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Release of an enantioselective nitrilase from *Alcaligenes faecalis* MTCC 126: a comparative study

Abstract Nitrilases constitute an important class of hydrolases, however, cheap and ready availability of enzyme sources limit their practical synthetic applications. The present investigation was directed to compare the applicability of various physical cell disintegration methods namely, solid shear, liquid shear and sonication, for the release of an enantioselective nitrilase from *Alcaligenes faecalis* MTCC 126. Different parameters associated with each method were optimized in order to ensure maximal release of active nitrilase. The methods were also compared under optimal conditions for their efficiency of nitrilase release and extent of cell disruption, and enzyme release were visualized under a differential interference contrast microscope (DIC) and SDS-PAGE, respectively. Maximum release of the enzyme protein from the cells was observed in case of liquid shear method employing high-pressure homogenization, however, the specific activity of nitrilase was highest in cell-free extract (CFE) generated by sonication. Both the solid shear and liquid shear proved to be equally effective for maximum release of intracellular enzymes, however, from the specific activity point of view, sonication was found to be a better one compared to other two methodologies. The generated cell-free extract can be further employed for the production of enantiopure chiral carboxylic acids, which are important chiral building blocks.

Keywords Cell disintegration · CFE ·
Nitrilase · Sonication · Liquid shear · Solid shear ·
Mandelonitrile · *Alcaligenes faecalis*

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Introduction

One of the bottlenecks in the development of a specific biotransformation is to find the appropriate biocatalyst [1]. Unfortunately, at present, very little enzyme preparations are commercially available, which are very expensive and therefore seriously limit their synthetic applications. If the enzyme preparations are not available commercially, the desired activities could be found by using methods like 'intelligent' screening [2, 3]. There is a considerable industrial interest in the enzymatic conversion of nitriles to their corresponding high-value carboxylic acids owing to the desirability of conducting such conversions under mild conditions that would not alter other labile reactive groups [4]. Additionally, the existence of nitrile-hydrolyzing enzymes that exhibit high degree of *enantio*- and *regio*-selectivity, offers synthetic possibilities that are difficult to achieve by conventional catalytic approaches [5].

(R)-$(−)$-mandelic acid is an important chiral building block for the production of semi-synthetic cephalosporins [6], penicillins [7], antitumor agents [8], antiobesity agents [9] etc., and is also used as a chiral resolving agent. Production of this important fine chemical can be achieved by chemical as well as by different enzymatic routes. Nitrilase-mediated pathway offers significant advantages over other routes because of the absence of the cofactor involvement, cheap starting material in the form of mandelonitrile and above all a possibility of carrying out a dynamic kinetic resolution which provides theoretically 100% yield of the product. Nitrilase from *Alcaligenes faecalis* MTCC 126 (corresponding to ATCC 8750) is known to enantioselectively hydrolyze racemic mandelonitrile to (R)-$(−)$-mandelic acid with high yield and enantiomeric excess [10]. The enzyme was purified to homogeneity and found to have a single subunit of molecular weight of 32 kDa. Whole-cell immobilization is an automatic choice for the biocatalytic reaction using this class of enzymes (nitrile

hydrolases) because of their intracellular nature. However, the immobilization of the whole cells would also increase the mass-transfer limitations already imposed by the cell membrane. Therefore, immobilizing the crude-protein extract could help to lift such diffusional limitations and at the same time allow reusability of the stabilized and 'activated' biocatalyst. Moreover, to gain access to molecular properties of the enzyme and define the various biocatalytic reaction parameters in a more better way, purification and further characterization of the enzyme is necessary. Cell disintegration is the first step for the downstream processing of intracellular enzymes. In the present investigation, we have attempted to compare various cell disruption methods, namely sonication, solid and liquid shear for the liberation of nitrilase from *A. faecalis* MTCC 126. The released nitrilase was further used for the production of (R)-$(-)$-mandelic acid (Fig. 1), an important chiral building block.

Materials and methods

Materials

Mandelonitrile and *n*-butyronitrile were obtained from Aldrich Chemical Company (Milawukee, USA). Growth media components were obtained from Hi-Media Inc. (Mumbai, India). Inorganic and buffer salts were obtained from Qualigens Inc. (Mumbai, India). Solvents, mineral acids and other chemicals of analytical grade were obtained from Ranbaxy Fine Chemicals Ltd. (Mohali, India) and S.D. Fine Chemicals Ltd. (Boisar, India). The microorganism, *A. faecalis* MTCC 126, was procured from Microbial Type Culture Collection (MTCC, IMTECH, India).

Microorganism and cultivation condition

Alcaligenes faecalis MTCC 126 was grown in a medium of the following composition (g/l): ammonium acetate 10, peptone 5, yeast extract 5, dipotassium hydrogen phosphate 5, magnesium sulphate 0.2, ferrous sulphate 0.03, sodium chloride 1 and *n*-butyronitrile 3 (pH 7.2) in a Bioflo 3000 bioreactor (New Brunswick Scientific, Edison, NJ, USA), using a 7l vessel with a working volume of 4.5 l. The cultivation was carried out at 30°C for 12 h (agitation 200 rpm and aeration 1 vvm). The seed culture was generated in 250-ml flask having the same medium composition, and was transferred to a 1-l flask to generate the inoculum (30°C for 18 h at 200 rpm). After the fermentation was over, cells were harvested by centrifugation at 10,000 g for 20 min at 4°C and washed with phosphate buffer (0.05 M, pH 7.0) containing 0.1 mM EDTA and 1 mM DTT and resuspended in the same buffer to obtain appropriate final cell concentration.
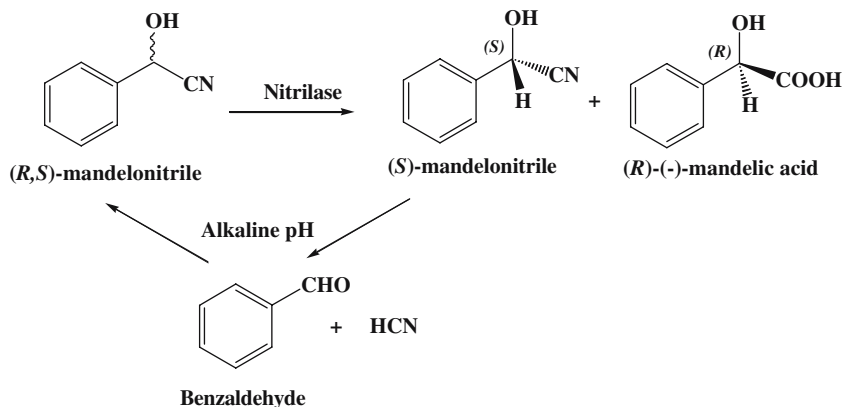
Enzyme assay

The reaction mixture containing the biocatalyst, suspended in phosphate buffer (0.05 M, pH 7.0) and the substrate (mandelonitrile) with a concentration of 12.5 mM, was incubated at 30°C for 20 min under shaking condition. The reaction was stopped by adding 0.1 N HCl. The reaction mixture was centrifuged at 10,000 g for 10 min, and the clear supernatant obtained was utilized for ammonia estimation by fluorimetric method [11].

Liberation of nitrilase from whole cells

Sonication

Aliquots of the resting cell suspension (200 mg/ml) were subjected to sonication with 30 s pulse-on and pulse-off time each using a Misonix ultrasonic processor XL 2020 (NY, USA) and the samples were collected at different time intervals to determine the enzyme activity and protein content. The effect of various parameters like sonication time (0.25–10 min), acoustic power (30–120 W) and cell concentration (50–500 mg/ml) was also

Fig. 1 Nitrilase mediated hydrolysis of mandelonitrile to enantiopure (R)-$(-)$-mandelic acid

measured. The enzyme activity was determined in both supernatant and debris as described earlier. The sample tubes were cooled in ice-bath while sonication and sampling were carried out.

Liquid shear

A Thermo Spectronic French Press (Rochester, USA) was operated at various pressures ranging from 1,000 to 18,000 psi in a 40 K cell to determine the effect of applied pressure on the release of nitrilase from the cell suspension (200 mg/ml). The effect of the other related parameters such as number of passages (1–4), flow rate of the slurry (0.25–8 ml/min) and cell concentration (50–500 mg/ml) were also investigated. Enzyme activity and protein content were estimated as described earlier. About 12 h prior to high-pressure homogenization, the 40 K cell was stored at 4°C and the cell suspensions were cooled by using an ice-bath after each passage.

Solid shear

A 300 ml chamber of Multi Lab Dyno-Mill (Willy A. Bachofen Maschinenfabrik, Basel, Switzerland) was charged with lead-free glass beads and cell suspension (200 mg/ml) and the enzyme release kinetics were determined. The effect of other parameters like, cell concentration (50–500 mg/ml), bead size (0.1–1.5 mm), bead volume (50–80%) and impeller tip speed (1,000–4,000 rpm) on the enzyme release were assessed. The chamber was cooled by using a mixture of glycerol and water at 0°C through the jacket of the vessel.

Differential interference contrast microscopy (DIC)

The bacterial cell debris obtained after centrifugation of the disrupted cell slurry at 15,000 g for 30 min at 4°C was appropriately diluted and the images were captured using DIC microscope model: E600 equipped with DIC module with a mounted Nikon camera (Japan). The images were visualized using Image Pro Express (Media Cybernetics, Madison, USA) at ×100 magnification.

Nitrilase purification

Alcaligenes faecalis cells (concentration 200 mg/ml) were disrupted using sonication under optimum conditions. Cell debris was removed by centrifugation (15,000 g, 30 min, 4°C) and CFE was subjected to ammonium sulphate fractionation (10%, w/v). The solution was stirred for 1 h and the precipitate formed was removed by centrifugation (15,000 g, 20 min, 4°C) and discarded. The supernatant was subjected to increased concentration of ammonium sulphate fractionation (35%, w/v) and the resulting precipitate was collected by further centrifugation. The precipitate was resuspended in

phosphate buffer (0.05 M, pH 7.5) containing 1 mM DTT and dialyzed overnight against the same buffer. The dialyzed solution was centrifuged at 100,000 g for 2 h (Beckman, Fullerton, CA, USA) and the supernatant obtained was loaded to phenyl-sepharose column, pre-equilibrated with same buffer containing 30% ethylene glycol. The column was flushed with a linear gradient (30–70%) of ethylene glycol in same buffer using fast protein liquid chromatography (Aktaprime, Amersham Biosciences, Uppsala, Sweden). The eluted active fractions were combined and concentrated by Centricon YM-10 membrane (Millipore Corporation, Bedford, USA), then dialyzed overnight against phosphate buffer (0.05 M, pH 7.5) containing 30% ethylene glycol. The dialyzed solution was loaded to DEAE-cellulose column equilibrated with buffer containing 30% ethylene glycol, and then eluted with a linear gradient of sodium chloride in the same buffer (0–0.5 M). The active fractions were concentrated by filtration on a Centricon YM-10 membrane and dialyzed overnight against the same buffer containing 30% ethylene glycol. The protein solution therefore obtained was stored in small aliquots at −80°C.

Protein determination

Protein was determined by the Bradford method [12] with bovine serum albumin as standard.

Gel electrophoresis

Gel electrophoresis was performed in 12% SDS-polyacrylamide gel with a Tris–Glycine buffer system [13]. The gel was stained with commassie brilliant blue (R 250) and the densitometric analysis of the gel was carried out on a Gel Doc 2000 and QuantityOne software (Bio-Rad Laboratories, Hercules, USA).

Sample preparation for high performance liquid chromatography (HPLC)

The pH of the reaction mixtures with CFE was brought down to 2.0 by the addition of 6 N HCl and the precipitated protein was removed by centrifugation (15,000 g, 20 min, 4°C). The clear supernatant obtained was subjected to reverse phase HPLC analysis.

For chiral HPLC analysis, after removal of the proteins the pH of the reaction mixture was adjusted to 8.5 with 2 N NaOH and washed with equal volume of diethyl ether. The pH of the aqueous layer was then readjusted to 1.5 with 6 N HCl and the desired product was extracted with equal volume of the same ether. The extract was concentrated under reduced pressure to yield mandelic acid.

High performance liquid chromatography

The amounts of mandelic acid, mandelamide and mandelonitrile were assayed by analytical high performance liquid chromatography (model 10AD VP) (Shimadzu, Japan) equipped with a LiChroCART RP-18 column (250×4 mm, 5 μm) (Merck, Germany) at a flow rate of 0.8 ml/min with a solvent system 0.01 M phosphate buffer (pH 4.8) and methanol (65:35, v/v). The retention times for mandelic acid, mandelamide and mandelonitrile were 2.6, 4.2 and 18.3 min, respectively. $A_{254 \text{ nm}}$ was measured.

The optical purity of the mandelic acid was determined by analysis of the enantiomers on CHIRALCEL-OD-H column (250 mm × 0.46 mm, 5 μm) (Daicel Chemical Industries, USA) at a flow rate of 0.5 ml/min with a mobile phase containing hexane, isopropanol and tri-fluoro acetic acid (90:10:0.2, v/v). The retention times for (S)-$(+)$ and (R)-$(-)$-isomers were 15.5 and 17.5 min, respectively. $A_{254 \text{ nm}}$ was measured.

Results

Sonication

Sonication is one of the most commonly employed techniques for cell disruption in the laboratory scale. This allows fast release of significant amount of intracellular protein in its active form. Enzyme release by sonication may be described using the following equation:

$$R = R_{\text{m}}(1 - e^{-kt}) \tag{1}$$

where R and R_{m} refer to the released enzyme activity and maximum enzyme activity that can be released, respectively (U mg^{-1} min^{-1}), k refers to disruption constant for sonication and t refers to time of sonication (min) [14]. The enzyme released (%) and enzyme retained (%) in the debris is the enzyme activity expressed relative to activity of the whole cells. The release of nitrilase from the whole cells was monitored over a period of 15 s to 10 min upon sonication at acoustic power of 120 W with 30 s pulse-on and 30 s pulse-off time. The total cellular protein release increased upto 7 min but the enzyme of interest was released within 2 min, beyond which the enzyme was found to be unstable under the conditions of release. It may be due to the release of active proteases, which might have degraded the desired enzyme or denaturation of the enzyme because of the heat generated by sonication. A simultaneous decrease in the enzyme activity retained in the cell debris was observed (Fig. 2a).

In order to release the intracellular nitrilase by sonication, various associated parameters were optimized such as sonication time, acoustic power and cell concentration. Since the horns and micro-tip probes of the sonicator amplify the longitudinal vibration of the convertor within the system, higher amplifications result in greater cavitation and hence greater disruption. The effect of acoustic power for nitrilase release was therefore determined by sonicating the cell suspensions in the range of 30–120 W for 2 min. Below 60 W, there was little release of protein, however, a steady increase in the enzyme released (%) was observed up to 120 W (Fig. 2b), which was used for further studies. The concentration of cells in the suspension (50–500 mg/ml) did not seem to affect the nitrilase release under the conditions of sonication (Fig. 2c).
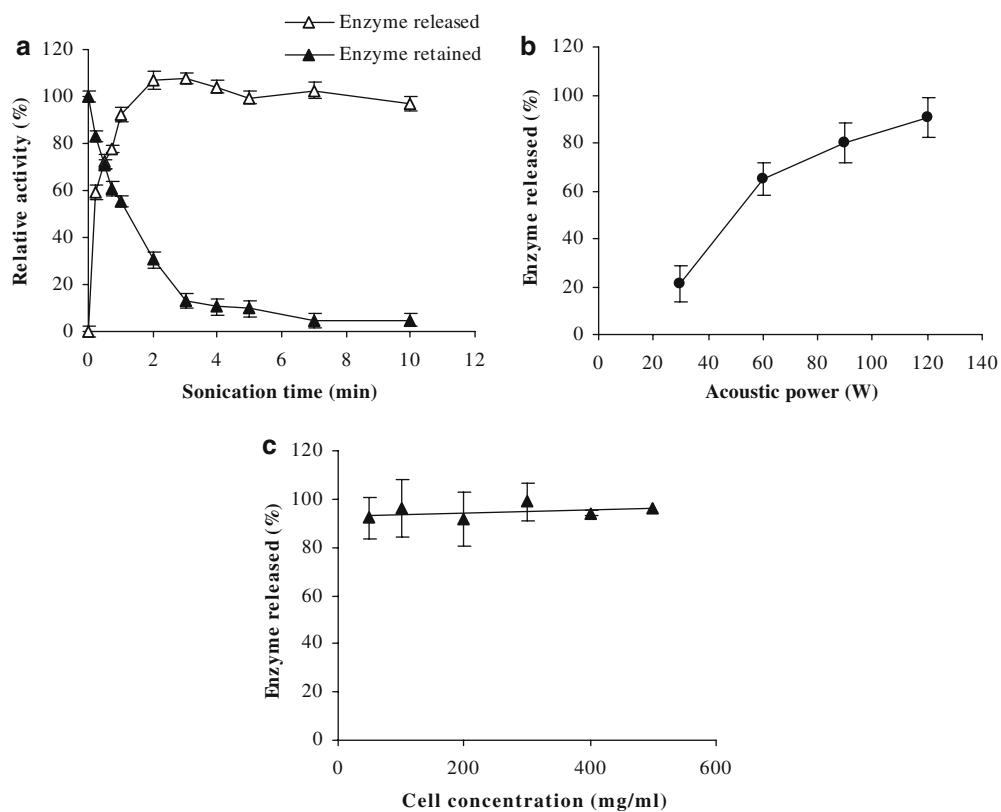
Liquid shear

High-pressure homogenization based on the liquid shear method is the most widely used method for the extraction of intracellular bacterial enzymes [15]. High-pressure homogenizer forces the cell suspension through a small orifice and the cells are disrupted because of the extremely high-shear forces generated by the pressure drop, ΔP ($P_{\text{appl}} - P_{\text{atm}}$). Enzyme release by high-pressure homogenizer can be described using the following equation:

$$\ln\left(\frac{R_{\text{m}}}{R_{\text{m}} - R}\right) = kt \tag{2}$$

where R_{m} is the maximum releasable protein, R is the protein released from the cells, k is the rate constant for disruption and is a function of homogenizer pressure and valve geometry [16].

In order to determine how the applied pressure affects the disintegration of the whole cells and release of intracellular nitrilase, cell suspensions (200 mg/ml) were subjected to different gauze pressures ranging from 1,000 to 18,000 psi. With the increase in applied pressure the nitrilase activity in the cell-free extract increased up to 12,000 psi after which it attained saturation (Fig. 3a). The figure indicated that maximum release of nitrilase took place at 12,000 psi, however, compared to sonication the enzyme seems to be more stable under the conditions of release by liquid shear method probably because of the less amount of heat caused denaturation of the nitrilase. The total protein release from the cells also increased up to 12,000 psi and thereafter attained saturation. Upon variation of number of passages of the cell suspension through the homogenizer, optimum release of nitrilase was observed after two cycles (Fig. 3b). Operation of the homogenizer for more than two passages resulted generation of very fine debris causing severe downstream clarification problems and no significant enhancement in the amount of enzyme released. The cell concentration of the suspension utilized for disintegration (50–500 mg/ml) did not seem to affect the nitrilase release from the whole cells (Fig. 3d), while increasing the flow rate above 1 ml/min of the cell slurry through the orifice severely lowered the release of

Fig. 2 Effect of different parameters on the release of nitrilase from *Alcaligenes faecalis* MTCC 126 by sonication, **a** sonication time, **b** acoustic power, **c** cell concentration (sample volume, 1 ml; microtip probe diameter, 1/2″)

Fig. 3 Effect of various parameters on the release of nitrilase from *A. faecalis* MTCC 126 by liquid shear employing a high pressure homogenizer, **a** applied pressure, **b** number of cycles, **c** flow rate, **d** cell concentration (sample volume, 2 ml; French pressure cell diameter, 3/8″)
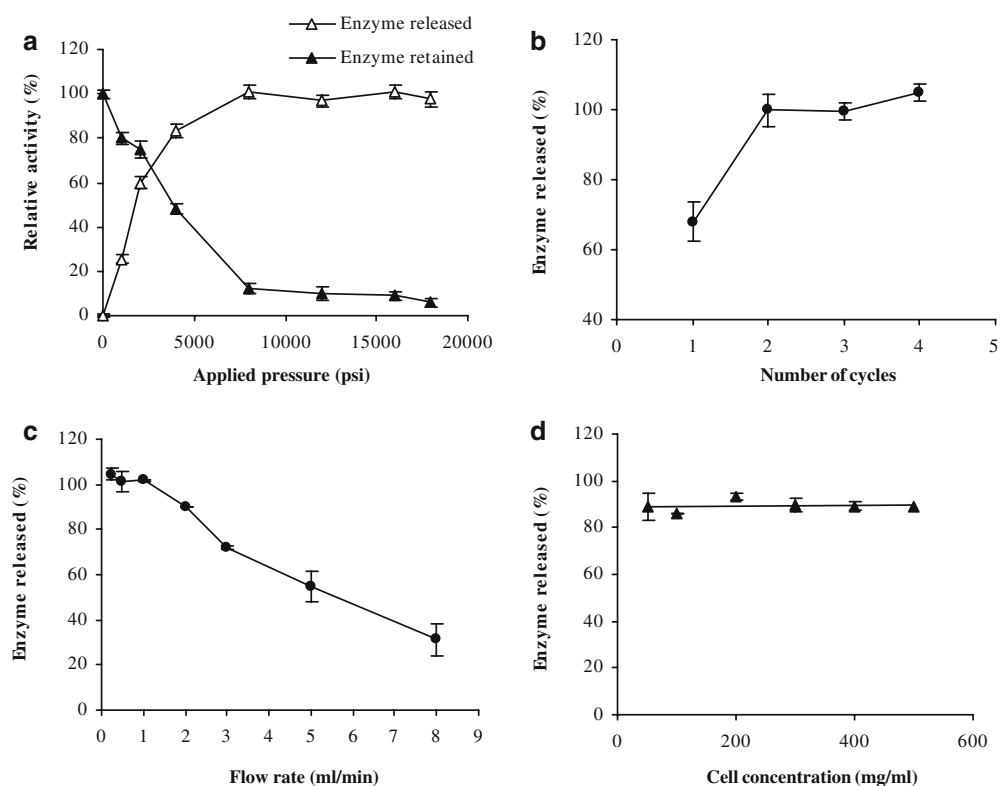
Fig. 4 Effect of various parameters on nitrilase release from *A. faecalis* MTCC 126 by solid shear method employing a bead mill, **a** milling time, **b** bead size, **c** bead volume, **d** impeller tip speed, **e** cell concentration (sample volume, 180 ml)
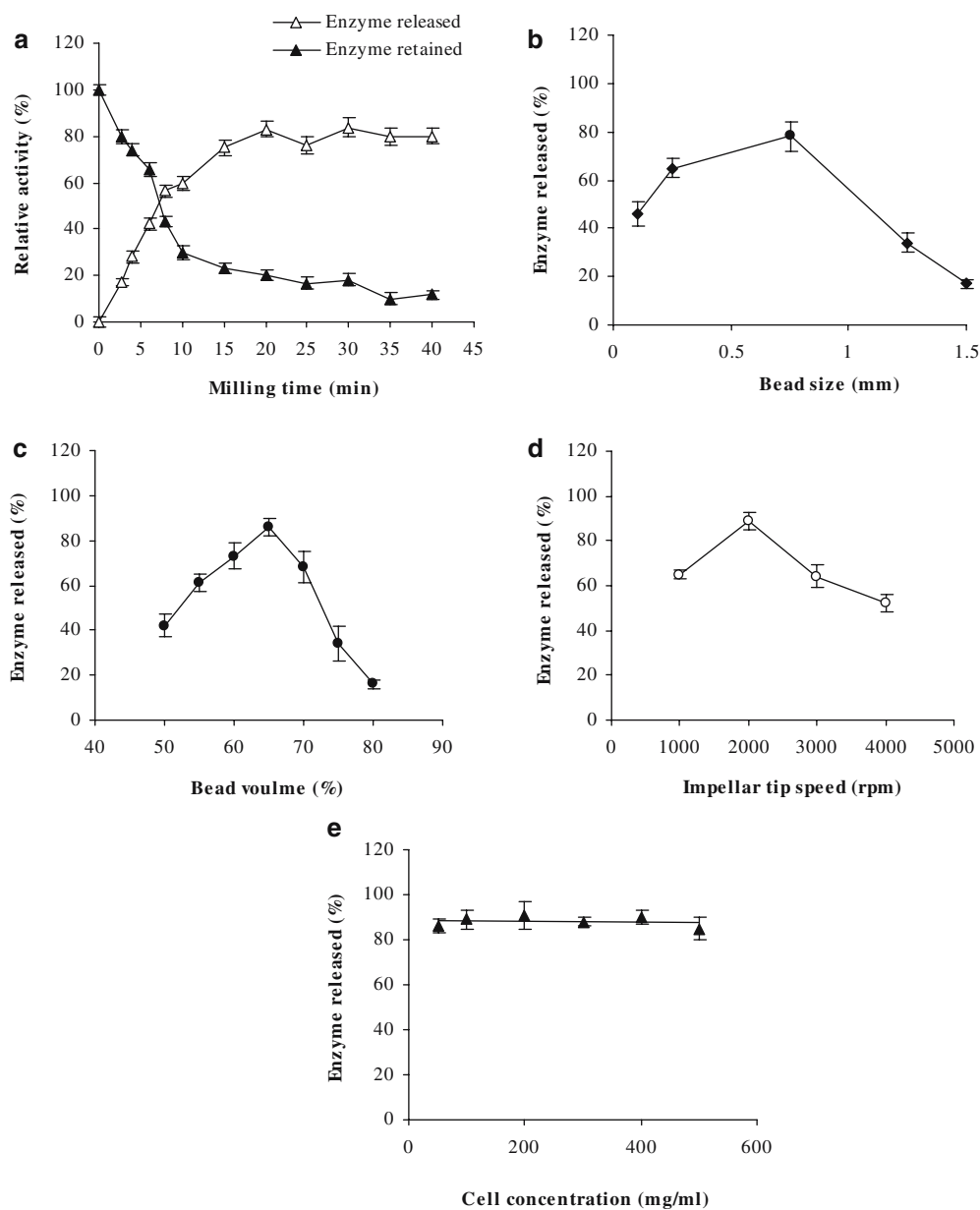
Table 1 Comparison of different disintegration methods employed for disruption of *Alcaligenes faecalis* MTCC-126 cells

| Methods employed	Nitrilase released (%)	Nitrilase retained (%)	Total protein in CFE (mg/ml)	Specific activity in CFE (U/mg)	Percentage breakage (%)	Disruption constant (k)
Sonication	88.80	11.2	9.73	125.98	67.65	0.52
Liquid shear	93.40	6.6	16.14	66.11	79.41	0.64
Solid shear	90.02	9.98	15.45	62.37	75.03	0.57

nitrilase (Fig. 3c). This is due to the fact that at higher flow rates (>1 ml/min) cells experienced the pressure drop for less period of time which caused incomplete disruption.

Solid shear

A bead mill consists of a chamber filled with small glass beads, 0.1–1.5 mm in diameter. With the cell suspension

Fig. 5 Differential interference contrast micrographs of disrupted cells of *A. faecalis* MTCC 126 generated by different cell disintegration methods, **a** culture prior to disruption, **b** cells after liquid shear, **c** cells after solid shear, **d** cells after sonication (*IC* Intact cells, *DC* Disrupted cells)

Control   Liquid shear

Solid shear   Sonication
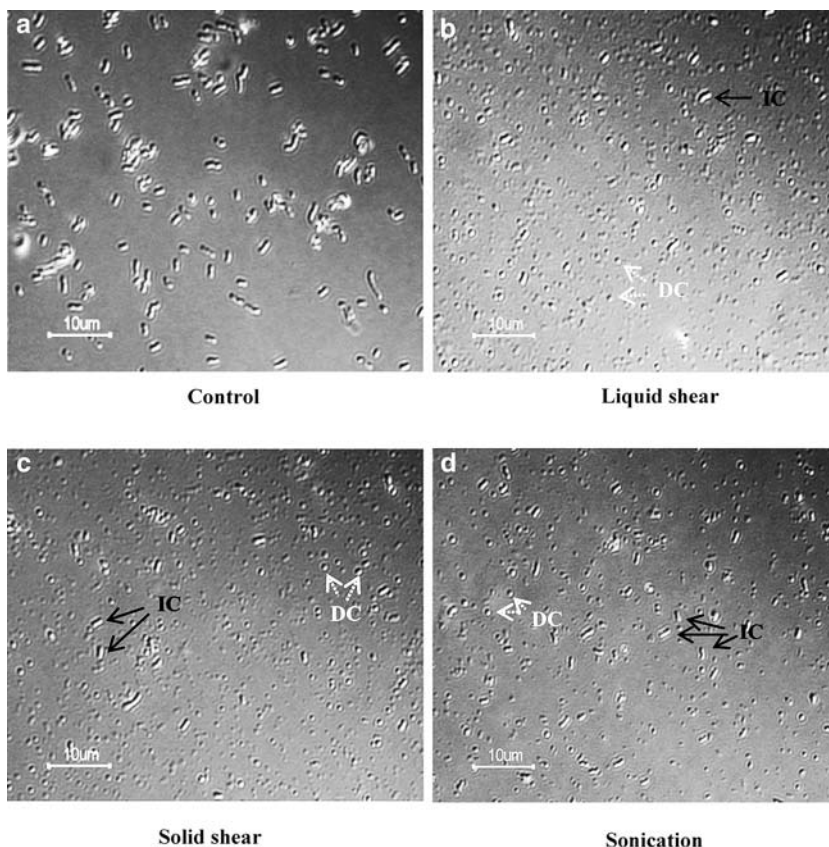
inside the chamber and the impeller rotating, the beads generate high shear forces, which disrupt the cell walls. Enzyme release by bead milling can be described using the following equation

$$\ln\frac{R_m}{R_m - R} = \frac{k(1 - \phi)V}{F} \times N \qquad (3)$$

where R and R_m refer to the released enzyme activity and maximum enzyme activity that can be released, respectively, (U mg^{-1} min^{-1}), t is the milling time, k is the disruption rate constant, V is the volume of the grinding chamber, ϕ refers to the fraction of the chamber volume occupied by the beads and F is the flow rate [17].

The variation of nitrilase release profile with time of milling was shown in Fig. 4a. It was evident that maximum amount of enzyme was liberated within 20 min after which it attained saturation. The extent of enzyme release also depended upon bead size and loading, with beads having a diameter of 0.75 mm (Fig. 4b) and 65% bead loading (Fig. 4c) producing best results. The poor disruption performance of the smallest beads (diameter 0.1 mm) was associated with fluidization effect of the slurry in the grinding chamber. The larger beads were too heavy to be significantly affected by the flow of the slurry, hence relatively less cell disintegration was achieved. The effect of impeller speed was monitored over a range of 1,000–4,000 rpm. The enzyme release increased up to 2,000 rpm but beyond this speed release

of nitrilase gradually declined (Fig. 4d). The rotor speeds greater than 2,000 rpm probably caused greater slip between the rotor and the grinding elements, hence effectiveness of the momentum transferred to the beads did not improve with further increase in rotational speeds. Decrease in the enzyme released at higher impeller speeds may be attributed to the denaturation of the nitrilase because of heat generation at those speeds. However, no effect of cell concentration on nitrilase release was observed similar to the observation in case of sonication and high-pressure homogenization (Fig. 4e).

Comparison of the different methods employed for cell disintegration and nitrilase release

The various methods employed for cell disintegration were compared at their optimal conditions of release to choose the most suitable method (Table 1). The cell disruption was also visualized by using DIC microscope (Fig. 5) and the release pattern of the nitrilase was analyzed by 12% SDS-PAGE (Fig. 6a) of the cell-free extracts produced by employing different methods. Cell breakage (%) was estimated by counting the number of intact cells in disrupted samples vs. the untreated samples in at least 50 different microscopic fields and the value represented the average of those readings. It can be concluded that the liquid shear method employing high-pressure homogenization resulted in greater cell
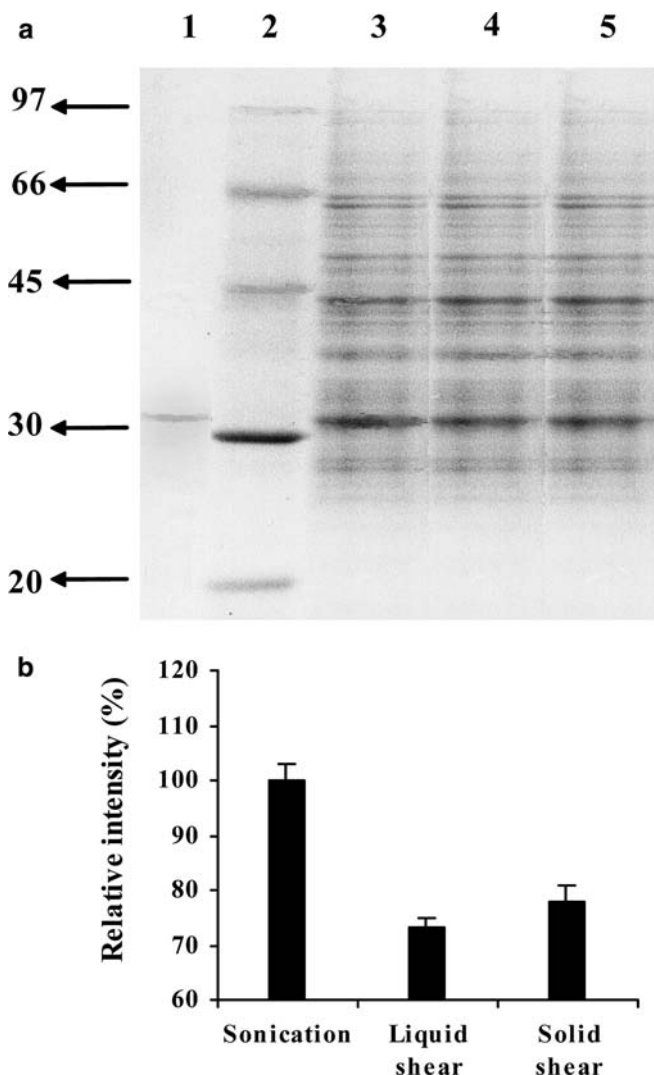
Fig. 6 a Protein profiles of cell free extracts generated by various disruption methods in 12% SDS-polyacrylamide gel *Lane 1* purified nitrilase, *Lane 2* standard marker proteins (kDa): Phosphorylase b (97), Albumin (66), Ovalbumin (45), Carbonic anhydrase (30), Trypsin inhibitor (20), *Lane 3* CFE released by sonication, *Lane 4* CFE released by liquid shear, *Lane 5* CFE released by solid shear (The direction is from top (cathode) to bottom (anode). Lanes 3, 4, and 5 were loaded with equal amount (30 μg) of protein) **b** Densitometric analysis of nitrilase band intensities, released by various cell disintegration methods

sample which was considered as 100%). Disruption constant (k) signifies the efficiency of a particular cell disintegration process. Higher disruption constant value depicts more efficient process. Based upon the value of disruption constants (k) obtained from the equations, high-pressure homogenization seemed to result in maximum protein release from the cells compared to other two techniques. This was also evident from the fact that higher percentage disintegration was obtained with high-pressure homogenization (Table 1). Therefore, although sonication resulted in less cell breakage but was efficient technique for the selective release of the nitrilase from the cells, which can be subsequently used for further immobilization and biocatalytic reactions, leaving the system free of competitive reactions due to the presence of other enzymes.
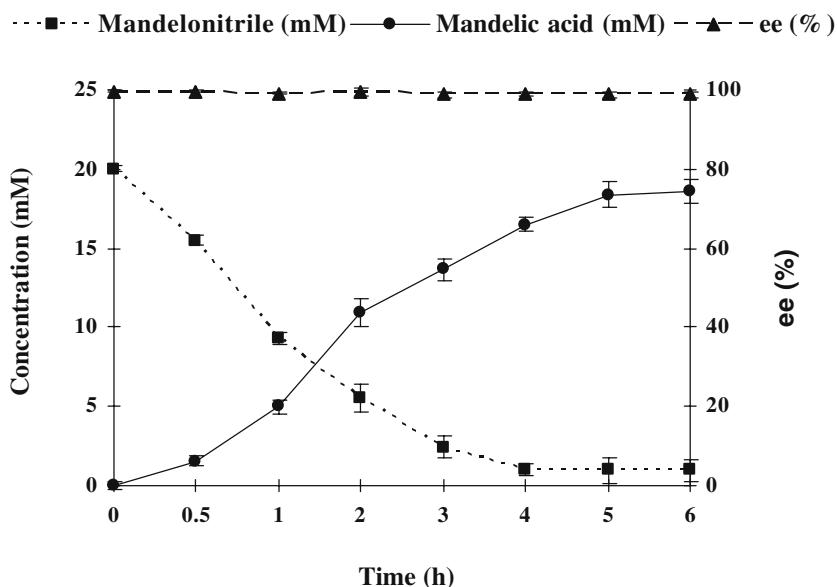
Biotransformation with CFE

The CFE liberated after sonication was utilized for hydrolysis of racemic mandelonitrile and the reaction course was monitored by taking samples at regular intervals which were analyzed by both reverse phase and chiral HPLC. The reaction was done at a scale of 20 ml in phosphate buffer (0.05 M, pH 7.5) utilizing 2 ml CFE and reaction was initiated by the addition of 20 mM mandelonitrile solubilized in ethanol. The results indicated that the enzyme retains its enantioselectivity upon its release to give 93% conversion with 98.89% ee for the *R* enantiomer within 6 h (Fig. 7).

Discussion

Cell disruption is the first and one of the most important steps in the downstream processing of intracellular enzymes from microorganisms. For releasing intracellular components, optimal choice of operating conditions for maximum release of the enzyme of interest is desired as it affects the overall cost and the enzyme yield. Limited sources of purified nitrile hydrolyzing enzymes have come up only recently, however for practical synthetic applications such sources cannot be utilized because of their expensive nature. Moreover, purification of the enzyme is essential for the classification of a definite reaction pathway, to discover new enzymes or new functionalities in previously known enzyme system. It is possible only after the purification of a particular protein to clearly identify the molecular mechanisms responsible for a discrete function. Characterization of a protein in crude form never conveys actual idea of the function of the entity, as rearrangement and other chemical changes of the substrate may take place in presence of the competitive enzymes present in the crude extract. Since, nitrilase from *A. faecalis* MTCC 126 was known to hydrolyze mandelonitrile to enantiopure (*R*)-(−)-mandelic acid [10], further purification and characterization of the nitrilase would allow to ascertain

disruption and maximum release of nitrilase (93.48%), however, the specific activity of the nitrilase in the cell-free extract was highest for sonication (125.96 U mg^{-1}), indicating that the method resulted in greater selective release of nitrilase than other cellular proteins, which may be desirable for purification. The relative band intensities of the nitrilase showed a 27% greater intensity for the sonicated samples as compared to samples that were treated by high-pressure homogenization and bead mill (Fig. 6b), which showed comparable band intensities as analyzed by densitometry (73 and 77.8%, respectively, compared to band intensity of the sonicated

Fig. 7 Time course of
enantioselective hydrolysis of
racemic mandelonitrile by CFE
of *A. faecalis*

the process parameters in a more defined manner and will also lead to a better understanding of the molecular mechanism responsible for such activity. With the aim to exploit this highly enantioselective nitrilase for the generation of chiral building blocks, the present investigation was attempted to compare and evaluate the effectiveness and practical applicability of various methods of physical disruption like sonication, solid and liquid shear, for optimal release of nitrilase from *A. faecalis* MTCC 126. The unstable nature of this class of enzymes implicates whole-cell immobilization as a suitable alternative to utilize purified enzyme for biotransformation, which would additionally also increase the operational stability and facilitate reusability of the biocatalyst. However, immobilization of the whole cells would also increase the mass-transfer limitations already imposed by the cell membrane. Therefore, immobilizing the crude-protein extract could help to lift such diffusional limitations and at the same time allow for reusability of the stabilized and 'activated' biocatalyst.

Our results suggest that the choice for the final method of cell disruption be made according to the objective of the experiments. In spite of the greater effectiveness of sonication for selective release of the nitrilase compared to the other methods employed, its applicability is limited to small bench-top volumes. Liquid and solid shear methods employed by high-pressure homogenization and bead mill, respectively, appear to be equally effective methods for liberation of nitrilase from *A. faecalis* MTCC 126, for useful synthetic applications, however, these methods may prove to be expensive if purification of the enzyme is desired because of the significantly higher release of the other intracellular proteins. The lower specific activity obtained in the case of high-pressure homogenization and bead mill, may be further increased by using chromatographic techniques. Hence, an economic analysis in terms of the

cost of the subsequent task to be undertaken (like purification or large scale release for biotransformation) need to be compared before making a decision on the method of disruption. Presently, we are attempting to investigate nitrile hydrolysis by immobilized whole cells and cell-free extracts. We are also of the opinion that immobilizing the permeabilized whole cells for enhanced biotransformation could serve as a suitable alternative to this study. The protective cell envelope would shield the enzyme and a suitable degree of permeabilization would help to lower the diffusional barrier.

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