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The use of immobilized penicillin acylase for estimation of penicillin G

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The microbial enzyme, penicillin acylase (EC 3.5.1.11) hydrolyses penicillin G to 6-aminopenicillanic acid and fenylacetic acid. This reaction has been recently widely used in the pharmaceutical industry for bioconversion of penicillin G and different matrices for immobilization of penicillin acylase were used [1], but this technique has not been used for analytical purposes. We have tried to apply immobilized penicillin acylase for quantitative determination of penicillin G in the industrial fermentation processes. So far, penicillin G was commonly assayed either by measuring inhibition of growth of sensitive microorganisms or by iodometric titration but both these methods are too time-consuming for efficient control of the fermentation process. On the other hand the method based on the enzymatic reaction is rapid and simple. The immobilized biocatalyst may be used several times without losing its activity, and this makes the method inexpensive.

The enzyme was entrapped in calcium alginate gel according to Vorlop [2]. The biocatalyst was in the form of beads (2.0 ± 0.2 mm in diameter); each bead contained about 0.1 unit of penicillin acylase, 0.2% of glutaraldehyde and 3.0% of calcium alginate. The beads were formed by dropping slowly the aqueous solution of all these components into a 1 M calcium chloride solution, with constant stirring at room temperature. The exchange of sodium to calcium ions led to gel formation.

Penicillin acylase (10 units/mg of protein) was isolated from the culture of a high-productive *Escherichia coli* strain.

A single bead of the biocatalyst was sufficient for penicillin G estimation. The bead was placed into a glass tube containing 1 ml of penicillin G solution, or appropriately diluted fermentation broth, pH 7.5 (100 - 2000 µg of antibiotic/ml) and incubated at 37°C for 20 min; by that time the enzymic reaction was completed and the bead was removed. The biocatalyst, after being washed with distilled water, was ready for the next test. The amount of penicillin G was calculated on the basis of the amount of 6-aminopenicillanic acid released, determined colorimetricaly according to Nys [3]: 5 ml of acetic acid:ethanol (2:8, v/v) and 1 ml of 1% *p*-dimethylaminobenzoic aldehyde solution in ethanol were added to the reaction mixture, and after 20 min the absorbance at 410 nm was read at room temperature.

The logarithmic relation between the amount of 6-aminopenicillanic acid released and penicillin G concentration was observed over the range of antibiotic concentration of 100 - 2000 µg/ml (Fig. 1).

To improve the physico-chemical properties of the biocatalyst the beads had been treated with sodium chloride, causing transient partial dissolution of the gel [4], followed by calcium chloride, to increase the rigidity and smoothness of the bead surface. This seems to be essential for the possibility to use the biocatalyst several times. One bead of the biocatalyst can be used for at least 50 estimations of penicillin G. The accuracy of antibiotic estimation is about 5% which is sufficient for rapid analysis of the fermentation broth and antibiotic solutions at different steps of fermentation and

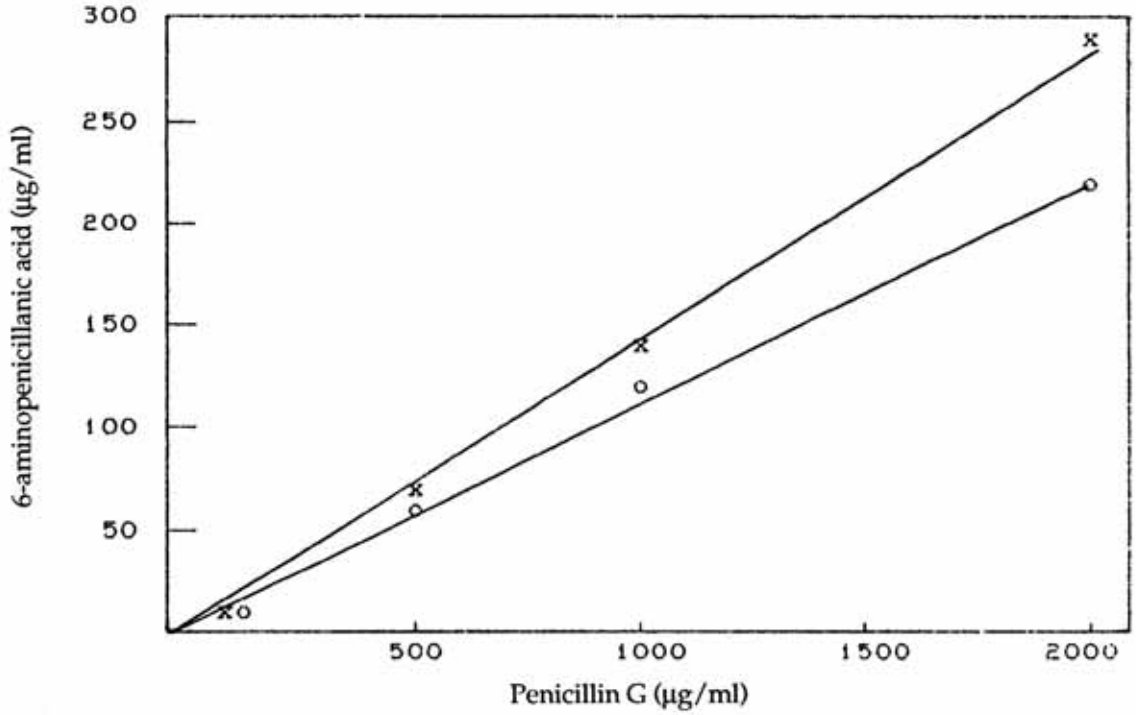

Fig. 1. Penicillin G assay based on the release of 6-aminopenicillanic acid by penicillin acylase.
×, Modified by the succesive NaCl and $CaCl_2$ treatment; ○, unmodified

purification of penicillin G in the production process.

REFERENCES

1. Vandamme, E.J. (1988) in *Bioreactor Immobilized Enzymes and Cells* (Moo-Young, M., ed.) pp. 261, Elsevier Applied Science, London, New York.
2. Vorlop, K.D. (1987) *Enzyme Eng.* **501**, 338 - 350.
3. Nys, P.S. (1973) *Antibiotiki* **18**, 270 - 275.
4. Vorlop, K.D. & Klein, J. (1984) in *Enzyme Technology* (Lafferty, R.M., ed.) pp. 219, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo.