RESEARCH PAPER

Received 20 November 1995
Indian J. Pharm. Sci., 1996, 58(6) pp. 226-229

Continuous Production of 6-amino Penicillanic acid from Benzyl Penicillin using Immobilized *E. coli* Cells

P. BHATTACHARJEE*, S. PATIL, M. TRIPATHI AND S.C. CHATURVEDI
Dept. of Pharmacy, S.G.S.I.T.S., 23 Park Road, Indore (M.P.)
*School of Life Sciences, Devi Ahilya Vishwavidyalaya, Indore (M.P.)

The effect of the type of matrix on the bio conversion of Benzyl penicillin to 6-Amino penicillanic acid (6-APA) using immobilized *E. coli* cells was studied. The *E.coli* strains selected were MTCC 476, MTCC 448, MTCC 521 and MTCC 66. The matrices selected for entrapping these cells were polyacrylamide, calcium alginate, K-carrageenin and agarose. Bioconversion was performed with free cells as well as immobilized cells, afterwards continuous production of 6-APA was carried out using a packed bed column.

FOR the production of 6-APA, the technique of enzyme immobilization is employed in industry.[1,2] Still this technology is subjected to further investigation for optimization, to develop an easy and cheap procedure for the production of 6-APA. The selection of a suitable strain and an appropriate immobilizing matrix are needed to get higher yields of 6-APA. There are other factors like pH, temperature, substrate concentration and flow rate for the continuous production which affect the extent of bioconversion. In the present investigation, *E.coli* MTCC 448 was selected for studying penicillin acylase activity after immobilization in different matrices. 6-APA produced continuously, using a packed bed reactor of immobilized cells was compared with free cell activity.

EXPERIMENTAL

Culture of *E.coli* cells

Four strains of *E.coli* i.e. MTCC 521, MTCC 476, MTCC 448 and MTCC 66 (procured from the Institute of Microbial Technology, Chandigarh, India) were grown aerobically in a media containing beef extract (0.1%), yeast extract (0.2%), peptone (0.5%) and sodium chloride (0.5%), pH 7.2 at 30° for 24 h and harvested by centrifugation (10,000 rpm) at 4°.

* For correspondence

Free cell activity

Time course of formation of 6-APA by free *E.coli* cells was determined. About 0.75 g wet *E.coli* cells were taken in a solution of 20 mg/ml benzyl penicillin in 1/15 M phosphate buffer (pH 8.5) and incubated at 30° with shaking, the amount of 6-APA formed was estimated periodically at an interval of 15 min. No activity was found with MTCC 521, therefore, the strain was rejected for further studies. The results are given in Table-1.

Estimation of Penicillin Acylase Activity

6-APA formed in the reaction mixture was estimated by withdrawing 2 ml aliquot and acidifying with 1N HCl to obtain pH 2.0. The acidified solution was mixed with 5 ml n-butylacetate, aqueous phase was separated and estimated using colorimetric hydroxylamine method.[3]

Table - 1 : Mole Percent Conversion of Benzyl Penicillin to 6-APA with Time

Time (min)	Mole Percent Conversion		
	MTCC 476	MTCC 448	MTCC 66
15	5.594	9.506	17.667
30	18.137	33.990	20.712
45	64.106	39.341	32.482
60	74.486	58.004	42.986
75	52.747	36.055	21.056
90	36.322	30.708	21.150

Immobilization of *E.coli* cells

Free cells were immobilized in granules of polyacrylamide using method given by Larson and Mosbach.[3] Entrapment of free cells in beads of calcium alginate, K-carrageenin and agarose was done according to methods given by Kierstan *et al.*[4], Tosa *et al*[5]. and Matsunaga *et al.*[6] respectively.

Immobilized cell activity

All the three strains which showed activity were immobilized in all the four matrices[7] but maximum retention of activity was observed in case of MTCC 448 in polyacrylamide by estimating the 6-APA produced. The results are shown in Table-2.

Therefore, MTCC 448 immobilized in polyacrylamide was selected for continuous production of 6-APA.

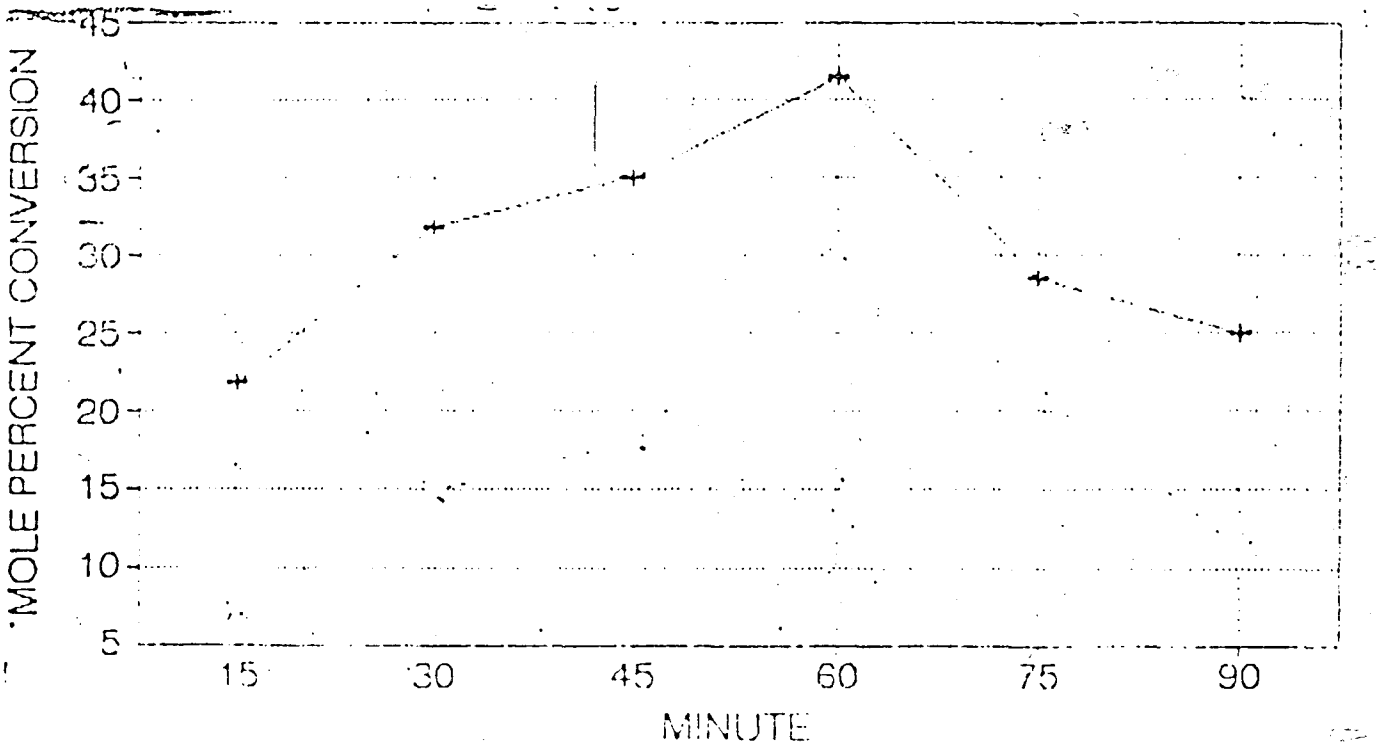
Continuous production of 6-APA by MTCC 448

Continuous production of 6-APA was carried out with *E.coli* MTCC 448 using a column (6 cm long, 2.5 cm diameter) packed with immobilized cells, a solution of benzyl penicillin (10 mg/ml) in phosphate buffer pH 8.5 was passed through the column continuously with flow rate of 0.15 ml/min.[7] 6-APA formed was determined collecting the effluent hourly. The result is shown in Fig. 1.

METHOD OF IMMOBILIZATION

About 5.0 g of wet cells were suspended in 25 ml of 0.05 M tris- HCl buffer pH 7.5, 7.23 g of acrylamide monomer and 6.37 g of N,N'-methylene bis acryl amide (BIS) were dissolved in 23 ml of 0.05 M tris-HCl buffer pH 7.5. 0.1 g of N,N,N',N'-tetramethylene diamine (TEMED) was dissolved in 1.0 ml of distilled water, 0.05 g of ammonium per sulphate was dissolved in 1.0 ml of distilled water. The cell suspension and all other solutions were cooled below 10°. A glass jar was filled with ice cooled water with some ice pieces. Two glass plates were clamped together with a rubber tube in between. Such that the distance between the glass plates is 2 mm. The solution of acrylamide and BIS was added to cell suspension and was shaken well. To the above suspension, solution of TEMED and ammonium per sulphate were added. It was shaken vigorously and added in between the glass plates immediately. The glass plate alongwith this suspension was kept in a glass jar for cooling until completion of polymerisation. The glass plates were taken apart and the stiff gel film formed was removed. The gel was pressed through a nylon mesh so as to get the granules of 2 mm^3 dimension and dissolved into

Fig. - 1 Bioconversion to 6-APA by immobilized *E.Coli* MTCC 448 in Polyacrylamide gel.



buffer solution of 0.05 M tris-HCl buffer, pH 7.5. The gel was washed thoroughly with buffer to remove excess of TEMED.

RESULTS AND DISCUSSION

Table-1 shows the time course of product formation by free cells, highest mole percent conversion was obtained by *E.coli* MTCC 476. After immobilization in different matrices MTCC 476 showed a considerable loss in activity but MTCC 448 showed retention of activity. Therefore, *E.coli* MTCC 448 was selected for the continuous production of 6-APA. MTCC 448 is an equivalent strain of ATCC 9637 and is reported to have high penicillin acylase activity.[2] Though in this study the penicillin acylase activity of *E.coli* MTCC 448 was not found considerably high, but the investigation was aimed at the selection of suitable immobilizing matrix which would retain the maximum free cell activity. Entrapment method was selected for immobilization as it least affected the cells.

Table - 2 : Percent Retention Penicillin Acylase Activity of E.coli MTCC 448 in Different Matrices

Entrapping Agent	Percent Retention of Penicillin Acylase Activity
Polyacrylamide gel	86
Calciumalginate gel	61
K-carrageenin	55
Agarose	Nil

Table-2 shows percent retention of penicillin acylase activity of *E.coli* MTCC 448 in different matrices. Cells immobilized in agarose show negligible activity probably due to leakage of cells from the get matrix for open structure of agarose.

Fig.1 shows bioconversion to 6-APA by immobilized *E.coli* cells of MTCC 448 in Polyacrylamide, which is between 40-45 mole percent.

REFERENCES

1. Vandamme, E.J., **Economic Microbiology**, 1981, 5, 467.

2. Sato, T., Tosa, T. and Chibata, I., **European J. Appl. Microbiol.**, 1976, 2, 153.

3. Larson, P.O. and Mosback, K., **Methods In Enzymobgy.**, 1976, 44, 183.

4. Kierstan, M. and Bucke, C., **Biotechnol. and Bioeng.**, 1977, 19, 387.

5. Tosa, T., Sato, T., Mori, T., Yamamato, K., Takata, I., Nishida, Y. and Chibata, I., **Biotechnol. and Bioeng.**, 1979, 21, 1697.

6. Matsunga, T., Karube, T. and Shuichi, S., **Biotechnol. and Bioeng.**, 1980, 22, 2607.

7. Bhatacharjee, P., **Continuous Production of 6-APA from Benzyl Penicillin Using Immobilized Cells of E.coli.**, M. Pharm. Thesis, 1994.