Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Immobilization of whole resting cell of *Bacillus* sp. APB-6 exhibiting amidotransferase activity on sodium alginate beads and its comparative study with whole resting cells

Pankaj Kumari, Duni Chand*

Department of Biotechnology, Multi Faculty Building (Phase-I), Gyan Path, HP University Campus, Summer Hill, Shimla - 171 005 (HP), India.

Key words: Amidotransferase, Butryamide, Butyrohydroxamic acid, Alginate, Immobilization.

***Corresponding Author: Duni Chand,** Department of Biotechnology, Multi Faculty Building (Phase-I), Gyan Path, HP University Campus, Summer Hill, Shimla - 171 005 (HP), India.

Abstract

Amidotransferase have considerable industrial interest due to potential applications in the production of useful hydroxamic acids. Hydroxamic acids (α-aminohydroxamic acid, acetohydroxamic acid, butyrohydroxamic acid etc.) have been investigated as anti-human immunodeficiency virus agents, antimalarial agents and have also been recommended for treatment of ureaplasma infections and anaemia. Amidotransferase from *Bacillus* sp. APB-6 is capable of butyramide conversion to butyrohydroxamic acid. Whole resting cells containing active amidotransferase enzyme were prepared and immobilized in the gel beads of sodium alginate, agar, polyacrylamide, Carrageenen and hydrogel. The beads were tested for amidotransferase using Iron (III) chloride reagents at 55°C and were found to be affected by substrate concentration, type of buffer, buffer pH, buffer molarity, bead size, time of incubation, solvents, metal ions and reaction temperature. These factors were optimized using sodium alginate immobilized beads. These immobilized beads were used repeatedly as biocatalysts in 10 reactions to test their reusability potential and cells retain 85.45 percent relative activity upto 7th cycle. The alginate entrapped cells were tested for the thermal in comparison to free cells and alginate immobilized cells were found more thermostable. This study proved useful in understanding the technique of immobilization of amidotransferase enzyme, its operational stability and its importance in the synthesis of butyrohydroxamic acid.

Introduction

Amidases (EC 3.5.1.4) are widespread in various organisms and they catalyze the hydrolysis of amides into the corresponding carboxylic acids and ammonia [1]. Amidase, an amide-hydrolyzing enzyme also shows acyltransferase activity. The use of acyltransferase or bacteria with microbial enzymes with acyltransferase activity may be used to convert amides to hydroxamic acids [2]. In recent years, amidases have gained considerable interest in industries for the synthesis of wide variety of carboxylic acids, hydroxamic acids and hydrazide, which find applications in commodity chemicals synthesis, pharmaceuticals agrochemicals, and waste water treatments [3]. This enzyme finds application as industrial catalyst in organic synthesis, in the treatment of industrial effluents containing toxic amides, and also as a therapeutic agent. Several hydroxamic acids are used as drugs and have been reported as tumor inhibitors, anti-HIV and anticancerous [4, 5]. Hydroxamic acids also show antimicrobial and antifungal activity [6]. In most of the industrial, analytical and clinical processes, enzymes are mixed in a solution with substrates and cannot be economically recovered after the exhaustion of the substrates. This single use is obviously quite wasteful when the cost of enzyme is considered. The needs for sustainable, efficient and cost effective processes are in demand for many chemical and biological industries [7]. Thus, there is an incentive to use enzyme or cells in an immobilized or insolubilized form so that they may be retained in a biochemical reactor to catalyze further the subsequently added substrate.

Biocatalysts with high amidase activity based on whole bacteria cells can be used to produce various carboxylic acids, including the ammonium salts of the acrylic and nicotinic acids and nonsteroid antiinflammatory preparations [8, 9, 10]. The use of an immobilized enzyme or cells makes them economically feasible to operate an enzymatic process in a continuous mode. Immobilization can alter such enzyme properties as activity, substrate specificity, and resistance to various environmental factors. Immobilized enzymes are more stable than the soluble ones, and they retain stability for long periods of time, which make them rather promising for use in industry [11]. This study was undertaken to determine the ability of the whole cells of immobilized

Bacillus sp. entrapped in different matrices for the biotransformation of butyramide to butyrohydroxamic acid. In order to simplify handling of the amidase and to improve its stability, a suitable immobilization method was searched. The immobilization technique using different matrices like agar, polyacrylamide and alginate was applied to a number of different enzymes [12]. Amidase from *R. erythropolis*, when coimmobilized on butilsepharose together with the nitrilase from *Aspergillus niger*, hydrolyzed 4-cyanopyridine to isonicotinic acid [13]. The amidase of *Rhodococcus rhodochrous* 4-1 was immobilized by covalent attachment to activated chitosan by physical sorption on carbon adsorbents and by the formation of cross linked aggregates [14].

Experimental

Material and Method

In the present study a nitrile metabolizing bacterium isolated in the Department of Biotechnology, Himachal Pradesh University, Shimla from the soil samples of Shimla (Himachal Pradesh, INDIA) during spring season in the month of March and April and the temperature was near about 25°C to 30°C has been explored for its amidotransferase (amidase) activity.

Chemicals

All the chemicals were of analytical grade. The nitriles and amides were from Alfa Aesar, A Johnson Matthey Company (earlier Lancaster Synthesis). Media components were from HiMedia (Mumbai) and the inorganic salts were of analytical grades.

Production medium for amidotransferase

A loopful of bacterial cells (*Bacillus* sp. APB-6*)* from the slant were seeded in 50 ml of modified nutrient broth containing 6 g peptone, 3 g beef extract, 1 g yeast extract, 10 g glucose per liter of distilled water [15] pH 7.5 and incubated at 30°C for 24 h in an incubator shaker (160 rpm). Four ml of this seed culture (OD_{600} ≈14, 3.31 mg dcw ml^{-1}) was added to 50 ml of media containing (g/l) 30 g acetamide, 3.5 g NaCl, 2 g glucose, 15 g yeast extract, 70 mM inducer (n-methyl acetamide), 7.0 pH and was incubated at 32.5°C for 27 h, at 150 rpm in an incubator shaker. The cells were harvested by centrifuging the culture broth at 10,000 g for 15 min and were suspended in 0.1 M Glycine-NaOH buffer (pH 7.0) and after two washings with the same buffer, the cell suspension was referred to as 'whole resting cells' and assayed for enzyme activity.

Amidotransferase assay

Reference: Brammar and Clarke (1964) [14].
Reagents: $FeCl_3$ reagent [6.0 % (w/v) in 2.0 % HCl (v/v)]
One unit (U) of amidotransferase activity was defined as that amount of enzyme in resting cell which catalyzed the release of one micromole of butyrohydroxamic acid per min under assay conditions.

Immobilization of resting cells of *Bacillus* sp. APB-6 exhibiting amidotransferase activity on different matrices

Entrapment in Agar

In case of immobilization in agar, 20 ml resting cells (33.39 mg dcw/ml) were added and mixed with 3% of agar solution and sterilized by autoclaving. The mixture was immediately poured into a petridish and kept for solidifying. The gel was cut into discs and stored in buffer at 4°C for further use.

Entrapment in polyacrylamide

For immobilization in polyacrylamide, 15% of acrylamide, 7.5% of bis-acrylamide and 0.5 % of ammonium persulfate were added thoroughly mixed and heated. 50 µl of TEMED was added to this test tube. In another test tube, 20 ml of resting cell suspension (33.39 mg dcw/mL) was taken. The contents of the two test tubes were mixed properly and immediately poured in petridish and covered. Polymerization was allowed to proceed for 1 h. The solidified gel was cut into discs and stored in buffer at 4°C for further use.

Entrapment in Alginate

In 4% of sodium alginate solution cell suspension 20 ml (33.39 mg dcw/ml) was added. The resultant suspension was dropped into a magnetically stirred solution of $CaCl_2$ (0.2 M) and immersed for about 2 h to form spherical entrapment alginate beads.

Entrapment in Hydrogel

5ml of HPMA and 0.23 ml EGDMA were mixed in water / ethylene glycol (1/1.5 ml). This solution was heated at 55°C for 5 min. After cooling 1 ml of cell suspension (33.39 mg dcw/ml), 0.5 ml of 15% sodium metasulphite and 0.5 ml of 40% APS were added. Polymerization was allowed to proceed for overnight at room temperature. The solidified gel was cut into discs.

Carrageenen entrapment

Carrageenan (4% w/v) was dissolved in distilled water by gentle heating and sterilized by autoclaving. To this solution 20 ml of resting cell suspension (33.39 mg dcw/ml) was mixed and poured into sterile petriplate, allowed to solidify and then cut into equal small blocks.

Assay of amidotransferase activity of immobilized cells

The immobilized cells were incubated with butyramide and hydroxyl amine HCl and glycine-NaOH buffer at

55°C for 5 min in a water bath shaker and the reaction was stopped by the addition of 4 ml of $FeCl_3$ reagent.
A control was also prepared by omitting the enzyme (beads), which was added after the reaction was stopped to consider the auto conversion of butyramide into racemic mixture of hydroxamic acid. The reaction mixture was centrifuged at 10,000 g for 10 min, discarded the pellet and clear supernatant was collected for estimation of butyrohydroxamic acid. The absorbance was read at 500 nm.

Optimization of process parameters for amidotransferase activity of both free and alginate immobilized resting cells for butyrohydroxamic acid synthesis

Effect of buffer pH and buffer concentration

In order to find out the optimum buffer pH, the reaction was performed in various buffers of different pH. The following buffers were used:

Citrate buffer	(pH 4.0-6.0)
Sodium phosphate buffer	(pH 6.0-8.0)
Potassium phosphate buffer	(pH 6.0-8.0)
Glycine-NaOH buffer	(pH 7.5-10.5)
Carbonate buffer	(pH 9.0-10.5)
Borate buffer	(pH 7.5-9.5)

Enzyme reactions were carried out at different concentrations of selected glycine NaOH buffer (25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300 mM) at pH 7.5 for immobilized cell and pH 9.5 for free resting cell suspension.

Incubation temperature and incubation time

The enzyme reactions for both immobilized, free resting cells were carried out at different temperatures ranging from 40°C to 70°C and to optimize optimum incubation time enzyme reactions were carried out at different time ranging from 5 to 45 minutes.

Enzyme concentration

Different amount of resting cells (0.263-2.63 mg dcw/ml), and alginate gel beads were used. Enzyme activity was assayed as described earlier.

Substrate concentration

Varied concentrations of butyramide and hydroxylamine hydrochloride were used in the range from 0.04 mM to 0.44 mM and 0.2 mM to 2.0 mM per reaction mixture respectively and enzyme activity was assayed as descried earlier.

Effect of various organic solvents, metal ions and inhibitors

Enzyme reaction was performed in the presence of various organic solvents e.g. methanol, acetone, propane-2-ol, benzene, ethylene glycol, 1,4-dioxan, carbon tetrachloride, isoamyl alcohol and toluene (at concentrations of 10% v/v). To work out the effect of $FeCl_3$, $MgCl_2.6H_2O$, $ZnSO_4.7H_2O$, $CoCl_2$, $CuSO_4.5H_2O$, NaCl, $AgNO_3$, $BaCl_2.2H_2O$, $HgCl_2$, NaN_3, $CaCl_2.2H_2O$, $CdCl_2.H_2O$, Pb $(NO_3)_2$, $MnCl_2.2H_2O$, Urea, dithiothreitol (DTT), Ethylene-di-amine tetra acetic acid (EDTA), phenyl methyl sulphonyl fluoride (PMSF) and polyethyleneglycol (PEG) on enzyme activity, these were preincubated with enzyme at 30°C for 20 min and were added to the reaction mixture at 1mM final concentration. Enzyme activity was assayed as described earlier.

Bead size optimization and reusability of alginate gel beads

To determine the optimum sized beads for the enzyme reaction, different sized beads (350-1000 nm) were prepared by using the encapsulator. Alginate beads were recycled as biocatalyst in the subsequent reactions to test their reusability potential. Removal of beads from the reaction mixture stopped first the enzyme reaction. Then the beads were thoroughly washed with 100 mM glycine-NaOH buffer for second reaction and so on.

Thermostability of enzyme and shelf life of enzyme

Thermostability of enzyme was studied by incubating the resting cells at various temperatures i.e. 40°C, 45°C, 50°C, 55°C and 60°C for five hours. The residual acyltransferase activity was assayed after an interval of 1 h as described earlier. Storage stability of enzyme was investigated both at 4°C and 30°C for 60 days (at an interval of 3 days). Enzyme activity was assayed as described earlier in section.

Results and Discussion

In present study bacterium isolated from soil samples has been identified and deposited as *Bacillus* sp. APB-6 at Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh (INDIA) with accession number MTCC-7540. Since the amidotransferase activity has been studied so the protein is being addressed as amidotransferase, though it is an amidase. Similarly, amidase from *Bacillus* sp. F-8 having acyltransferase activity was isolated from soil samples collected from effluent sites of dye and pharmaceutical industries located in urban Sanganer and Sitapura areas of Jaipur, Rajasthan, India [2]. Gorbunova *et al.* (2015) reported amidase having Acyltransferase activity from *Rhodococcus rhodochrous* 4-1 and immobilized by covalent attachment to activated chitosan by physical sorption on carbon adsorbents and by the formation of cross linked aggregates in the absence of carrier [14].

Immobilization of resting cells of *Bacillus* sp. APB-6 exhibiting amidotransferase activity on different matrices

Resting cells of *Bacillus* sp. APB-6 exhibiting amidotransferase activity were immobilized by various methods on alginate, PVA-alginate, polyacrylamide, agar, carrageenan and hydrogel matrices as discussed earlier. Initially, entrapped resting cells exhibited about 66.3 %, 58.3 %, 60.93 %, 48.31 % and 55.39 % residual enzyme activity in alginate, polyacrylamide, agar, carrageenan and hydrogel matrices respectively. Alginate immobilized (Figure 2) cells were of higher amidotransferase activity (66.31 U/mg dcw) and these were chosen for further comparable study. Figure 1 Showed whole resting cells entrapped in different type of matrices, beads and their size and morphology obtained after immobilization. Resting cells of *Bacillus megaterium* F-8 having acyltransferase activity were efficiently immobilized by entrapment in agar, polyacrylamide, alginate and PVA-alginate gel and assessed for their reusability potentials [2]. Similarly Nonthasen *et al.* studied the efficient entrapment of *Kluyveromyces marxianus* DBKKUY-103 in polyvinyl alcohol hydrogel for ethanol production from sweet sorghum juice [17]. Intact cells from *Pseudomonas aeruginosa* strain L10 containing amidase were used as biocatalysts both free and immobilized in a reverse micellar system for production of hydroxamic acid [18].

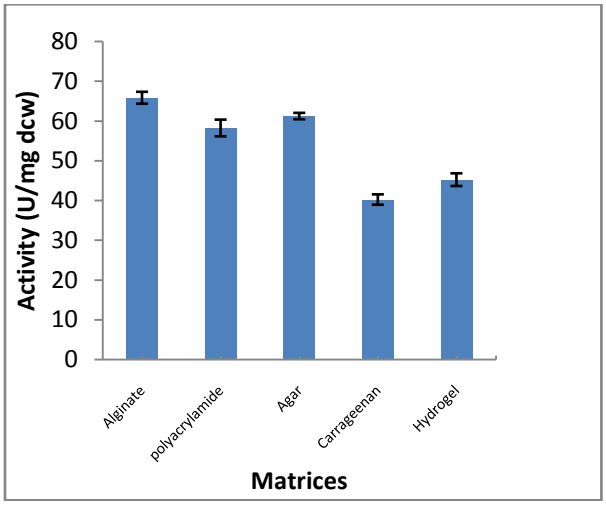


Figure 1. Enzyme activity on different matrices used for the immobilization.

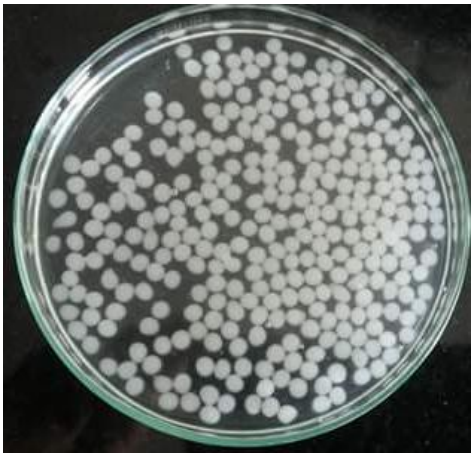
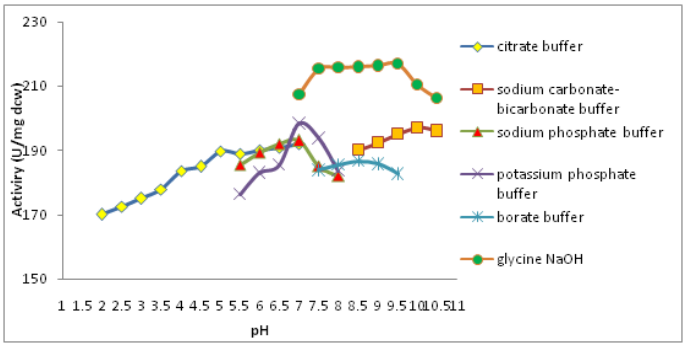


Figure 2. Alginate entrapped resting whole cell of *Bacillus* sp. APB-6.

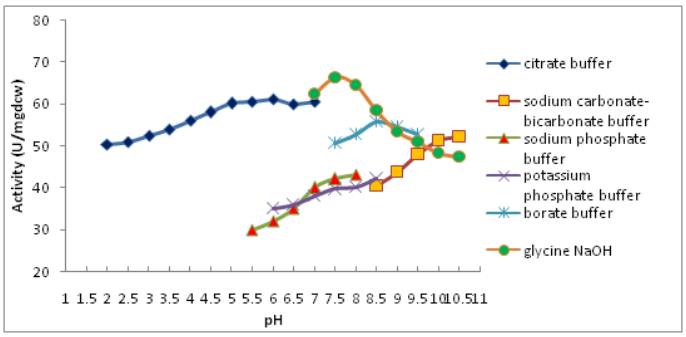
Optimization of process parameters for amidotransferase activity of both free and immobilized resting cells for butyrohydroxamic acid synthesis

Effect of buffer pH Buffer concentration

Maximum enzyme activity was recorded in 200 mM glycine NaOH at pH 9.5 for free cells and enzyme activity was maximum in 200 mM glycine NaOH at pH 7.5 for alginate (68.03 U/mg dcw) entrapped cell (Figure 3 a, b). Similarly Sogani *et al.* (2012) [2] also observed that there was no significant change in type of buffer and buffer pH of alginate immobilized cells of *Bacillus megaterium* F-8 exhibiting acyltransferase activity w.r.t. free cells. Maximum acyltransferase activity was found at pH 7.5 while free cells exhibited 8.0 as pH optima for amide hydrolysis. Bhatia *et al.* reported maximum acyl transfer activity in sodium phosphate buffer (104.44±0.03 U/mg dcm, pH 7.5) at 100 mM (110.21± 0.03 U/mg dcm) from amidase of *Pseudomonas putida* BR-1 [19].



(a)



(b)

Figure 3. (a) Effect of buffer and pH on free cell suspension amidotransferase activity. (b) Effect of buffer and pH on amidotransferase activity in alginate entrapped cell.

Incubation temperature and incubation time

Maximum amidotransferase activity of *Bacillus* sp. APB-6 free cells was recorded at 50°C (220.14 U/mg dcw) and at 55°C for alginate entrapped cell (Figure 4). Maximum acyltransferase activity was exhibited at 55°C by both alginate immobilized and non-immobilized *Bacillus megaterium* F-8 cells [2]. The amidase of *G. Subterraneus* RL-2a was active at a broad range of temperature (40–90°C) and has maximum activity at 70°C [20]. *P. putida* BR-1 produces maximum amidase acyl transfer activity 138 U/mg dcm at 50°C, with highest conversion (95%) of nicotinamide to nicotinyl hydroxamic acid [19]. Maximum amidotransferase activity (220.34 U/mg dcw) was observed at the incubation time of five minutes in case of free cells, 15 min incubation time for alginate (92.13 U/mg dcw) (Figure 5). Similar results were observed in *Geobacillus pallidus* BTP-5x MTCC 9225 by [21].

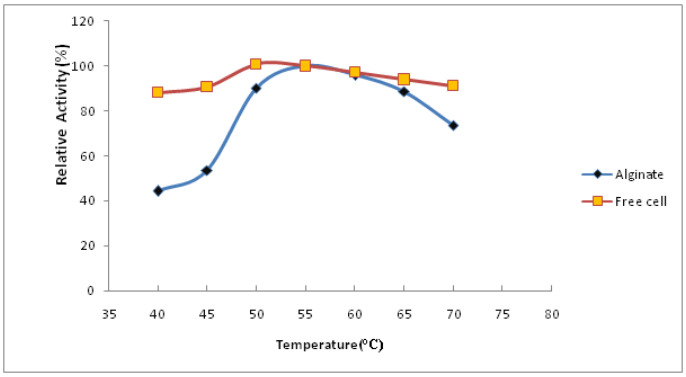


Figure 4. Effect of temperature on amidotransferase activity of *Bacillus* sp. APB-6.

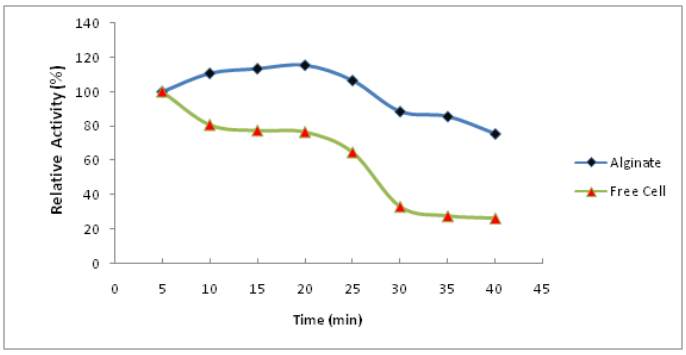


Figure 5. Effect of incubation time on amidotransferase activity of *Bacillus* sp. APB-6.

Enzyme concentration

Enzyme activity was increased up to 0.789 mg dcw/ml and further increase in cell concentration led to decrease in activity. For alginate entrapped cells maximum amidotransferase activity was recorded at 1.052 mg dcw/ml (93.14 U/mg dcw). Maximum acyl transfer activity 114.42±0.01 U/mg dcm of amidase of *P. putida* BR-1 was observed with 0.12 mg dcm/ml resting cells [19].

Substrate concentration for amidotransferase of *Bacillus* sp. APB-6

Maximum amidotransferase activity was recorded in the 0.24 m moles and 0.32 m moles of butryamide for free and immobilized cells respectively. The concentration optima of acyl group acceptor hydroxyl amine HCL was found to be 1.6 mM for both free cells and alginate immobilized cells. Maximum amidase production (5.84 U/mL) by alginate immobilized *E. coli* NCIM 2569 cells was obtained with 1.0% (w/v) acetamide [22]. Optimum substrate concentration (acetamide) for alginate gel entrapped cells of *Bacillus megaterium* F-8 exhibiting acyltransferase activity was 1.0 mmoles which was higher compared to the optimum substrate concentration of 0.850 mmoles for free cells [2].

Effect of various organic solvents, metal ions and inhibitors

Amyl alcohol strongly inhibited enzyme activity, relative activity in alginate and PVA-alginate entrapped cell was 47.49 %, 52.32 % respectively. Free cells activity was inhibited more in comprasion to immobilized cells (Figure 6). Among all the metal ions used, only copper sulphate had substantially inhibited the activity of both free and immobilized enzyme at 1 mM concentration, which indicated the presence of free thiol residues at the active site of the enzyme. Mehta *et al.* (2013) observed inhibition of acyltransferase activity of *Geobacillus subterraneus* RL-2a in the presence of metal ions, such as Co, Hg, Cu, Ni ions and thiol reagents [20].

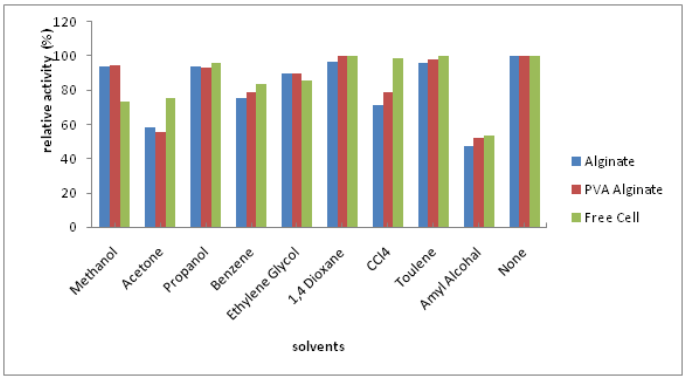


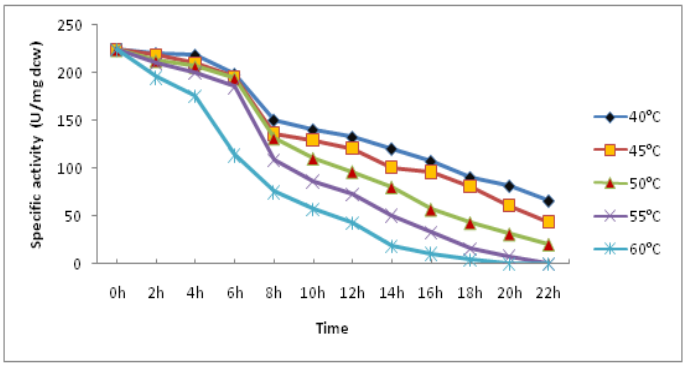
Figure 6. Effect of various solvents on amidotransferase activity.

Bead size optimization and reusability of alginate gel beads.

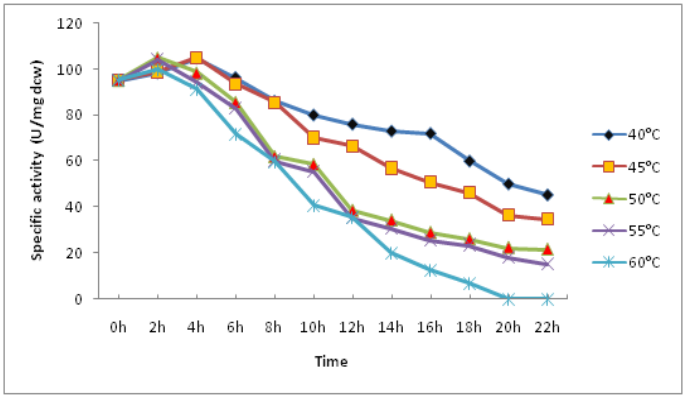
Among different size bead used alginate beads having 1000 nm bead size and control having 4 mm show nearly same enzyme activity. Alginate immobilized cells retain 85.45 percent relative activity upto 7th cycle. Alginate immobilized *E. coli* NCIM 2569 cells could be recycled successfully for three batches [22]. Alginate gel beads containing resting cells of *Bacillus megaterium* F-8 were recycled for ten cycles and it showed maximum reusability [2].

Thermostability of enzyme and shelf life of enzyme

It has been found that resting cells of *Bacillus* sp. APB-6 exposed to 40°C, 45°C, 50°C, 55°C retained the amidotransferase activity upto 8 h but alginate entrapped resting cells of *Bacillus* sp. APB-6 exposed to 40°C, 45°C retained 50% the amidotransferase activity upto 14 h (Figure 7 a,b). With increase the time of incubation at high temperature enzyme activity of immobilized cells increased, this may be due to increase in the membrane permeability for substrate and product in alginate gel bead. Cells exposed at 4°C having $t^{1/2}$ of 36 days and cells exposed 25°C having $t^{1/2}$ of 24 days. Alginate entrapped resting cells of *Bacillus* sp APB-6 exposed at 4°C and 25°C retained the amidotransferase activity for a long time and no significant loss in enzyme activity upto 22 days. Alginate entrapped cells exposed at 4°C retained 74% of amidotransferase activity upto 38 days and cells exposed 25 °C having t ½ of 32 days. So alginate entrapped cells can be stored at 4°C for longer duration. Alginate entrapped resting cells retained very good activity at 55°C in comparison to free cells [2]. The amidase of *G. Subterraneus* RL-2a was active at a broad range temperature (40–90 °C) and had a half-life of 5 h and 54 min at 70°C [20]. Bhatia *et al.* (2013) reported that the cells of *Alcaligenes* sp. MTCC 10674 exposed to 4°C and 30°C retained most of the acyl transfer activity but the cells stored at 40°C and 50°C exhibited fast decrease in acyl transfer activity as compared to the cells stored at 4°C and 30°C [23]. Biocatalyst thermostability is of paramount importance for any bioprocess [24].



(a)



(b)

Figure 7. (a) Thermostability profile of whole cells of *Bacillus* sp. APB-6 at different temperature (b) Thermostability profile of alginate entrapped cells of *Bacillus* sp. APB-6.

Conclusion

The results obtained in this study show that that alginate gel beads entrapped whole cell of *Bacillus sp.* APB-6 express thermostable amidotransferase activity that may be used repeatedly for the hydrolysis of amides and synthesis of hydroxamic acids. Biotransformation at commercial scale has a very high potential in contrast to chemical processes for the synthesis of this commodity compound which is a key compound in medicine, analytical chemistry, phytochemistry, agronomy and in nuclear technology and waste water treatment studies.

Acknowledgements

The authors are highly grateful to the University Grant Commission (UGC), India for providing financial assistance in the form of Senoir Research Fellowship to Miss. Pankaj Kumari and Department of Biotechnology Himachal Pradesh University Shimla, for providing research facilities.

References

1. Pertsovich SI, Guranda DT, Podchernyaev DA, Yanenko AS and Svedas VK: Aliphatic amidase from Rhodococcus rhodochrous M8 is related to the nitrilase/cyanide hydratase family. Biochemistry 2005; 70:1280–1287.
2. Sogani M, Mathur N, Sharma P1 and Bhatnagar P: Comparison of immobilized whole resting cells in different matrices vis-a-vis free cells of Bacillus megaterium for acyltransferase activity. Journal of Environmental Research And Development 2012; 6: 695-701.
3. Muri EMF, Nieto MJ, Sindler RD and Williamson JS: Hydroxamic acids as pharmacological agents. Current Medicinal Chemistry 2002; 9:1631–1653.
4. Fournand D, Vaysse L, Dubreucq E, Arnaud A and Galzy P: Monohydroxamic acid biosynthesis. Journal of Molecular Catalysis B: Enzymatic 1998; 5:207-211.
5. Ramakrishna C, Dave H and Ravindranathan M: Microbial metabolism of nitrilesand its biotechnological potential. Journal of Scientific and Industrial Research 1999; 58: 925-947.
6. Shankar B, Tomar R, Kumar R, Godhara M and Sharma VJ: Antimicrobial activity of newly synthesized hydroxamic acid of pyrimidine-5-carboxylic acid and its complexes with Cu(II), Ni(II), Co(II) and Zn(II) metal ions. Journal of Chemial and Pharmaceutical Research 2014; 6:925-930.
7. Vaghari H, Eskandari M, Sobhani V, Berenjian A, Song Y and Malmiri JH: Process Intensification for Production and Recovery of Biological Products. American Journal of Biochemistry and Biotechnology 2015; 11:37-43.
8. Chen J, Zheng RC, Zheng YG and Shen YC: Microbial transformation of nitriles to high-value acids or amides. Advances in Biochemical Engineering / Biotechnology 2009; 113:33–77.
9. Hirrlinger B, Stolz A and Knackmuss HJ: Purification and properties of an amidase from Rhodococcus erythropolis MP 50 which enantioselectively hydrolyzes 2-arylpropionamides. Journal of Bacteriology 1996; 178:3501–3507.
10. Yamamoto K, Otsubo K, Matsuo A, Hayashi T, Fujimatsu I and Komatsu KI: Production of R-(-)-Ketoprofen from an Amide Compound by Comamonas acidovorans KPO-2771-4. Applied and Environment Microbiology 1996; 62:152–155.
11. Berezin IV, Klyachko NL, Levashov AV, Martinek K, Mozhaev VV and Khmel'nitskii YuL: Immobilizovannye fermenty (Immobilized Enzymes) 1987 Moscow: Vysshaya shkola.
12. Banerjee A, Sharma R and Banerjee UC: The nitriledegrading enzymes: current status and future prospects. Applied Microbiology and Biotechnology 2002; 60:33–44.
13. Vejvoda V, Kaplan O, Kubá D, K en V and Martinková L: Immobilization of fungal nitrilase and bacterial amidase-two enzymes working in accord. Biocatalysis and Biotransformation 2006; 24:414–418.
14. Gorbunova AN, Maksimova YuG, Ovechkina GV and Maksimov AYu: Catalytic and Stereoselective Properties of the Immobilized Amidase of Rhodococcus rhodochrous 41. Applied Biochemistry and Microbiology 2015; 51:539–545.
15. Bhalla TC, Miura A, Wakamoto A, Ohba Y and Furuhashi K: Asymmetric hydrolysis of α -aminonitriles to optically active amino acids by a nitrilase of Rhodococcus rhodochrous PA-34. Applied Microbiology and Biotechnology 1992; 37:184– 190.
16. Brammar WJ and Clarke PH: Induction and repression of Pseudomonas aeruginosa amidase. Journal of General Microbiology 1964; 37:307–319.
17. Nonthasen K, Piyatheerawong W and Thanonkeo P: Efficient entrapment of Kluyveromyces marxianus DBKKUY-103 in polyvinyl alcohol hydrogel for ethanol production from sweet sorghum juice. Turkish Journal of Biology 2015; 39:119-128.
18. Bernardo M, Pacheco R, Minhalma M and Pinho MN: Hydroxamic Acids Production in Reverse Micelles and Recovery by Membrane Separation Processes. Journal of molecular catalysis B-Enzymatic 2013; 93:28-33.
19. Bhatia RK, Bhatia SK, Mehta PK and Bhalla TC: Biotransformation of nicotinamide to nicotinyl hydroxamic acid at bench scale by amidase acyl transfer activity of Pseudomonas putida BR-1. Journal of Molecular Catalysis B: Enzymatic 2014; 108: 89–95.
20. Mehta PK, Bhatia SK, Bhatia RK and Bhalla TC: Purification and characterization of a novel thermo-active amidase from Geobacillus subterraneus RL-2a. Extremophiles 2013; 17:637–648
21. Sharma M, Sharma NN and Bhalla TC: Biotransformation of Acetamide to Acetohydroxamic Acid at Bench Scale Using Acyl Transferase Activity of Amidase of Geobacillus pallidus BTP-5x MTCC 9225. Indian. J. Microbiol 2012; 52:76-82.
22. Nampoothiri MK, Roopesh K, Chacko S and Pandey A: Comparative Study of Amidase Production by Free and Immobilized Escherichia coli Cells. Applied Biochemistry and Biotechnology 2005; 120:97-108.
23. Bhatia RK, Bhatia SK, Mehta PK and Bhalla TC: Bench scale production of benzohydroxamic acid using acyl transfer activity of amidase from Alcaligenes sp. MTCC 10674. Journal of Industrial Microbiology and Biotechnology 2013; 40:21–27.
24. Bhalla T, Kumar J, Kumar H and Agarwal H: Amidase production by Rhodococcus sp. NHB-2. National Academy Science Letters 1997; 20:139-142.