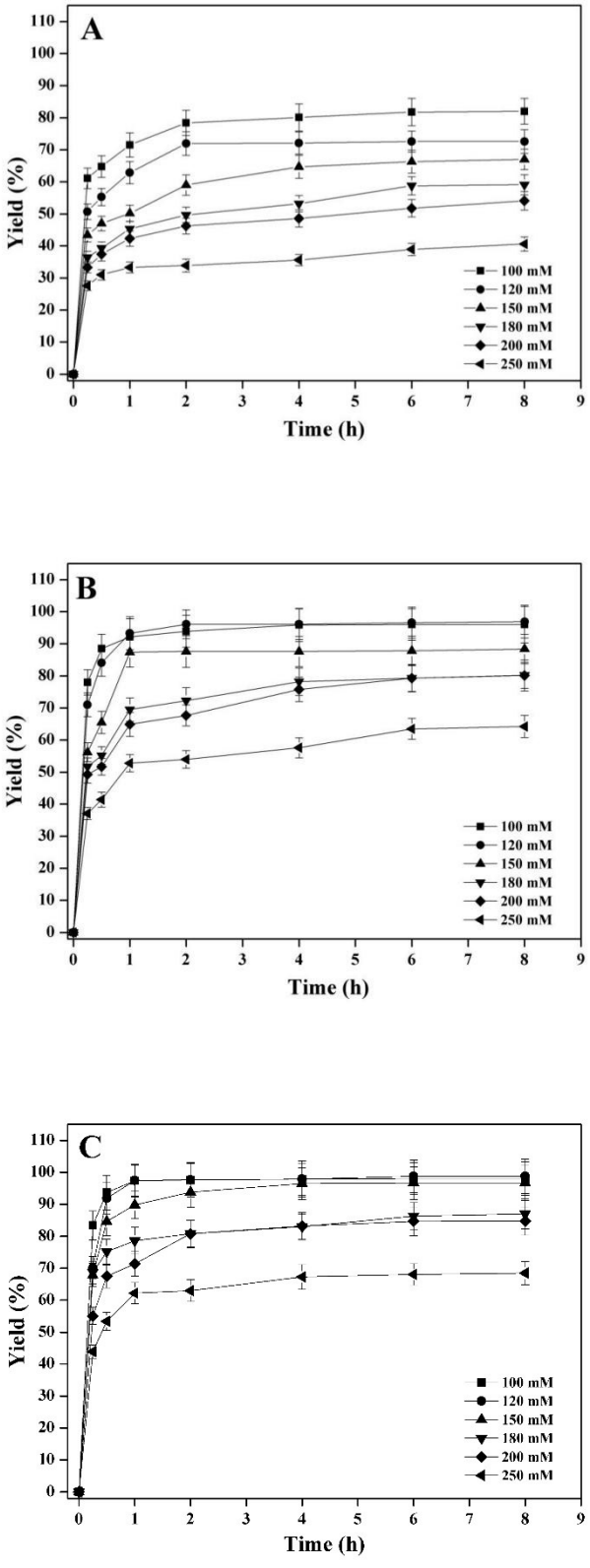
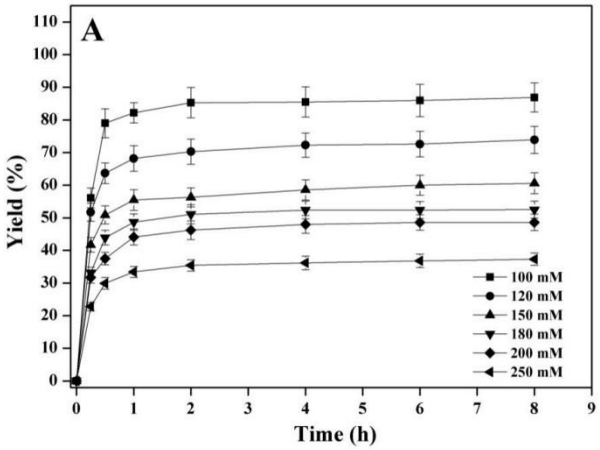
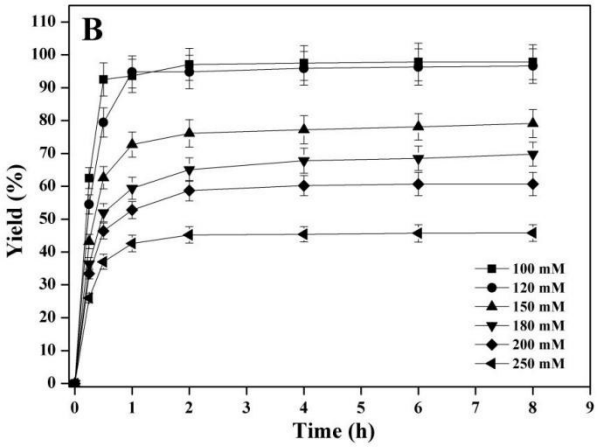


Figure S2. Effects of ADBN concentrations with different enzyme amounts on ADBA yield in HFE-

7100/H_2O (10 %, v/v) biphasic system.Reaction conditions: temperature 20 ^oC, pH 7.0, reaction volume 400 µL and shaking speed (200 rpm). (A) 15 U/mL , (B) 20 U/mL and (C) 30 U/mL.





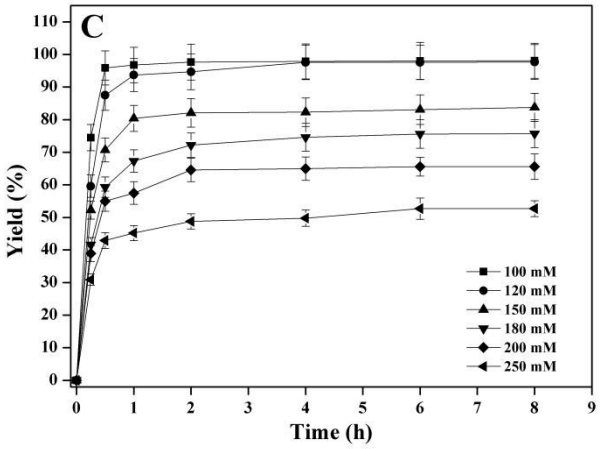


Figure S3. Effects of ADBN concentrations with different enzyme amounts on ADBA yield in glycerol carbonate/H_2O (5 %, v/v)homogeneous system.Reaction conditions: temperature (20 °C), pH 7.0, reaction volume (400 μL) and shaking speed (200 rpm). (A) 15 U/mL, (B) 20 U/mL and (C) 30 U/mL.

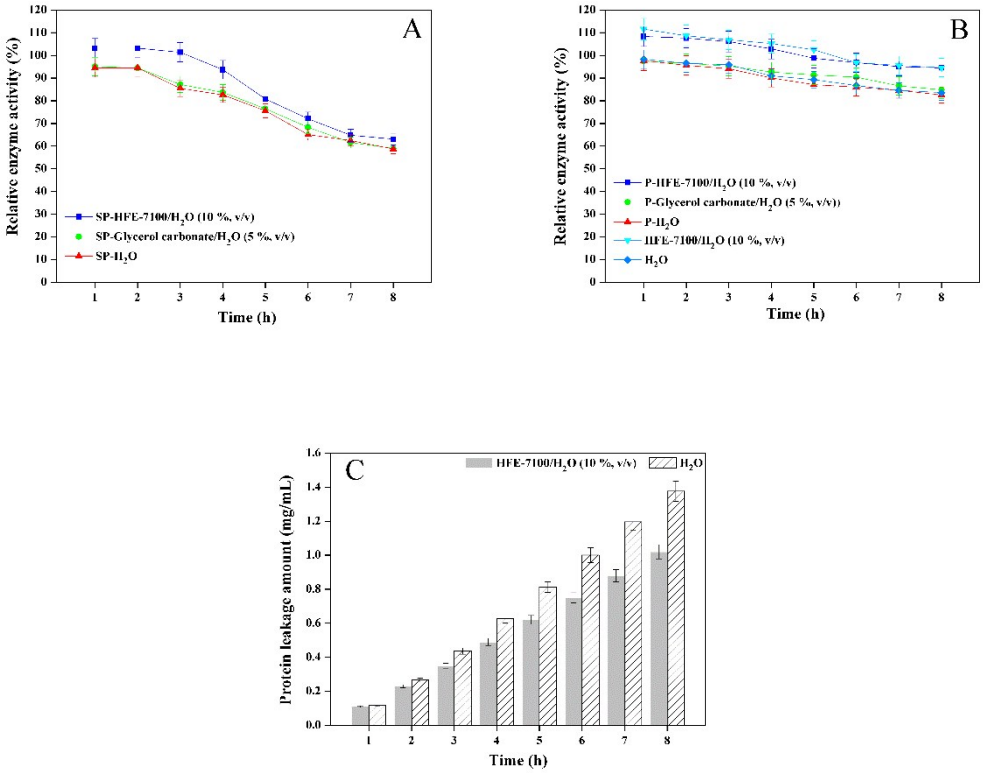


Figure S4 (A) Effects of substrate toxicity on the enzyme activities of whole cells in HFE-7100/H_2O (10 %, v/v) biphasic system, glycerol carbonate/H_2O (5 %, v/v) homogeneous system and H_2O (SP: substrate and product), 180 mM, 130 mM and 120 mM of ADBA with 120 mM of ADBN were added in the above mentioned reaction systems at 200 rpm and 20 °C for 8 h; **(B)** Effects of product toxicity on the enzyme activities of whole cells in HFE-7100/H_2O (10 %, v/v) biphasic system, glycerol carbonate/H_2O (5 %, v/v) homogeneous system and H_2O (P: product), 180 mM, 130 mM and 120 mM of product ADBA were added in the above mentioned reaction systems at 200 rpm and 20 °C for 8 h; Effects of the toxicity of HFE-7100/H_2O (10 %, v/v) biphasic system on the enzyme activities of whole cells, a certain amount of whole cells was added in HFE-7100/H_2O (10 %, v/v) biphasic system to a enzyme amount of 30 U/mL at 200 rpm and 20 °C for 8 h; Effect of protein leakage on enzyme activity of whole cells in single aqueous phase, a certain amount whole cells was added in single aqueous phase to a enzyme amount of 30 U/mL at 200 rpm and 20 °C for 8 h. The enzyme relative activity (%) used to evaluate the level of ADBN toxicity to the enzyme activity of whole cells was defined as the percentage of the residual enzyme activity to the initial enzyme activity of wet whole cells (approximately 109 U/g wet cells). **(C)** The leaked protein content of supernatants in HFE-7100/H_2O (10 %, v/v) biphasic system and single aqueous phase at 200 rpm and 20 °C for 1-8 h.

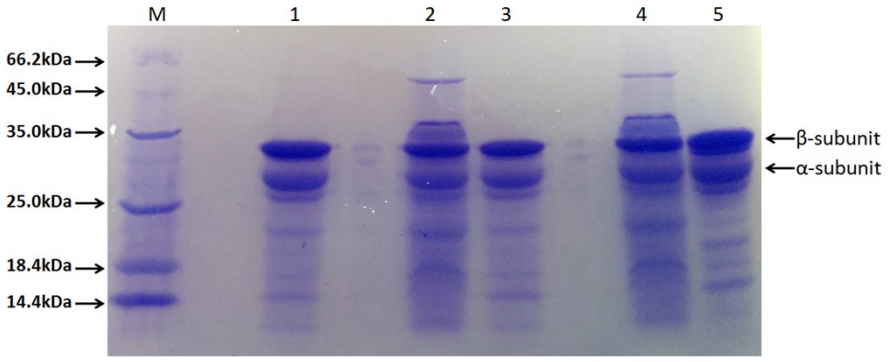


Figure S5. SDS-PAGE analysis of leaked protein in the supernatants of HFE-7100/H_2O (10 %, v/v) biphasic system and single aqueous phase at 200 rpm and 20 °C for 8 h. M: molecular mass markers (Fermentas), from top to bottom, 116.0, 66.2, 44.0, 35.0, 25.0, 18.4 and 14.4kDa, respectively. Certain amount of whole cells (30 U/mL in reaction mixture) used for hydration of ADBA from ADBN were disrupted using the ultrasonic cell disruptor (JY92-2D, Ningbo Scientz, China) on ice at 400 W for 60 cycles (working 5 s and intervals 5 s as one cycle). The disrupted cell debris was collected by centrifugation at 12, 000 rpm and 4 °C for 10 min. The supernatants obtained from sonication, HFE-7100/H_2O (10 %, v/v) biphasic system and single aqueous phase were used for partly purification of NHase according to the previously reported methods, [3] and were employed to SDS-PAGE analysis. Lane 1: partly purified supernatants from sonication; Lane 2 and Lane 3: supernatants obtained from HFE-7100/H_2O (10 %, v/v) biphasic system at 200 rpm and 20 °C for 8 h and its partly purified enzyme; Lane 4 and Lane 5: supernatants obtained from single aqueous phase at 200 rpm and 20 °C for 8 h and its partly purified enzyme.

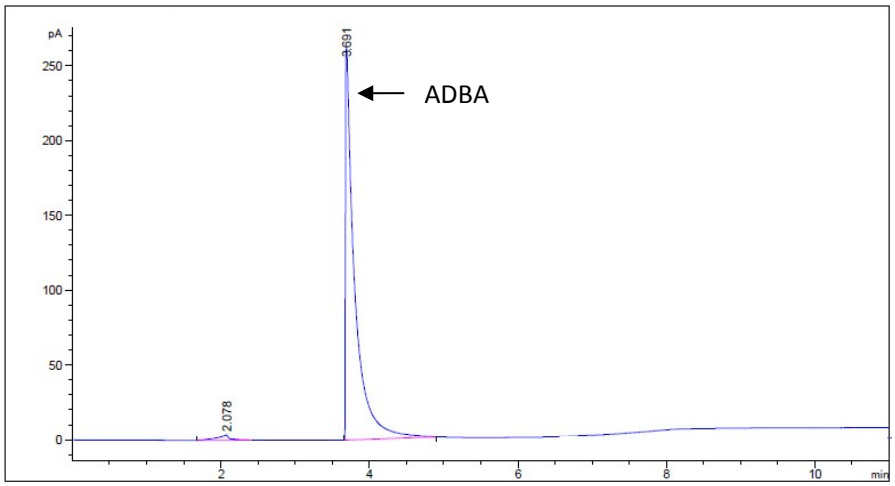


Figure S6. GC analysis of purified product. GC was carried out using Agilent 7820N equipped with AT·FFAP column (30m×0.25mm×0.25mm). The temperatures of injector and the detector were set as 250 °C. The carrier gas nitrogen flow was 1.2 mL/min. After a 3 min solvent delay time at 150 °C, the oven temperature was increased at 10 °C/min to 200 °C, followed by an additional 5 min delay. The injection volumn was 0.2 μL under a splitless mode.

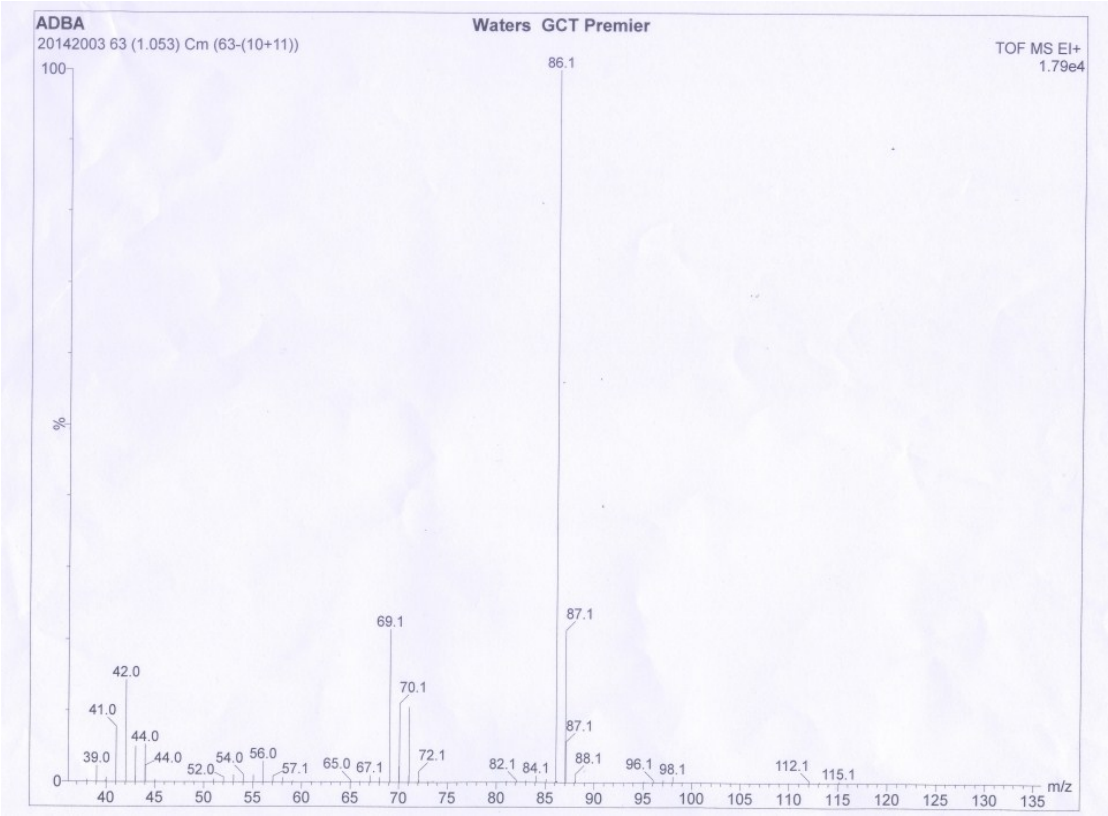


Figure S7. MS (EI) identification of purified product. MS(EI) identification parameters: Mass spectra were obtained in the mass range m/z=35-800 with a source temperature of 250 ^{o}C and an ionization voltage of 70eV.

Table S1. Chemical and enzymatic methods for hydration of ADBN to ADBA

Chemical methods	Reaction conditions	Environmental friendliness/ Catalyst recyclability	Yield (%)	Specific productivity (mM $KU^{-1}h^{-1}$) [a]	References
1	Reaction system: extreme acid+ dichloromethane Catalyst:concentrated sulfuric acid. Temperature: 100°C Substrate concentration: 3.54 M	Hardly	81.7	NS	4, 5
2	Reaction system: extreme alkaline + palladium Catalyst: H_2O_2 Temperature: <30°C Substrate concentration: 3.85 M	Hardly	75	NS	6
Enzymatic methods					
1	Reaction system: n-hexene/H_2O (30 %, v/v) Catalyst: wild *R.boritolerans* CCTCC M208108 whole cell Temperature: 10°C Substrate concentration: 22.3 mM Operation cycle: 2 batches	Partially	91	0.376	2
2	Reaction system: HFE-7100/H_2O (10 %, v/v) Catalyst:recombinant *E. coli* whole cells Temperature: 20°C Substrate concentration: 120 mM Operation cycle: 7 batches	Totally	97.3	3.76	This study

[a] Specific productivity was defined according to the ADBA concentration (mM), the enzyme amount (KU) and reaction time (min) that required to obtain the highest yield provided in references and this study.

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