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Bioconversion of Cephalosporin-G to 7-Amino Deacetoxy Cephalosporanic acid

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Cephalosporin -G (Ceph-G) was converted to 7-amino deacetoxycephalosporanic acid (7-ADCA) using immobilized penicillin-G acylase (ImPGA). Effect of different variables on the rate of hydrolysis using a recirculated packed bed reactor (RPBR) was studied. A comparison between a RPBR and a stirred tank reactor (STR) was carried out. The RPBR was found to be superior to STR.

SEMISYNTHETIC antibiotics are more useful because of development of resistance with natural antibiotics. Therefore, the intermediates of semisynthetic antibiotics draw a great deal of importance. 7-ADCA is the starting compound for production of various semisynthetic cephalosporins. It is obtained by hydrolysis of Ceph-G[1-4].

Conversion of Ceph-G to 7-ADCA can be brought about either chemically or enzymatically. Previously chemical process was the only method available for this conversion, but due to recent advances, enzymatic methods are now preferred for this conversion. The enzymatic method is more advantageous as it proceeds at room temperature,gives higher yield, do not disrupt the β-lactam ring and is more economical.

In the present study, effect of modified enzyme kinetics on bioconversion was studied. A comparison between RPBR and a STR has also been carried out.

EXPERIMENTAL

All the chemicals used were of synthetic and analytical grade. Bio- conversion of Ceph-G to 7-ADCA was carried out using the ImPGA. Ceph-G and ImPGA were procured from Hindustan Antibiotics Ltd., Pimpri, Pune, India.

**For correspondance

Estimation of 7-Amino deactoxycephalosporanic acid

The 7-ADCA was estimated by a colorimetric method using PDAB (p-dimethyl amino benzaldehyde)[5]. This method is based upon the principle of Schiff's base formation between free amino group of 7-ADCA and PDAB (dissolved in methanol) in acidic environment to produce a yellow coloured complex which is measured at 415 nm.

The Immobilized Penicillin-G Acylase Activity[6]

The ImPGA activity was determined using Pen-G as substrate. In the present study enzyme activity was found to be 300 IU/g dry weight of enzyme.

Hydrolysis of Ceph-G to 7-ADCA Using a Recirculated Packed Bed Reactor (RPBR)[7]

The substrate solution was prepared in phosphate buffer (0.05 M, pH 7.8). The pH of Ceph-G solution was adjusted to 7.8 using 2 N ammonia solution and transferred to a reaction vessel. pH of the reaction mixture was maintained at pH 7.8 during the course of reaction by the addition of 2 N ammonia solution. The rate of hydrolysis of Ceph-G to 7-ADCA in a RPBR is influenced mainly by flow rate of reaction liquor through the enzyme column, substrate concentration, enzyme to substrate ratio, and the diameter to height ratio of enzyme bed.

Table - 1
Effect of flow rate on hydrolysis of CEPH-G in RPBR

S.No.	Flow Rate (ml/min)	Hydrolysis (%)	Time (min)
1.	75	88.97	120
2.	100	94.28	120
3.	140	96.28	90
4.	160	96.28	90

d:h ratio – 1:1
Substrate concentration – 4%
Optimum flow rate – 140 ml/min.

Table – 2
Effect of substrate concentration on hydrolysis of CEPH-G in RPBR

S.No.	Substrate Conc. (%)	Hydrolysis (%)	Time (min)
1.	3	100	60
2.	4	96.24	90
3.	5	97.20	90
4.	6	90.03	150

d:h ratio – 1:1 Flow rate – 140 ml/min
Optimum substrate concentration – 5%

During optimization, all these parameters were studied by varying flow rate, substrate concentration, enzyme to substrate ratio and diameter to height ratio of enzyme bed. The optimum value for each parameter was determined. The optimized RPBR was then compared with a conventional STR for Ceph-G to 7-ADCA bioconversion.

Hydrolysis of Ceph-G to 7-ADCA Using Stirred Tank Reactor (STR)

The substrate solution was prepared in phosphate buffer (pH 7.8). The pH of solution, after dissolving Ceph-G in buffer was adjusted to 7.8 using 2 N ammonia solution, and transferred to the reaction tank. The specified amount of ImPGA was added and mixture was kept in a state of continuous agitation. During the course of reaction pH of the medium was maintained at pH 7.8 by the addition of 2 N ammonia solution. After completion of the process, the reaction liquor was filtered and filtrate was processed for 7-ADCA isolation.

The rate of hydrolysis of Ceph-G was determined by following the rate of alkali consumption during bioconversion[7]. The 7-ADCA was precipitated by adjusting the pH of the solution 3.5, which is its iso-electric point by the addition of 6 N Hydrochloric acid. After precipitation it was kept at low temperature and filtered.

RESULTS AND DISCUSSION

The main factors affecting bioconversion of Ceph-G to 7-ADCA in a RPBR are flow rate of substrate solution,

TABLE-3
Effect of enzyme to substrate ratio on hydrolysis of CEPH-G in RPBR

S.No.	E:S Ratio	Hydrolysis (%)	Time (min)
1.	1:2.25	99.15	60
2.	1:3.75	97.20	90
3.	1:5.25	92.65	150

E:S ratio – Enzyme Substrate ratio.
d : h ratio – 1:1
Substrate concentration – 5%
Flow rate – 140 ml/min.
Optimum E:S Ratio – 1:3.75

Table-4
Effect of diameter to height ratio on hydrolysis of CEPH-G in RPBR

S.No.	d/h ratio	Hydrolysis (%)	Time (min)
1.	1:0.5	97.74	90
2.	1:1.0	97.20	90
3.	1:2.0	96.14	150
4.	1:3.0	86.05	150

Substrate concentration – 5%
Flow rate – 140 ml/min.
E:S Ratio – 1:3.75
Optimum ratio of enzyme bed diameter:height is 1:1

substrate concentration, enzyme to substrate ration and diameter to height ratio of the enzyme bed. The effect of flow rate of substrate solution on rate of hydrolysis in a RPBR is shown in table 1. It is apparent from the table that the optimum flow rate is 140 ml/min which gave 96.28% hydrolysis in 90 min. Table 2 shows the effect of substrate concentration on rate of hydrolysis of Ceph-G, the optimum substrate concentration being 5% which resulted in 97.2% conversion of Ceph-G to 7—ADCA in 90 min. Table 3 and 4 show the effect of enzyme to substrate ratio on rate of hydrolysis and diameter to height ratio of enzyme bed respectively. The optimum enzyme to substrate ratio is 1:3. 75 and diameter to height ratio is 1:1. The comparison of a RPBR and a STR is shown in Table No. 5.

The results show that enzyme concentration per unit volume in a RPBR is far more than in a STR. The RPBR has other advantages over a STR in 7-ADCA production, such as the enzyme is not subjected to shear by stirrer, least chances of contamination due to a closed system and convenient to stop at any time. The immobilized enzyme, in addition, never comes in contact with alkali and thus is more stable.

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Table – 5 : Comparison of RPBR and STR

S.No.	Parameter	RPBR	STR
1.	Ceph-G conc. w/v	5%	5%
2.	ImPGA:Ceph-G Ratio (w/w) (optimum)	1:3.75	1:2.5
3.	Hydrolysis	97.2%	95.0%
4.	Time	90 min	90 min
5.	7-ADCA/kg of ImPGA	2.417 kg.	1.611 kg.

RPBR: Recirculated packed bed reactor
STR : Stirred tank reactor

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REFERENCES

1. Sykes, P. and Todd, A. R. In; "The Chemisry of Penicillin", Princeton University Press, Princeton, N. J., 1949, 156.
2. Chow, A. W., Hall N. M. and Hoover, J. R. E., **J. Org. Chem.**, 1962, 27, 1387.
3. Gaeldal, E., Morch P. and Tysbring, L., **Tetrahedron Lett.**, 1962, 2, 381.
4. Essery, J. M., Dadabo, K., Gottstein, W. J., Halls Frand, A. and Cheney, L. C., **J. Org. Chem.**, 1965, 30, 4388.
5. Shewale, J. G., Kumar, K. K. and Ambekar, G. R.; **Biotechnol. Tech.**, 1987, 1, 69.
6. Borkar, P. S., Thadani, S. B. and Ramchandran, S.; **Hind Antibiot. Bull.**, 1978, 20, 81.
7. Deshmukh, P., In; **"Bioconversion of Cephalosporanic acid-G to 7-Aminodeacetoxy cephalosporanic acid and Purification of the Product"**, M. Pharm. Thesis, D. A. V. V., Indore, 1995, 31.