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Immobilized Penicillin V Acylase

Development of an Industrial Catalyst

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Index Entries: Immobilized penicillin V acylase; penicillin V, immobilized acylase, immobilized cells.

Most companies using enzymic splitting of penicillin (generally pen-G) to 6-APA developed their immobilized penicillin acylases—for in-house use—about 10 years ago. Often the enzymes are first purified and then subsequently coupled to such carriers as Sephadex, ion exchange beads, or acrylic polymers (*1–4*).

NOVO's approach has been different since its aim has been to develop and market a cheap immobilized enzyme that can produce 6-APA from penicillin-V.

At first a conventional screening for a new enzyme source yielded an aerobic gram-negative bacteria with high acylase and low β-lactamase activities (the latter could be completely removed by mutant selection). This penicillin V acylase was characterized as a membrane-bound thiol enzyme with high specificity for the phenoxyacetyl (V) side chain. Attempts to purify and then immobilize the enzyme were, however, not very successful because of the instability of the enzyme, and a new mild immobilization method had to be developed that permitted the stabilization of the whole cell material (*5*).

Such an immobilized preparation, denoted SP 194, was tested for batch-wise production of 6-APA in a stirred tank or recycled column. Although addition of mercaptoethanol to the reaction mixture stabilized the enzyme, the processing lifetime of the preparation (about 30 batches) was judged too short to be interesting (cfr. Table 1).

With the knowledge that the enzyme processing stability had to be improved, we started the next iteration by choosing a fungal pen-V acylase that was not a thiol

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TABLE 1
Processing Lifetime (Until 25% Residual Activity) in Hours at Conversion of 4% w/v pen-V in 50 mM Phosphate pH 7.8 in a Packed-Bed Reactor

Degree of conversion	10%	20%	35%
External pH drop	0.6	1.1	2.1
SP 194, processing lifetime	170	100	50
SP 217, processing lifetime	3000	1900	1800

enzyme. Again the strain was free from β-lactamase activity, and the enzyme could be immobilized as crude cell material. An immobilized preparation, denoted SP 217, was produced at a very early stage to permit comparison of its performance to that of SP 194.

The graphs 1 and 2 show that the enzymes are indeed very different. SP 217 is less product inhibited (Fig. 1) and performs, e.g., 90% conversion of 4% w/v penicillin V with only about a 50% increase in time compared to the theoretical time with unchanged initial activity ($\tau = 1.4$), while SP 194 requires more than doubled conversion time at optimum substrate concentration ($\tau = 2$).

Furthermore, SP 217 has a broader pH activity profile (Fig. 2) and retains high activity at lower pH values. This is very important since the pen-V acylase reaction produces a severe pH drop in the microenvironment of the immobilized enzyme unless high buffer concentrations and a well-stirred pH-stat regulated reactor are used.

Most important is that the processing stability is very much better for SP 217 activity than for SP 194. Table 1 shows processing lifetimes ($2T^{1/2}$) that have been

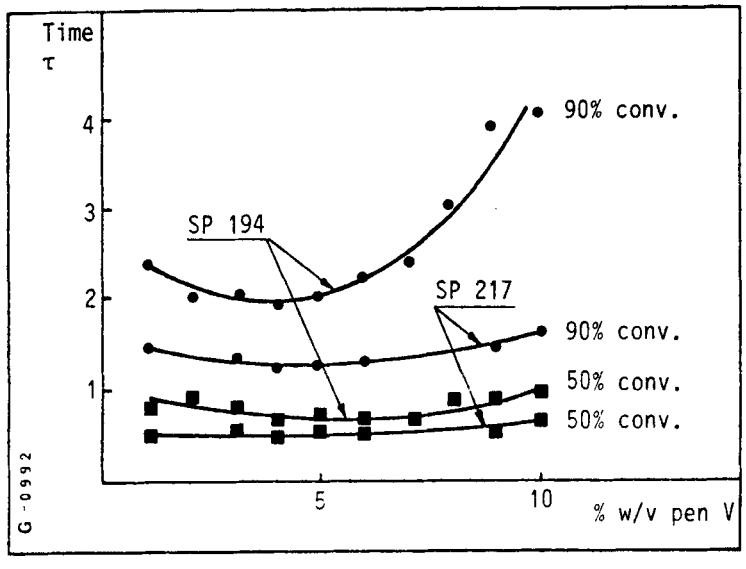

Fig. 1. Dimensionless time for 50% and 90% conversion as a function of substrate concentration. 180–340 μ particles in a well-stirred tank with pH-stat = 7.5 and pen V dissolved in 0.2 *M* phosphate; $\tau = t_{real}/t_{theor.}$ with unchanged initial activity.

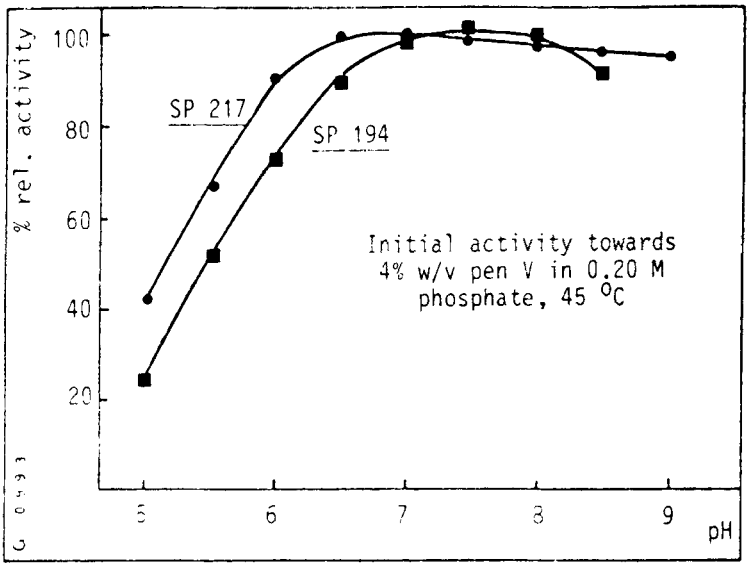

Fig. 2. pH activity optima for SP 194 and SP 217.

determined by running packed-bed reactors to different degrees of conversion and thus with different external pH drops over the beds. In fact, the stability of SP 217 (now with commercial name NOVOZYM 217) together with its moderate product inhibition and broad pH activity/stability profile makes it possible to produce 6-APA continuously from penicillin V in a series of packed-bed reactors with intermediary pH regulation (*6*).

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