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Biocatalytic hydrolysis of cyanohydrins: an efficient approach to enantiopure α-hydroxy carboxylic acids

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Abstract

Rhodococcus erythropolis NCIMB 11540 was found to have a highly active nitrile hydratase/amidase enzyme system present which accepts the nitrile function of α-hydroxynitriles (cyanohydrins) as substrates. This biocatalytic hydrolysis using whole bacterial cells leads to α-hydroxy carboxylic acids which are much valued chiral building blocks in organic synthesis. Employing enantiopure cyanohydrins, which are easy available using (*R*)- or (*S*)-hydroxynitrile lyases, the products were obtained in high yield without racemization, decomposition or side reactions. Herein, the application of this biotransformation for preparative scale applications is described. To clarify the substrate acceptance of the nitrile hydrolyzing enzymes of *R. erythropolis* NCIMB 11540, several selected model compounds were subjected to biocatalytic hydrolysis. Reaction conditions were optimized to enable preparative scale conversions. In this manner, (*R*)-2-chloromandelic acid and (*R*)-2-hydroxy-4-phenylbutyric acid, two important pharmaceutical intermediates, were prepared in a gram scale. The substrate concentrations used were 9.3 and 13 g/l, respectively. The process yielded both acids in high optical (ee > 99 and 98%) and chemical (98%) yield after short reaction times (3 and 1.5 h).

Keywords: Nitrile hydratase; Amidase; Cyanohydrin hydrolysis; α-Hydroxy carboxylic acids; Biotransformation

1. Introduction

Enantiopure α-hydroxy carboxylic acids are versatile building blocks in organic synthesis [1] and several biocatalytic approaches to this class of compounds have been described [2]. The most general approach is the biocatalytic synthesis of enantiopure cyanohydrins using (*R*)- or (*S*)-hydroxynitrile lyases (HNL) [3] with subsequent chemical nitrile hydrolysis. This two-step procedure provides simple access to this class of compounds because of the broad substrate spectrum of the HNLs [3–5]. In addition, these enzymes are available with both *R*- and *S*-selectivity. Nevertheless, chemical nitrile hydrolysis has several disadvantages especially for highly functionalized chiral molecules. The required drastic reaction conditions may cause racemization, decomposition or side reactions. Especially for industrial processes, long reaction times at high temperature are unfavorable due to high energy consumption. An alternative to such processes would be to use a highly active biocatalyst which can hydrolyze enantiomerically pure cyanohydrins in a very efficient manner. Requirements on such a biocatalyst would be ready availability for the organic chemist, for example simple and reliable fermentation procedures,

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high activity for short reaction times and a broad substrate spectrum to ensure general applicability. Enantioselectivity is not required because the chiral center of the molecules has already been generated using the well investigated HNL-enzyme systems [3–5].

Enzymatic hydrolysis of nitriles is a well established biocatalytic method, for recent reviews see [6–8]. Enzymatic nitrile hydrolysis can proceed via two different pathways: The action of a nitrilase (EC 3.5.5.1), with some exceptions [9], leads directly to the corresponding carboxylic acid. The second pathway involves a nitrile hydratase (NHase; EC 4.2.1.84)/amidase (EC 3.5.1.4) enzyme system. Initially the amide is formed and then hydrolyzed to the acid by an amidase. Although a large number of nitrile hydrolyzing enzymes accepting different substrate types is known, only a few reports describe the acceptance of the nitrile function of the unstable cyanohydrins [10–15]. Furthermore, in most cases only lactonitrile and mandelonitrile were investigated [16,17], maybe due to their commercial availability. In contrast, several patents describe the hydrolysis of the nitrile function of cyanohydrins [18–20]. Most of them deal with the conversion of racemic cyanohydrins by enantioselective nitrilases to optically active α-hydroxy carboxylic acids. Disadvantages resulting from the kinetic resolution procedure were circumvented by in situ racemization of the remaining cyanohydrin [21].

We have found *Rhodococcus erythropolis* NCIMB 11540 to have a highly active NHase/amidase enzyme system which accepts various cyanohydrins as substrates. The biocatalyst can be used as a whole cell system and the present paper describes our investigations of this biotransformation regarding substrate spectrum and optimization of reaction conditions. The main goal was to show the applicability of this approach in preparative scale reactions. Consequently, the present method was applied to an efficient two-step biocatalytic synthesis of two important pharmaceutical intermediates, namely (*R*)-2-chloromandelic acid, the chiral building block of an antithrombotic agent [22], and (*R*)-2-hydroxy-4-phenylbutyric acid, the key intermediate for the synthesis of ACE-inhibitors [23]. Both target compounds were prepared in a gram scale.

2. Experimental

2.1. Substrates and reference materials

2.1.1. General remarks

The used aldehydes, mandelic acid (**3c**) and mandelamide (**3b**) were commercially available. *Rac*-mandelonitrile (**3a**) and (*R*)-2-chloro-mandelonitrile (**4a**, ee > 99%) were a kind gift of DSM Fine Chemicals, Austria. Reactions were monitored by TLC (Merck silica gel 60 F_{254}), compounds were visualized with UV (254 nm) and by spraying with Mo-reagent (10% H_2SO_4, 10% $(NH_4)_6Mo_7H_{24}{\cdot}4H_2O$ and 0.8% $Ce(SO_4)_2{\cdot}4H_2O$). Flash chromatography was performed on Silica gel 60 (Merck, 70-230 mesh) using mixtures of ethyl acetate and petroleum ether. ^1H and ^{13}C NMR spectra were recorded on a Gemini 200 BB (Varian). For analytical methods vide infra.

2.1.2. Synthesis of racemic cyanohydrins

To a solution of freshly distilled aldehyde (5%) in TBME (*t*-butylmethyl ether), weakly basic ion-exchange resin (Amberlyst A-21) and freshly generated HCN (5 equiv.) [24] were added. The mixture was stirred at room temperature, and after quantitative conversion (5–24 h), the mixture was filtered over a bed of Na_2SO_4. Evaporation of the solvent and flash chromatography (petroleum ether:EtOAc, 10:1) yielded the pure cyanohydrins. Structures were proved by NMR analysis. Spectroscopic data were identical with those previously reported. 2-Hydroxy-4-phenyl-butyronitrile (**1a**) [25]; *E*-2-Hydroxy-3-pentenenitrile (**2a**) [26]; 4-methyl-mandelonitrile (**5a**) [27]; 3-phenoxymandelonitrile (**6a**) [28].

2.1.3. Synthesis of racemic α-hydroxy amides

The respective cyanohydrin was protected with *t*-butyldimethylsilyl chloride according to standard methods. The crude silylated cyanohydrin and K_2CO_3 (8–10 equiv.) were stirred in a mixture of 30% H_2O_2/methanol (1:1) at room temperature. After the reaction had reached completion water was added and the product was extracted with CH_2Cl_2. The organic phase was dried, evaporated under reduced pressure and the crude product dissolved in THF. After addition of tetrabutylammonium fluoride (1.5 equiv.), the solution was stirred at room temperature

for several hours. Subsequently, the solvent was evaporated and the product was isolated by flash chromatography (EtOAc). Spectroscopic data of **1b** were identical with those previously reported [29]. NMR data of compounds **2b** and **4–6b** are given below. *E*-2-hydroxy-3-pentenoic acid amide (**2b**): ^{1}H NMR: $\delta = 1.72$ (3H, d, $J = 6$), 4.41 (1H, d, $J = 6$), 5.54–5.59 (1H, m), 5.82–5.90 (1H, m). ^{13}C NMR: $\delta = 16.7$, 72.4, 128.6, 129.2, 177.7; **2**-chloromandelamide (**4b**): ^{1}H NMR: $\delta = 5.50$ (1H, s), 7.23–7.51 (4H, m). ^{13}C NMR: $\delta = 70.8$, 127.1, 128.9, 129.4, 129.45, 133.9, 138.3, 176.6; 4-methylmandelamide (**5b**): ^{1}H NMR: $\delta = 2.32$ (3H, s), 4.96 (1H, s), 7.16 (2H, d, $J = 8$), 7.34 (2H, d, $J = 8$). ^{13}C NMR: $\delta = 20.0$, 74.1, 126.8, 128.8, 137.5, 137.8, 177.6; 3-phenoxymandelamide (**6b**): ^{1}H NMR: $\delta = 4.98$ (1H, s), 6.90–7.36 (9H, m). ^{13}C NMR: $\delta = 73.8$, 117.0, 118.2, 118.8, 121.7, 123.3, 129.6, 129.7, 142.7, 157.4, 157.6, 177.1.

2.1.4. Synthesis of racemic α-hydroxy acids

The respective cyanohydrin was refluxed in concentrated HCl. After quantitative conversion, HCl was removed under reduced pressure and to remove residual water, portions of toluene were added and evaporation was continued. Filtration over activated charcoal (EtOH), removal of the solvent and recrystallization from toluene yielded the desired α-hydroxy acids. Their spectroscopic data were identical with those previously reported. 2-Hydroxy-4-phenylbutyric acid (**1c**) [30]; *E*-2-hydroxy-3-pentenoic acid (**2c**) [31]; 2-chloromandelic acid (**4c**) [14]; 4-methylmandelic acid (**5c**) [32]; 3-phenoxymandelic acid (**6c**) [32].

2.1.5. Enzymatic synthesis of (*R*)-2-hydroxy-4-phenylbutyronitrile (*1a*)

A solution of freshly distilled 3-phenylpropanal in TBME (5%) was stirred with the same volume of recombinant (*R*)-HNL lysate (150 IU/ml) [33]. To this emulsion 5 equiv. of freshly prepared HCN [24] were added dropwise at 0 °C. After stirring over night in a sealed vessel at ambient temperature, the reaction mixture was extracted with TBME. Drying over Na_2SO_4 and removal of the solvent in vacuo gave crude (*R*)-2-hydroxy-4-phenylbutyronitrile (**1a**) in 91% yield and 98% ee (GC). NMR spectra were identical to literature values [25].

2.1.6. Synthesis of the acetonide derivatives of the α-hydroxy acids

The respective α-hydroxy acid and a catalytic amount of conccentrated H_2SO_4 were stirred in acetone. After 1 h saturated $NaHCO_3$ was added and the product was extracted with ethyl acetate. After drying (Na_2SO_4), the derivatives were analyzed by GC. NMR data of these compounds are given below.

2,2-Dimethyl-5-(2-phenylethyl)-1,3-dioxolan-4-one: ^{1}H NMR: $\delta = 1.57$ (3H, s), 1.66 (3H, s), 2.00–2.23 (2H, m), 2.77–2.85 (2H, m), 7.22–7.36 (5H, m). ^{13}C NMR: $\delta = 26.0$, 27.5, 31.1, 33.6, 73.4, 110.8, 126.5, 128.8 (2C), 140.7, 173.4.

5-(2-Chlorophenyl)-2,2-dimethyl-1,3-dioxolan-4-one: ^{1}H NMR: $\delta = 1.61$ (3H, s), 1.69 (3H, s), 5.70 (1H, s), 7.22–7.34 (4H, m). ^{13}C NMR: $\delta = 26.0$, 27.0, 74.2, 111.2, 127.5, 129.6, 130.5, 130.9, 132.0, 134.3, 170.7.

2.2. Bacterial strain and culture conditions

R. erythropolis NCIMB 11540 was maintained on agar plates using the medium described below (15% agar). Sub-culturing was performed every 12 weeks and plates were stored at 4 °C. *R. erythropolis* NCIMB 11540 was grown in Erlenmeyer flasks containing 250 ml medium on a rotary shaker at 30 °C and 130 rad/min (per liter: 4.0 g Na_2HPO_4, 2.0 g KH_2PO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.02 g $CaCl_2{\cdot}2H_2O$, 0.05 g ammonium ferric(III) citrate (brown), 1.0 g yeast extract, 10 g meat peptone, 10 g glucose, 1 ml trace element solution [34]). The flasks were inoculated with 5 ml of a pre culture (100 ml medium in Erlenmeyer flasks inoculated with cell material from agar plates, growth for 20–24 h at conditions described above). After 20 h of growth (OD about 7–8) the cells were harvested by centrifugation (20 min, 6370 × *g*, 4 °C) and washed with phosphate buffer (pH 6.5, 50 mM). These cells were then re-suspended in buffer. This cell suspension was either used directly (resting cells) for the biocatalytic transformations or lyophilized after freezing in liquid nitrogen.

2.3. Analytical methods

Cyanohydrins and their corresponding aldehydes, hydroxy amides and hydroxy acids were examined by HPLC (HP 1100) using a reversed-phase

column Nova-Pak C_{18} (5 μm, 3 mm × 150 mm, Waters). Mixtures of 0.1% H_3PO_4/acetonitrile were employed at a flow rate of 0.9 ml/min. Absorbance was measured at 210 nm with the exception of 2-hydroxy-3-pentenenitrile and its derivatives (**2a–c**) which were analyzed at 204 nm.

Enantiomeric purities were analyzed on a Chrompack Chirasil-Dex CB column (25 m × 0.32 mm, 0.25 μm film) using a HP 6890 gas chromatograph equipped with an FID. 2-Hydroxy-4-phenylbutyronitrile (**1a**) was acetylated using standard methods prior to analysis (1 bar H_2, 140 °C, 4.5 min (*R*)/5.3 min (*S*)). The enantiomers of 2-chloromandelic acid (**4c**) and 2-hydroxy-4-phenylbutyric acid (**1c**) were separated after derivatization to the acetonides as described above (2.1.6.). **4c**: 1 bar H_2, 115 °C, 14.8 min (*R*), 15.4 min (*S*); **1c**: 1 bar H_2, 130 °C, 7.9 min (*R*), 8.6 min (*S*).

2.4. Enzyme activity and substrate acceptance

2.4.1. Method A

Lyophilized cells (106 mg) were rehydrated for approximately 1 h in phosphate buffer (50 mM, pH 6.5, 10 ml), if not stated otherwise. Portions of this cell suspension (475 μl) were mixed with 25 μl of substrate solution (200 mM cyanohydrin in dimethylsulfoxide (DMSO)) and then agitated in a thermomixer (30 °C, 1000 rad/min). Periodically, a reaction was terminated by the addition of 0.5 ml 1N HCl. After removing the cells by centrifugation, cyanohydrin and products were examined using HPLC. NHase activity was determined on the basis of the formation of hydroxyamide and hydroxy acid within the first 10 min (nearly linear slope). Amidase activity was estimated in qualitative manner during the course of the reaction.

2.4.2. Method B

Portions of resting cell suspensions (OD 40–80, 4.75 or 4.85 ml) were mixed with a solution of (*R*)-2-chloromandelonitrile (**4a**, dissolved in an organic cosolvent) to a total volume of 5 ml. Substrate concentration, cosolvent and reaction temperature were changed depending on the experiment. The mixture was agitated at 150 rad/min on a rotary shaker. After different time intervals, aliquots were taken (200 μl each) and 200 μl 1N HCl were added. The cells were removed by centrifugation and products were analyzed using HPLC. In some cases, after complete conversion, the product was extracted from the acidified mixture with TBME in order to determine enantiomeric purity.

2.5. General procedure for the biocatalytic hydrolysis of cyanohydrins in a preparative scale

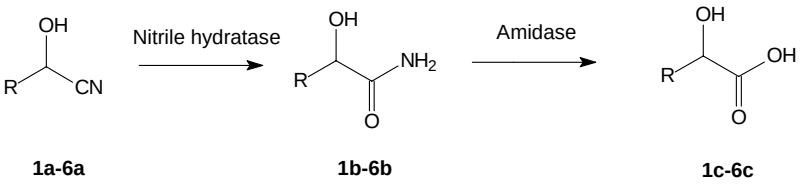
To a suspension of *R. erythropolis* NCIMB 11540 resting cells in phosphate buffer (50 mM, pH 6.5, OD 60–80), the enantiopure cyanohydrin (final concentration 10–15 g/l), dissolved in DMSO was added. The mixture (final DMSO content 2% (v/v)) was agitated at 50 °C using an orbital shaker (150 rad/min). The course of the reaction was monitored by HPLC (0.5 ml samples) as described above. After the conversion had reached completion, the cells were removed by centrifugation and washed once with distilled H_2O. The resulting aqueous solution was acidified to pH 2 with aqueous HCl (1N). Extraction with TBME followed by drying (Na_2SO_4) and concentration yielded the desired product as a pale yellow oil. After filtration over activated charcoal (EtOH) and removal of the solvent pure α-hydroxy acid was obtained in good yields (90–98%) as a white solid. If necessary further purification or ee-enhancement is possible by recrystallization from toluene.

3. Results and discussion

3.1. Enzyme activity and course of the reaction

Several aromatic and aliphatic cyanohydrins were hydrolyzed with lyophilized cells of *R. erythropolis* NCIMB 11540 in 50 mM phosphate buffer, pH 6.5 according to Method A (Table 1). The relative activities of the NHase were determined by monitoring the course of the reaction using HPLC-analysis, a typical reaction course is shown in Fig. 1. In all cases, the amide appeared as an intermediate which proved the presence of a NHase/amidase enzyme system. Aliphatic cyanohydrins were hydrolyzed at higher rates than mandelonitrile and derivatives thereof. Extremely low activity was obtained for the sterically demanding 3-phenoxymandelonitrile. Especially amide hydrolysis was found to proceed slower for aromatic cyanohydrins but fast enough for preparative

Table 1
Cyanohydrin hydrolysis catalyzed by *R. erythropolis* NCIMB 11540



1a-6a **1b-6b** **1c-6c**

Substrate	R	Activity of Nhase[a] (%)	Rate of amide hydrolysis[b]
1	$PhCH_2CH_2$	100	High
2	*E*-$CH_3CH{=}CH$	180	High
3	Ph	90	Low
4	2-Cl-C_6H_4	80	Low
5	4-CH_3-C_6H_4	120	Low
6	3-Oph-C_6H_4	10	Very low

The reaction mixtures contained in a total volume of 0.5 ml phosphate buffer (50 mM, pH 6.5) 5 mg lyophilized cells, 25 μl DMSO and 10 mM substrate.

[a] NHase activity with 2-hydroxy-4-phenylbutyronitrile (**1a**, 0.17 ± 0.02 μmol mg^{-1} (dry cells) min^{-1}) was taken as 100%.

[b] Amidase activity was not determined separately, the given values resulted from qualitative observations during the course of the reaction.

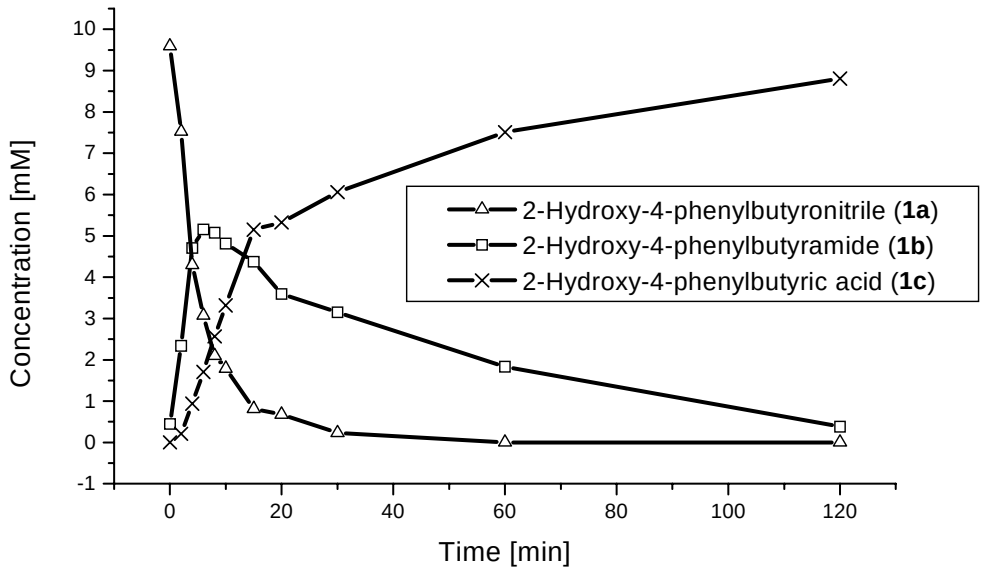


Fig. 1. Typical course of the reaction during the hydrolysis of a cyanohydrin using lyophilized cells of *R. erythropolis* NCIMB 11540. The conversion of *rac*-2-hydroxy-4-phenylbutyronitrile (**1a**, 10 mM) by resting cells was assayed in 50 mM phosphate buffer (pH 6.5) containing 5% (v/v) DMSO at 30 °C.

applications. Enantioselectivity was not observed during our investigations. In contrast to many other nitrile-hydrolyzing enzymes, enzyme induction was not required to develop nitrile-hydrolyzing activity. Whole bacterial cells could be used for this biotransformation which has the advantage that cell disruption is not required and the sensitive NHase remains within the protective "natural environment".

3.2. Optimization of reaction conditions for preparative scale applications

3.2.1. General

Employing enantiopure cyanohydrins as substrates (available via HNL catalyzed synthesis) [3,4], an easy access to both enantiomers of α-hydroxy carboxylic acids could be provided. For this reason, efforts focused on optimizing this biotransformation for preparative scale applications. One of the challenges of this approach is the reversibility of the cyanohydrin formation which causes loss of enantiopurity and reduced yields. To avoid this, the first reaction step has to proceed very quickly which means that highest enzyme activity is required. Consequently, the use of freshly prepared resting cells is favored for preparative applications because we found that lyophilization caused a decrease of enzyme activity. Suspensions of resting cells can be stored at 4 °C for 1 day and at −18 °C for several days without significant loss of activity.

3.2.2. Buffer and pH

To determine the most suitable buffer system the pH was changed to lower (pH 5, acetate buffer) and higher (pH 8, tris buffer) values (Method A). Especially lowering the pH would be favorable with respect to the stability of the cyanohydrins. However, the experiments showed that all deviations from 50 mM phosphate buffer, pH 6.5 reduced the activity to 10–20% and only incomplete conversions were obtained.

3.2.3. Substrate concentration

An important point regarding the scale up of this hydrolysis reaction is the enhancement of the substrate concentration. However, as mentioned above, a rapid first hydrolysis step is necessary due to the instability of cyanohydrins in this aqueous buffer system. To meet this requirement, the enzyme amount (cell density) has to be adapted to the substrate concentration. Starting from a substrate concentration of 10 mM which was used during the first analytical experiments (Method A), the substrate concentration was increased in several increments to determine the optimal cell density and time needed for nitrile hydrolysis (Method B). It was found that up to 10–15 g/l substrate could be used and high reaction rates were obtained using cell suspensions with an OD of approximately 60–80. At higher concentrations problems such as substrate decomposition and incomplete conversion arose. This could be due to enzyme deactivation or substrate/product inhibition. The use of higher cell densities turned out to be impractical because of the viscosity of the resulting mixture.

3.2.4. Cosolvent

A small amount of an organic cosolvent is often used to ensure sufficient solubility of the lipophilic, and in some cases, solid cyanohydrin substrate. Several organic solvents were examined in this respect (5% (v/v), Method B) and the hydrolysis of (*R*)-2-chloromandelonitrile (**4a**) was monitored (Fig. 2). Dimethylsulfoxide (DMSO) was found to be the most suitable cosolvent followed by *N*,*N*-dimethylformamide (DMF). Ethanol and 2-propanol clearly gave slower conversions, which suggests that NHase and amidase are sensitive to solvent change. The use of all other solvents resulted in incomplete conversion and low reaction rates due to enzyme deactivation and low substrate solubility. Another problem which appeared was agglutination of the cells. Regarding the amount of cosolvent, experiments showed better results using low solvent volumes. About 2% DMSO ensured sufficient substrate solubility for concentrations up to 15 g/l.

3.2.5. Temperature effect

The biocatalytic hydrolysis of (*R*)-2-chloromandelonitrile (**4a**, 60 mM) was assayed at 30, 40 and 50 °C (Method B) to determine if an acceleration of the reaction was possible by employing higher temperatures. This was not found to influence nitrile hydrolysis, this reaction always being complete in 30 min. The rate of amide hydrolysis was clearly enhanced using higher reaction temperatures (Fig. 3). No decrease in optical purity was observed.

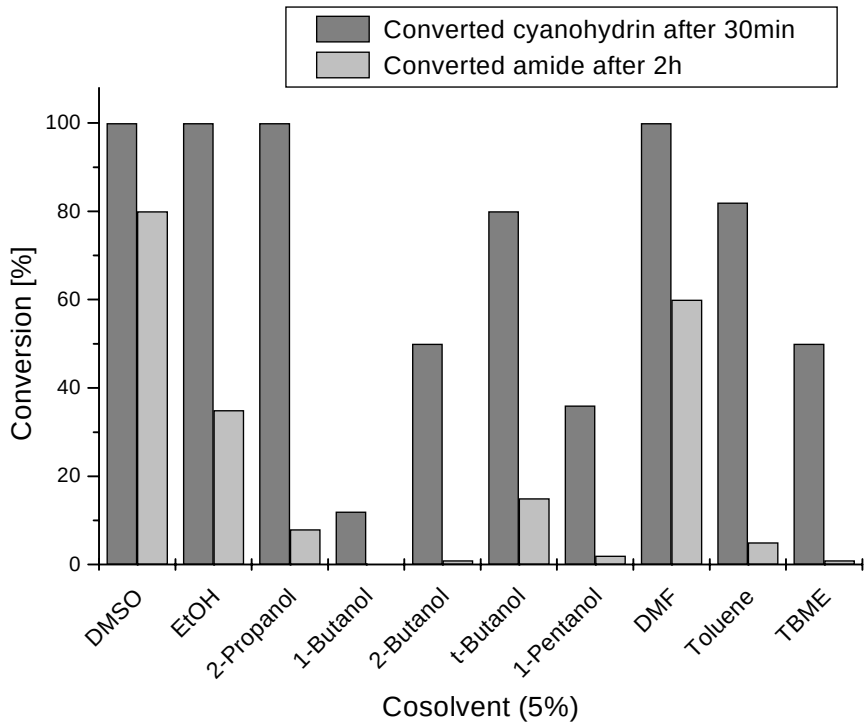


Fig. 2. Effect of organic cosolvents (5% (v/v)) on the activity of the NHase/amidase enzyme system of *R. erythropolis* NCIMB 11540. Whole resting cells were assayed in 50 mM phosphate buffer (pH 6.5) with (*R*)-2-chloromandelonitrile (**4a**, 60 mM) at 30 °C.

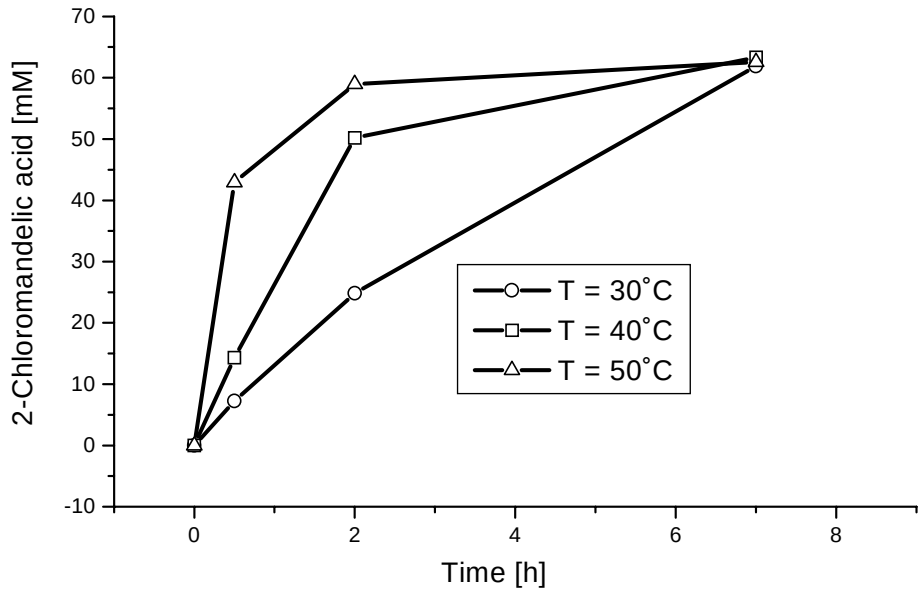


Fig. 3. Enhancement of the reaction rate using higher temperature. The hydrolysis of (*R*)-2-chloromandelonitrile (**4a**, 60 mM) was assayed using whole resting cells of *R. erythropolis* NCIMB 11540 in phosphate buffer (50 mM, pH 6.5). Only the hydroxy acid **4c** formed is indicated because nitrile hydrolysis is quantitative after 30 min.

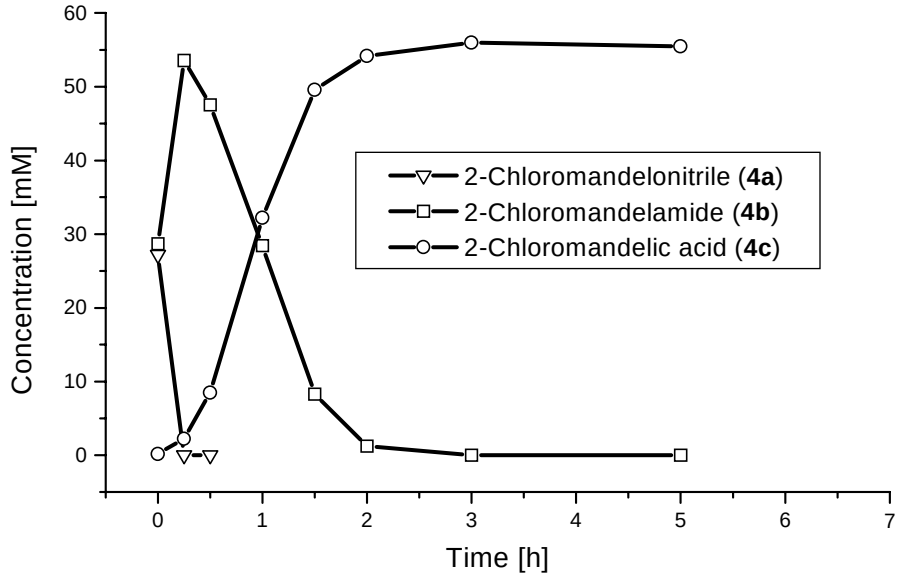


Fig. 4. Reaction course during the hydrolysis of 1.3 g (*R*)-2-chloromandelonitrile (**4a**) employing whole resting cells of *R. erythropolis* NCIMB 11540 in phosphate buffer (50 mM, pH 6.5) 50 °C.

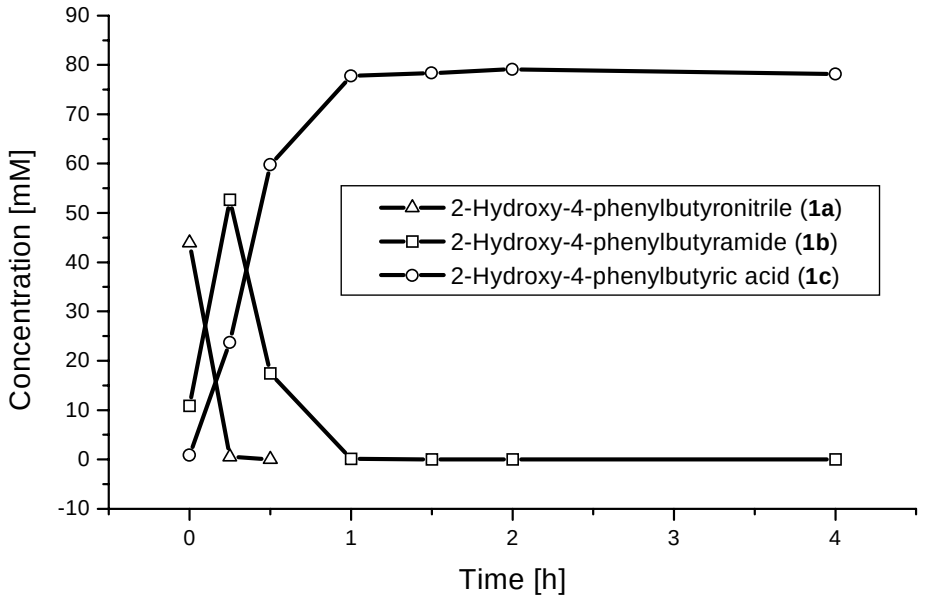


Fig. 5. Reaction course during the hydrolysis of 2.0 g (*R*)-2-hydroxy-4-phenylbutyronitrile (**1a**) employing whole resting cells of *R. erythropolis* NCIMB 11540 in phosphate buffer (50 mM, pH 6.5) at 50 °C.

3.3. Application of the system to the preparation of α-hydroxy carboxylic acids

The applicability of this system was demonstrated with the synthesis of two important pharmaceutical intermediates. (*R*)-2-chlormandelic acid (**4c**) was prepared from (*R*)-2-chloromandelonitrile (**4a**) in excellent yield and enantiomeric purity. In a typical experiment, 1.3 g **4a** (ee > 99%) were completely hydrolyzed using a suspension of resting cells (140 ml, OD 63, substrate concentration 9.3 g/l) within 3 h (Fig. 4). After work up, 1.4 g pure (*R*)-2-chloromandelic acid (**4c**, 98%, ee > 99%) were obtained. As a second example, (*R*)-2-hydroxy-4-phenylbutyric acid (**1c**) was also prepared by the use of this procedure. The substrate, (*R*)-2-hydroxy-4-phenylbutyronitrile (**1a**) was obtained by HNL catalyzed cyanohydrin formation using (*R*)-HNL from *Prunus amygdalus*. The reaction yielded the cyanohydrin quantitatively with an ee of 98%, this being in contrast to previously reported results [35]. This crude cyanohydrin (2.0 g) was subjected to biocatalytic hydrolysis, again using a suspension of *R. erythropolis* cells (150 ml, OD 75, substrate concentration 13 g/l). Complete conversion was reached within 90 min (Fig. 5). After work up, pure (*R*)-2-hydroxy-4-phenylbutyric acid (**1c**) was obtained in high yield (2.2 g, 98%) without loss of enantiomeric purity (ee 98%). In both cases, an extremely high hydrolysis rate of the nitrile function with these substrate concentrations (9.3 and 13 g/l) was observed. The first sample was taken immediately after substrate addition and mixing, subsequent analysis showed that at least half of the substrate had already been consumed.

4. Conclusions

In conclusion, we have shown that bacterial cells of *R. erythropolis* NCIMB 11540 can be used as a convenient catalyst for the nitrile hydrolysis of cyanohydrins. The preparation and use of this highly active biocatalyst is simple, thus offering the synthetic organic chemist a practical alternative to chemical methods. The required enantiopure substrates are readily accessible via HNL-catalyzed cyanohydrin synthesis. This biocatalytic approach, employing a sequence of two enzyme catalyzed transformations, provides a mild and environmentally benign method for the preparation of enantiopure α-hydroxy carboxylic acids.

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