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NOVEL DEVELOPMENTS FOR THE PRODUCTION OF 6APA IN THE PENICILLIN G FERMENTATION PLANT BY USING FIBER-ENTRAPPED PENICILLIN AMIDASE

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The production of 6APA by enzymatic hydrolysis of penicillin G can be integrated with the production of penicillin G. The penicillin G solutions obtained during the extraction from the fermented broth can be directly hydrolyzed to 6APA and phenyl acetic acid. The 6APA is then recovered while the phenyl acetic acid can also be recovered and recycled to the fermentation plant. Some of the results obtained by the research work performed by SNAM Progetti in their Microbiological Laboratories with the advice of Biochem Design are presented and analyzed. The economic potential of the integrated process is discussed.

GENERAL DEVELOPMENT OF AN INTEGRATED PROCESS

In the last few years, because of improvements in enzyme immobilization techniques, enzymatic hydrolysis of penicillin G for the production of 6APA has become a viable alternative to chemical hydrolysis. This article will present a novel development for producing 6APA by an enzymatic hydrolysis which is integrated between the penicillin and 6APA production stage.

The penicillin G is extracted from the fermented broth by solvent extraction; the end product of the solvent extraction is generally a crude concentrated solution of penicillin G, from which the penicillin is crystallized by azeotropic distillation with butanol. In the conventional 6APA enzymatic process, the dried crystals of penicillin G are dissolved in water to prepare a pure solution of penicillin G which is sent to the hydrolysis

stage to yield 6APA and phenyl acetic acid. It would appear that if the 6APA plant is located adjacent to the penicillin fermentation plant, then it would be possible to hydrolyze directly the crude solution of penicillin G obtained from the solvent extraction, bypassing the crystallization stage. In this stage about 8–10% of penicillin G is lost either in the mother liquor or through degradation during the azeotropic distillation.

In addition it should also be possible to recover the phenyl acetic acid, which can be used as precursor in the penicillin fermentation, and so recycled for refuse in the fermentation plant itself. Research work was started at the Monterotondo Microbiological Laboratory of SNAM Progetti with the aim of investigating this possibility.

A crude solution of penicillin was obtained from an industrial plant of a leading penicillin G manufacturer and was hydrolyzed directly using fiber-entrapped penicillin amidase. The results obtained were initially very disappointing as shown in Fig. 1. The residual activity of the fiber-entrapped enzyme was drastically reduced when the solution of penicillin G prepared from the crystallized product was replaced with a crude penicillin solution; the loss of activity of the enzyme was very apparent. It was found that, despite the fact that the crude solutions used had been stored at low temperature, they showed signs of product degradation. Thus initially it was believed that the loss of activity of the enzyme was due to these degradation products of penicillin G. Subsequent research work, however, has shown that this is not the case. The probable reason for loss of activity is the presence of compounds which are transferred from the fermented broth to the crude solution during the extraction stage and are subsequently removed during the crystallization; hence the pure crystals do not show this loss of activity.

Finally we discovered a method to remove these inactivating agents from the crude penicillin solution which has been successful. We have now succeeded in hydrolyzing the treated crude penicillin solution without a sensible loss in residual activity of the fiber-entrapped amidase. We show in Fig. 2 the variation of the residual activity plotted against time. When a pretreated crude solution is hydrolyzed, the residual activity is reduced by about 10%, compared to a pure penicillin solution. In addition, the initial residual activity can be restored any time the crude treated solution is replaced by the pure solution of penicillin G. The kinetics of the hydrolysis of the pure and of the pretreated penicillin solutions have been compared, and it is seen in Fig. 3 that there are no substantial differences when the consumption of caustic soda during the hydrolysis is plotted against time for both solutions.

The recovery of 6APA from the treated solution of penicillin G was then performed with only minor modification for the usual operating

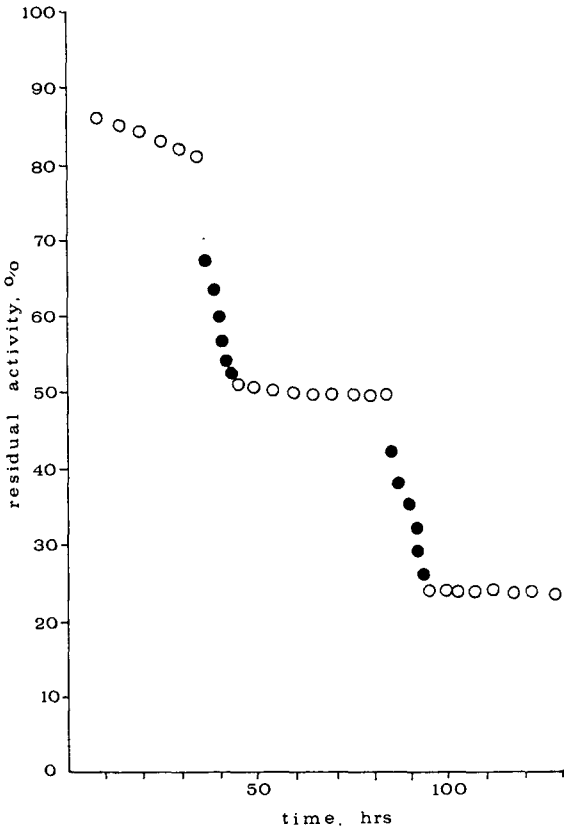

FIG. 1. Hydrolysis of penicillin G. Residual activity of fiber-entrapped penicillin amidase versus time. ○, pure penicillin solution; ●, treated penicillin solution.

procedure used in the conventional process. The characteristics of the 6APA produced from the treated solution are identical to those of 6APA obtained from the pure penicillin solution; in addition, these 6APAs have the same degree of purity.

The yield of 6APA obtained through bypassing the crystallization is shown in Table 1, where the conventional process, starting from pure penicillin solution, is compared with the integrated process utilizing a pretreated solution of crude penicillin. From this table it can be seen that the hydrolysis yield remains unchanged and that the integrated process presents a yield about 5% higher than that of the conventional process. If we now translate these results into economics and compare the con-

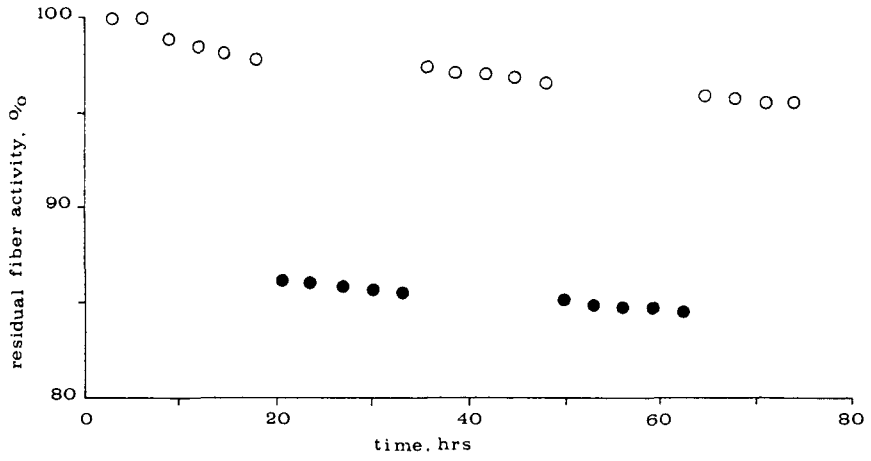

FIG. 2. Hydrolysis of penicillin G. Residual activity of fiber-entrapped penicillin amidase versus time. ○, pure penicillin; ●, treated penicillin solution.

ventional process, the integrated enzymatic processes, and the chemical process, we can observe the following:

1. The yield of the chemical process is slightly higher than that of the enzymatic process, which is due to higher losses of 6APA in the mother liquor during the recovery of 6APA from the enzymatically hydrolyzed solution since this solution has a lower concentration of

TABLE 1. **Comparison of the Yields of 6APA Produced by Conventional and Integrated Processes**

	Conventional process (mol)	Integrated process (mol)
Crude penicillin G solution	100	100
Crystallization losses	7.3	—
Treating losses	—	0.5
Penicillin G feed to the hydrolysis	92.7	99.5
6APA residue in the fiber	8.3	8.32
6APA in the hydrolyzed solution	79.8	85.80
6APA losses in the mother liquor	7.4	8.78
Total 6APA produced	80.7	85.34
Hydrolysis yield (%)	95.0	94.6
Crystallization yield (%)	91.6	90.0

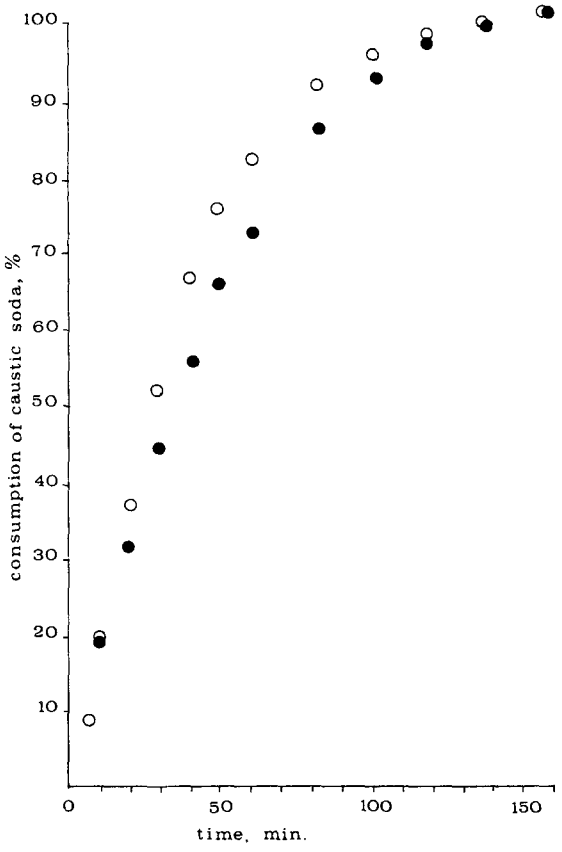

FIG. 3. Consumption of NaOH during the hydrolysis versus time. ○, pure penicillin; ●, treated penicillin solution.

6APA. This is because it is not possible to hydrolyze enzymatically a concentrated solution of penicillin G since it causes inhibition of the enzyme.

2. Less expensive raw materials are involved in the enzymatic process owing to its very simple mechanism of reaction.
3. The cost of the utilities is lower in the enzymatic hydrolysis because the hydrolysis is performed at ambient temperature and so refrigeration is not required.
4. The investment cost is lower in the enzymatic hydrolysis owing to a simpler processing scheme.

These observations have been quantified in Table 2 which indicates a breakdown of the manufacturing cost of the 6APA for the three processing alternatives.

The manufacturing costs of the 6APA, which are shown in Table 2, are totally dependent on the price of the penicillin G. The price of penicillin G is cyclical in the market and subject to the fluctuation. Figure 4 shows the manufacturing cost of the 6APA as a function of penicillin price. For the integrated process, the cost of the penicillin G can be discounted by the cost of the utilities, chemicals, and operating labor encountered in the crystallization stage which have been estimated at about $2.0/BU. The enzymatic processes also show lower manufacturing costs of 6APA both in low and high penicillin G price ranges, which means that in the small-scale production of 6APA where higher prices have to be paid for the supply of the penicillin G raw material, the enzymatic hydrolysis can be competitive with the chemical hydrolysis. The integrated process in particular appears to be very attractive for those manufacturers of penicillin G who are able to integrate the production of penicillin G with that of 6APA. In addition, the values shown in Fig. 4 have not taken into consideration the possibility of recycling the phenyl acid, which could contribute an estimated saving of 10% toward the cost of the raw materials needed for the production of the penicillin G. This assumes that 60% of the phenyl acetic acid is recovered and recycled. The chemical route has been well developed over the years and is nearly a practical maximum while the enzymatic routes have considerable room for improvement. Crystallization losses can be reduced by higher fiber loading, thereby allowing more concentrated penicillin G solutions to be used.

TABLE 2. **6APA Manufacturing Costs ($/kg)**[a]

	Chemical process	Conventional enzymatic process	Integrated enzymatic process
Penicillin G	43.72	46.01	39.65
Chemicals	7.49	0.78	1.35
Fiber-entrapped amidase	—	2.67	2.67
Utilities	4.64	0.91	0.91
Labor	3.12	3.95	3.95
Operating supplies	0.29	0.36	0.36
Depreciation	6.16	4.99	3.85
Other overhead	2.99	2.82	2.54
Total	68.41	62.49	55.28

[a]Basis: plant capacity, 40,000 kg/yr; penicillin G price, $14.35/BU.

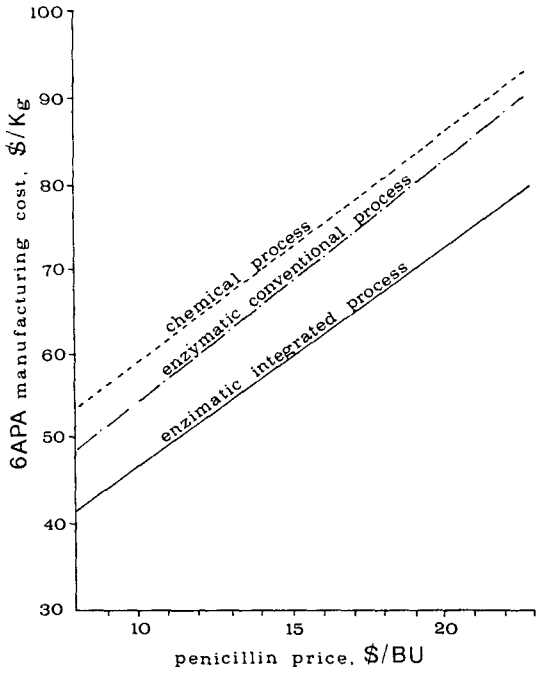

FIG. 4. 6APA manufacturing cost versus penicillin G. Price basis: plant capacity, 400,000 kg/yr.

We have concluded that the integrated process shows a great potential. The economics of this integrated process are sufficiently attractive to study the potential for revamping existing chemical 6APA plants located adjacent to penicillin fermentation facilities or to allow a penicillin manufacturer, which is not yet producing 6APA, to consider entering the market. For some time now, penicillin G has been considered a bulk commodity; with this process it is now conceivable that 6APA will achieve commodity status.

EXPERIMENTAL PROCEDURES USED

Preparation of Entrapped Penicillin Acylase

Throughout the series of experiments necessary to evaluate the viability of the system, the standard procedure described by Dinelli (1) to immobilize the penicillin acylase has been used. Ten kilograms of a solution of penicillin acylase containing glycerol, which had been phosphate-

buffered at pH 8, was added to a solution containing 5 kg of cellulose triacetate in 71.4 liters of methylene chloride which had been cooled to 4°C and slowly stirred. The emulsion which was then formed was extruded through a spinneret in a coagulation bath containing toluene. The fibers formed were then dried to remove all organic solvents.

Determination of Enzymatic Activity of the Entrapped Penicillin Acylase

The fibers containing the entrapped enzymes were divided into five equal portions. A single portion, which corresponded to 1 kg of cellulose acetate, was then strung along a jacketed column (43 × 14 cm), parallel to the column's longitudinal axis, and then fixed at the bottom. The column was then connected, via a pump, to a jacketed tank. Twenty liters of 6% wt/vol penicillin G (potassium salt), which had been buffered to pH 8.2 by a 0.02 M phosphate buffer was then continually pumped through the jacketed column, until a 97% or more conversion had been achieved. The pH was maintained at 8.2 by the automatic addition of NaOH solution. The penicillin hydrolysis curve so determined is illustrated in Fig. 3. Solutions of the potassium salt of the penicillin G and the crude solutions, extracted from the fermentation broth, were alternatively hydrolyzed. In order to determine the residual activity of the penicillin acylase, determinations of times necessary to achieve 50% conversions of the penicillin G solution 6APA have been measured and then compared to the times taken when the fiber has not been previously used.

Analytical Methods Used

The amount of 6APA in the hydrolyzed penicillin G solutions was measured by the hydroxylamine method (2). The purity of the 6APA extracted from the final reaction mixture was determined by the same method. Iodometric (3) and *p*-dimethylaminobenzaldehyde (4, 5) methods were also used.

REFERENCES

1. DINELLI, D. (1972) Process Biochem. 7 : 9.
2. BATCHELOR, F. R., CHAIN, E. B., HARDY, T. L., MANSFORD, K. R. L., and ROLINSON, G. H. (1961) Proc. Roy. Soc. B154 : 498.
3. ALICINO, J. F. (1961) Anal. Chem. 33 : 648.
4. BOMSTEIN, J. (1965) Anal. Chem. 37 : 576.
5. BALASINGHAM, K., WARBURTON, D., DUNNILL, P., and LILLY, M. D. (1972) Biochem. Biophys. Acta 276 : 250.