Penicillin Production in Corn Steep Media with Continuous Carbohydrate Addition[1]

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The work reported here presents the results of an investigation into the replacement of lactose by glucose or by sucrose in a corn steep liquor medium for the production of penicillin. Previous work has established (Johnson, 1946) that glucose and sucrose are metabolized by *Penicillium chrysogenum* at a more rapid rate than is lactose. Also, it has been shown that a maximum rate of penicillin production is obtained under fermentation conditions which support a slow rate of growth, such as those of the conventional lactose fermentation. In order to approximate those conditions when glucose or sucrose was used as a carbohydrate source, these sugars were fed at rates chosen so as to restrict their utilization. The optimum feed rate of each sugar for penicillin production was then determined. This work was done exclusively in 30-liter stirred and aerated stainless steel fermentors. Work of a similar nature employing a synthetic medium has been reported elsewhere (Hosler and Johnson, 1953).

Experimental Methods

Fermentation Techniques

Penicillium chrysogenum W49-133 was used throughout these experiments. Previous studies on penicillin production by this strain, and its derivation from the parent strain *Penicillium chrysogenum* Q176, have been reported elsewhere (Anderson *et al.*, 1953).

A vegetative inoculum for the 30-liter fermentors was prepared through several stages. Firstly, in a 500-ml Erlenmeyer flask a mixture of 5 g bran and 5 g crushed corn, moistened with tap water and sterilized by autoclaving at 15 pounds pressure for 45 minutes, was inoculated with a small quantity of a soil stock and incubated at 25 C for 7 days, by which time sporulation was abundant. Several flasks were prepared in this way at one time, and stored at 5 C until required. Secondly, to the sporulated corn-bran flask was added 100 ml of a solution containing 6 g dextrin and 2 g corn steep solids, previously sterilized by autoclaving. The spore suspension thus obtained was incubated at 25 C on a rotary shaker operating at 250 rpm and describing a 2-inch circle. After 24 hours the contents of the flask were added aseptically to a 30-liter seed fermentor, similar in design to the fermentors described by Brown and Peterson (1950). This seed fermentor contained 16 liters of a medium comprising (in grams per liter) corn steep solids 25, lactose 30, glucose 2.0, KH_2PO_4 0.25, $MgSO_4 \cdot 7H_2O$ 0.1, and $CaCO_3$ 1.0. To control foaming, 1.0 g per liter of a 6 per cent solution of Alkaterge C[3] in lard oil was added, and the pH of the medium was adjusted to 6.2 with NaOH before sterilization.

The seed fermentor was incubated in a water bath at 25 C, agitated at 380 rpm and aerated at the rate of 0.5 volumes of air per volume of medium per minute. After 24 hours' growth the required volume of inoculum for the 30-liter fermentors was withdrawn into sterile 1-liter Erlenmeyer flasks. Each fermentor received 5 per cent by volume of this inoculum.

The medium used in the 30-liter fermentors contained (in grams per liter) corn steep solids 30, glucose 5, $CaCO_3$ 5, Na_2SO_4 1.0, and Alkaterge C in lard oil 1.0, made up to a volume of 15 liters. The sugar to be added during the run was dissolved separately in tap water, the quantity of potassium phenylacetate (precursor) solution equivalent to a concentration in the fermentor of 3 g phenylacetic acid per liter, recommended by Brown and Peterson (1950), was added to it, and the volume made up to 3 liters. For the control runs, in which all of the sugar was added initially, the potassium phenylacetate was made up in 3 liters of solution and fed during the fermentation as for a sugar solution.

An antifoam reservoir filled with the solution of Alkaterge C in lard oil was fixed to the fermentor head, and a metering valve attached after sterilization, to permit the addition of antifoam to the fermentor on demand. The construction of this antifoam metering valve been described by Anderson *et al.* (1953) and a general description of the fermentors given by Brown and Peterson (1950).

Each fermentor was incubated at 25 C in the water bath, agitated at 500 rpm and aerated at the rate of 1.0 volumes of air per volume of medium per minute. No provision for pH control during the runs was found

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necessary. After sterilization, the pH of the medium was in the range 5.6 to 5.8, and 20 to 24 hours after inoculation, when sugar feeding was begun, the pH had risen to about 7.0 in almost every run.

The sugar and precursor solution was fed to the fermentors by means of a small 1-rpm motor-operated positive displacement pump shown in figure 1. The rubber tubing connecting the sugar reservoir to the fermentor passed along the semi-circular channel of the pump so that the tubing was always compressed by at least one of the rollers which travelled along the channel. The pump was caused to operate intermittently by means of a timer adjusted so that the sugar solution was fed at the desired rate.

Samples were withdrawn from the fermentors at intervals for penicillin assays and chemical analyses, and were handled in the manner described by Gailey *et al.*, (1946).

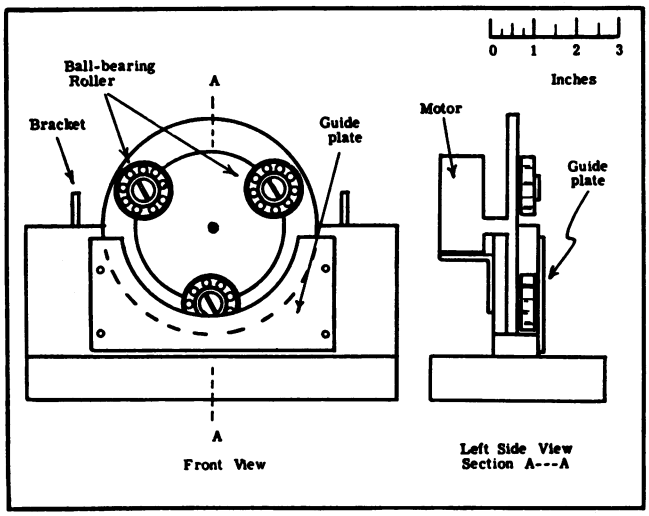

FIG. 1. Rotary positive displacement pump

Analytical Procedures

The pH of each sample was determined immediately after its withdrawal by means of a glass electrode.

The total penicillin titer was measured by a modification of the cylinder-plate method of Schmidt and Moyer (1944). The test organism used was *Micrococcus pyogenes* var. *aureus*.

All sugars were determined by the Shaffer and Somogyi (1933) method, with reagent 50 containing 5 g KI, and titrations were referred to standard curves prepared for each sugar. Lactose was hydrolyzed in 0.5 N HCl in an autoclave at 15 pounds pressure for 20 minutes. Sucrose was similarly hydrolyzed, except that the heating time was reduced to 5 minutes.

Soluble Kjeldahl nitrogen was determined by the micro-method of Johnson (1941) and the corresponding mycelial nitrogen content for any sample calculated by difference from the soluble nitrogen value of the sample taken at the time of inoculation.

For the determination of ammonia, a small aliquot of the undiluted sample filtrate was made alkaline, the solution then gently aerated, and the ammonia evolved absorbed in an aliquot of standard sulfuric acid.

RESULTS AND DISCUSSION

It was found that, with a concentration of 0.5 per cent glucose present initially in the fermentor medium, growth from the inoculum was rapid and the growth phase was virtually complete in 24 hours. The mold appeared to be indifferent to the presence of another sugar during this phase. Continuous sugar feeding was begun at 20 to 24 hours after inoculation and continued for 72 hours; extension of the feeding time beyond this period appeared to have no advantage, as a decrease in penicillin titer was found to occur at about 100 hours in most runs even when the sugar supply was not limiting.

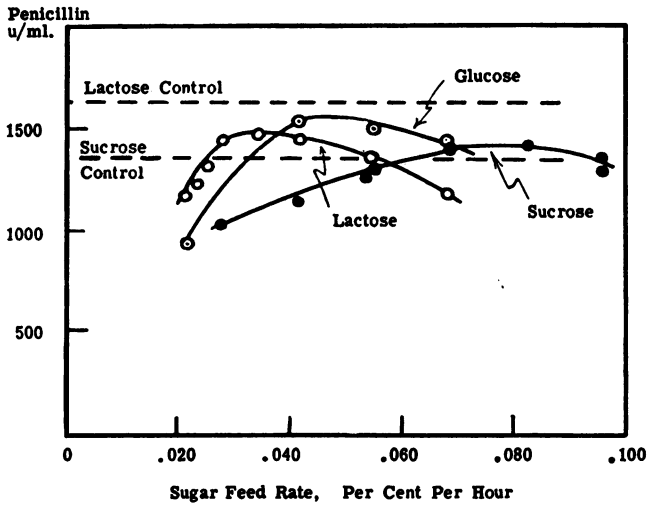

FIG. 2. Effect of sugar feed rate on penicillin production

For purposes of comparison of the value of the feeding technique for the three sugars tested, the conventional fermentation with 3 per cent lactose present initially was taken as a standard. None of the fed runs reached a penicillin titer as high as that in the lactose standard, which was 1610 units per ml (average of 3 runs). The penicillin yields from fermentations fed glucose, sucrose or lactose are shown in figure 2.

For glucose, the best feed rate tested was found to be 0.042 per cent per hour, equivalent to 3 per cent overall, giving a maximum penicillin titer of 1520 units per ml at 83 hours.

In the series of sucrose-fed runs it was found that the total concentration of sucrose required to give penicillin yields comparable with those from glucose or lactose-fed media was considerably higher than for those sugars.

The penicillin titer of control runs, in which 5 per cent sucrose was present initially, was 1360 units per

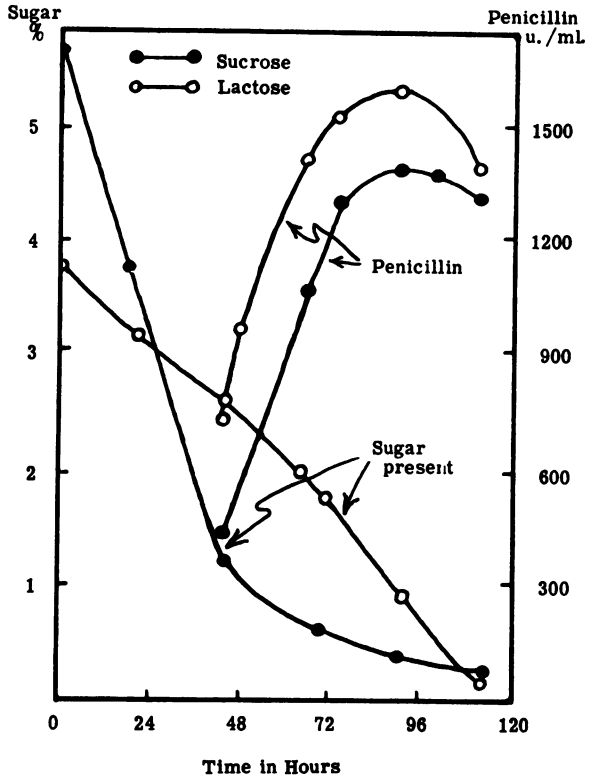

FIG. 3. Comparison of rates of sugar utilization and penicillin production for lactose and sucrose control fermentations

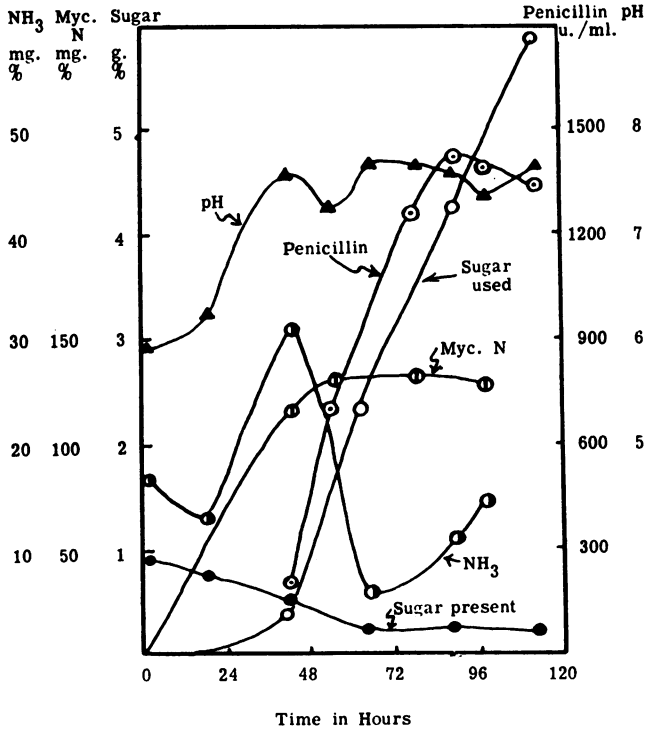

FIG. 4. Chemical changes in a fermentation fed 5 per cent sucrose

ml (average of three). While there was close agreement in the chemical changes occurring in media containing lactose or sucrose respectively, as regards ammonia and mycelial nitrogen levels, there was a striking difference in the rates of sugar utilization. This is shown in figure 3. Apparently the sucrose is assimilated so rapidly by the mold that the condition of semi-starvation typical of the lactose fermentation, and which appears to be necessary to a high penicillin yield, does not occur. The 5 per cent sucrose was used almost entirely by the mold in 48 hours. However, when the same total concentration of sucrose was fed slowly over a period of 72 hours the 5 per cent level was not reached before 90 hours and the penicillin yield was higher, 1410 units per ml, although still not as high as that in the lactose-fed fermentations. The chemical changes occurring in the sucrose-fed run are shown in figure 4.

For the lactose-fed series, the optimum feed rate was found to be 2.4 per cent overall, or 0.034 per cent per hour, giving a penicillin titer of 1470 units per ml. The chemical changes in the fermentation to which 2.4 per cent lactose was fed revealed no differences from the changes in the control runs sufficient to explain the lower yield in the fed fermentation.

TABLE 1. *Effect of time of addition of lactose on penicillin yield*

LACTOSE CONCENTRATION	TIME OF ADDITION TO MEDIUM	MAXIMUM PENICILLIN YIELD
%		*units per ml*
3.0	Before sterilization	1610
2.4	Before sterilization	1590
2.4	With inoculum	1630
2.4	After 16 hours growth	1542
2.4	After 22 hours growth	1490
2.4	Fed from 16th hour	1560
2.4	Fed from 22nd hour	1470

Some further experiments with lactose-fed runs were conducted in an attempt to clarify the anomaly apparent in figure 2; namely, that lactose when slowly fed would not give as high a penicillin yield as when present initially in the medium. The results of these experiments, which are shown in table 1, suggest that the lactose solution carried some factor which was effective during the growth phase of the fermentation in stimulating a higher yield of penicillin during the later phase. The effect was not due to the earlier addition of the precursor, as this was present initially in the media of all these experiments. Samples of the commercial grade of lactose used in this work assayed 95 to 97 per cent lactose against a standard USP lactose monohydrate.

SUMMARY

With a slow feeding technique, more rapidly assimilated sugars, such as glucose or sucrose, have been employed successfully in place of lactose as the carbohydrate source in a corn steep medium for the production of penicillin. The work was done with 30-liter fermentors, and the penicillin-producing organism used was *Penicillium chrysogenum* W49-133. For the three sugars studied, the optimum feed rates found were:

for glucose, 0.042 per cent per hour (3.0 per cent total); for lactose, 0.034 per cent per hour (2.4 per cent total); and for sucrose, 0.070 per cent per hour (5.0 per cent total). The optimum yields of penicillin obtained, corresponding to these feed rates, were: for glucose, 1520 units per ml; for lactose, 1470 units per ml; and for sucrose, 1410 units per ml.

Penicillin yields in media containing commercial lactose as the carbohydrate source were found to be improved when the lactose was present early in the growth phase of the fermentation.

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Bacteriostatic and Bactericidal Actions of Laurylamine Saccharinate Against Certain Bacteria of the Naso-Pharyngeal Tract and Other Bacteria[1]

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A previous paper by the authors (Gray and Taylor, 1952) reported the bacteriostatic effects of laurylamine saccharinate[4] in cultures of several common bacteria from the Macdonald College Collection. The present paper reports the results of more critical tests of bacteriostatic action against eight of the species of bacteria previously tested, and of tests of the bactericidal power of the compound against species of bacteria freshly isolated from the naso-pharyngeal tract. The bacteria used for the latter test included a strain of *Diplococcus crassus*, four species of *Neisseria*, and one strain of *Streptococcus salivarius*.

MATERIALS AND METHODS

The naso-pharyngeal bacteria were cultivated by streaking cotton swabs infected by contact with the surface of the nasal passage or the back of the mouth, onto the surface of suitable agar media in Petri plates. The solid medium found to be the most suitable for isolating *Diplococcus* and the *Neisseria* species was brain-veal agar (Difco). The *Streptococcus* was isolated from plates of brain-heart infusion (Difco) with agar (1.75 per cent) and methylene blue 1:50,000; it developed green colonies on the medium containing the dye at that concentration. A study of the morphology, staining by Gram's method, growth of colonies, and actions on carbohydrates yielded results which suggested that the characters of the *Neisseria* and *Streptococcus* species agree closely with those described under the chosen binomials in Bergey's Manual of Determinative Bacteriology (Breed *et al.*, 1948). The binomial *Diplococcus crassus* was applied by reason of close agreement of the culture's actions on carbohydrates, as reported by Mackie and McCartney (1942).

Before proceeding to test the bactericidal power of the laurylamine saccharinate against the freshly isolated bacteria, it was necessary to ascertain the concentrations which would prevent their growth. It was also necessary to repeat such tests with the Macdonald College Collection species (hereinafter designated by the letters MCC), to ascertain more critically the bac-

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[4] The laurylamine saccharinate was supplied by Delmar Chemicals Ltd., Lachine, Quebec, Canada.