[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

The Role of the Constituents of Synthetic Media for Penicillin Production[1]

BY F. G. JARVIS AND MARVIN J. JOHNSON

In any fermentation a close relationship exists between the environment of the organism and the nature of its metabolic processes. A thorough knowledge of this relationship is ordinarily required to obtain optimal results from the fermentation. The object of the work presented in this paper was to obtain, in part, an understanding of this relationship for the *P. chrysogenum* Q176 penicillin fermentation. The advantages of using chemically defined media in such studies are self evident. In addition, the reproducibility and relative freedom from complex extraneous compounds obtainable with a synthetic medium might be of considerable importance in penicillin manufacture.

Although several publications have appeared concerning the use of synthetic media for the production of penicillin in submerged culture[2,3,4,5] information concerning the metabolic behavior of penicillin-forming molds is still far from adequate. Practically no work dealing with the growth and metabolism of *P. chrysogenum* Q176 on synthetic media has, to the authors' knowledge, been published. Because of the importance of this organism in the field of penicillin production, more information concerning its metabolic behavior is particularly desirable.

Experimental Methods

Fermentation Techniques.—*P. chrysogenum* Q176 was used exclusively in these experiments. Data concerning the origin of this strain and preliminary studies on penicillin yields obtained by its use have already been published.[6,7]

All fermentations were conducted on a rotary type shaker in 500-ml. Erlenmeyer flasks. The shaker operated at about 275 r. p. m., imparting a motion such that all points on each flask described a horizontal circle one inch in diameter. Eighty ml. of medium was used in each flask. All fermentations were conducted at 25°.

Samples for penicillin assays and chemical analyses were taken under aseptic conditions and handled in the manner described by Gailey, *et al.*[6]

The standard salt mixture used in the experiments contained in grams per liter: KH_2PO_4, 3.0; $MgSO_4 \cdot 7H_2O$, 0.25; $FeSO_4 \cdot 7H_2O$, 0.10; $CuSO_4 \cdot 5H_2O$, 0.005; $ZnSO_4 \cdot 7H_2O$, 0.02; Na_2SO_4, 0.50; $MnSO_4 \cdot H_2O$, 0.02; and $CaCl_2 \cdot 2H_2O$, 0.05. A variation of this mixture which omitted the Na_2SO_4, $MnSO_4 \cdot H_2O$ and $CaCl_2 \cdot H_2O$ and included 3 μg. per liter of chromium was employed in our early experiments. No significant differences between the two mixtures could be detected under our standard fermentation conditions in which U. S. P. calcium carbonate was used in the inoculum medium and U. S. P. lactose[8] and lactic acid in the fermentation medium. On highly purified media, however, manganese and calcium were found to be required and were subsequently added to all media. No requirement could be demonstrated for chromium. The sodium sulfate was added to ensure sufficient sulfur for the high penicillin yielding fermentations encountered.

Vegetative inoculum was used throughout the experiments. In order to eliminate the use of corn steep liquor completely, a synthetic medium was used for the growth of all inoculum. This medium contained in grams per liter: glucose 40, ammonium sulfate 13, calcium carbonate 13 and the standard salt mixture.

The calcium carbonate was autoclaved separately (in suspension in distilled water) and added to the rest of the medium immediately before inoculation. A suspension of spores from a bottle plate, prepared from soil stock by the method described by Gailey, *et al.*,[6] was used to inoculate the medium. A heavy well-dispersed growth, which is easily handled in wide-mouth pipets, develops in about forty hours. A 3% inoculum was used in all fermentations.

All fermentations were run in triplicate, the penicillin values reported being the average of three flasks in every case.

Analytical Procedures.—Penicillin was assayed by the Oxford cup method with the use of *Staph. aureus* H as the test organism[9,10] and penicillin G as a standard.

The *p*H of each sample was determined immediately after removal by means of a gas electrode.

All sugars were determined by the Shaffer and Somogyi method[11] using their reagent 50 with 5 g. of potassium iodide. Titrations were referred to standard curves prepared for each sugar. Lactose-glucose combinations were referred to a standard hydrolyzed-lactose curve. Sucrose was hydrolyzed in 0.5 *N* sulfuric acid at 100° for ten minutes, lactose in 0.75 *N* hydrochloric acid in an autoclave at 15 lb. pressure for thirty minutes.

Lactic acid was determined by the method of Barker and Summerson.[12] The results obtained by this method checked with those obtained by the Friedemann method[13] within ±5%.

To determine acetic acid, appropriate aliquots of culture filtrate were steam distilled and the distillate titrated with a standard barium hydroxide solution to the phenolphthalein end-point.

The method used for ammonia-nitrogen was that described by Gailey, *et al.*[6] In some of our more recent experiments the ammonia was steam distilled rather than aerated.

Soluble Kjeldahl nitrogen was determined by the method described by Johnson.[14] The difference between the Kjeldahl nitrogen level and the ammonia-nitrogen level is reported as soluble organic nitrogen.

The mycelial nitrogen was determined by subtracting the soluble nitrogen present at the time of sampling from the soluble nitrogen present at the time of inoculation.

(1) Published with the approval of the Director of the Wisconsin Experiment Station. Supported in part by grants from the Heyden Chemical Corporation and the Bristol Laboratories.

(2) H. Koffler, S. G. Knight, W. C. Frazier and R. H. Burris, *J. Bact.*, **51**, 385 (1946).

(3) R. Pratt and Jean Dufrenoy, *Science*, **102**, 428 (1945).

(4) R. W. Stone and M. A. Farrell, *ibid.*, **104**, 445 (1946).

(5) J. W. Foster, H. B. Woodruff and L. E. McDaniel, *J. Bact.*, **51**, 465 (1946).

(6) F. B. Gailey, J. J. Stefaniak, B. H. Olson and M. J. Johnson, *J. Bact.*, **52**, 129 (1946).

(7) K. Higuchi, F. G. Jarvis, W. H. Peterson and M. J. Johnson, THIS JOURNAL, **68**, 1669 (1946).

(8) In this paper, the term "lactose" always refers to the monohydrate.

(9) J. W. Foster and H. B. Woodruff, *J. Bact.*, **47**, 43 (1944).

(10) W. H. Schmidt and A. J. Moyer, *ibid.*, 199 (1944).

(11) P. A. Shaffer and M. Somogyi, *J. Biol. Chem.*, **100**, 695 (1933).

(12) S. B. Barker and W. H. Summerson, *ibid.*, **138**, 535(1941).

(13) T. E. Friedemann and J. B. Graeser, *ibid.*, **100**, 291 (1933).

(14) M. J. Johnson, *ibid.*, **137**, 575 (1941).

Results and Discussion

General Nutritional Requirements.—The known inorganic requirements of the organism are reflected qualitatively by the salt mixture employed. Quantitative investigations on these requirements are now in progress, but are at present too incomplete to justify inclusion in this publication. In order to increase the buffering capacity of the media used, the potassium dihydrogen phosphate level employed is somewhat greater than would be required for purely nutritive purposes.

It has been previously shown[2] that the ammonium ion is more available to *P. chrysogenum* than the nitrate ion as a source of nitrogen. As there are no data which show organic nitrogen to be in any way superior to ammonia nitrogen for this organism our basal synthetic media have been designed to contain the latter as the sole source of nitrogen.

Carbohydrates were used in the experiments as the major carbon source. As only slightly greater penicillin yields were obtained in our early experiments by the use of 4% or higher sugar levels as compared with 3% levels, the latter quantity has been employed throughout our investigations. Details concerning the use of various types of carbon compounds in the fermentation media are given later in this paper.

Fermentation Rate.—The total amount of penicillin formed during a fermentation depends on the quantity of mycelium present, the rate of penicillin production per gram of mycelium, and the length of time during which penicillin formation can be sustained. It has been realized for some time that superior yields of penicillin are obtained only on media which support relatively slow fermentations. The higher yields obtained in such fermentations are, in part, due to the longer time period available for penicillin formation. A second reason for these higher yields is offered by the data presented in Fig. 1, which show that a maximum rate of penicillin production is obtained under fermentation conditions which support a very slow rate of growth. As the rate of growth during these fermentations varied in a continuous manner with the fermentation time, corresponding *p*H curves could also be drawn. These *p*H curves partially explain the difference in the shape of the penicillin production rate curves obtained in the two media. Thus, in the autolytic phase, the sharp decrease in rate of penicillin production encountered in experiment B is accompanied by a *p*H trend away from the optimum (*p*H 7.3), while the slower decrease encountered in experiment A is accompanied by a *p*H trend toward this optimum. We have not been able to devise a medium on which rapid mycelium growth and rapid penicillin production occur simultaneously.

Although conditions which support a very low hourly mycelium increase are desirable for penicillin formation, the growth phase of the fermentation should be characterized by the rapid development of a high concentration of mycelium. Thus an ideal medium should support two distinct fermentation rates: a rapid rate throughout the growth phase and a much slower rate during the remainder of the fermentation.

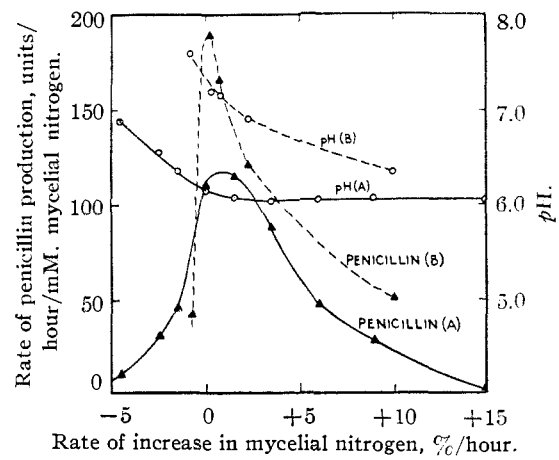

Fig. 1.—Relationship of rate of growth to rate of penicillin production. Negative growth rates indicate autolysis. (A) Medium contained in grams per liter: sucrose 30, ammonium acetate 8, and the standard salt mixture. (B) Medium contained in grams per liter: lactose 22.5, glucose 7.5, ammonium acetate 3, ammonium lactate 5 and the standard salt mixture.

A synthetic medium capable of producing this ideal performance can be approximated by properly choosing and balancing its constituents. Under a given set of conditions, the major factor affecting the rate of fermentation is the nature of the carbohydrate used in the medium. Glucose is fermented at a very rapid rate (Fig. 2), and lactose at a much slower rate (Fig. 3). Sucrose and galactose are utilized at about the same rate as glucose.

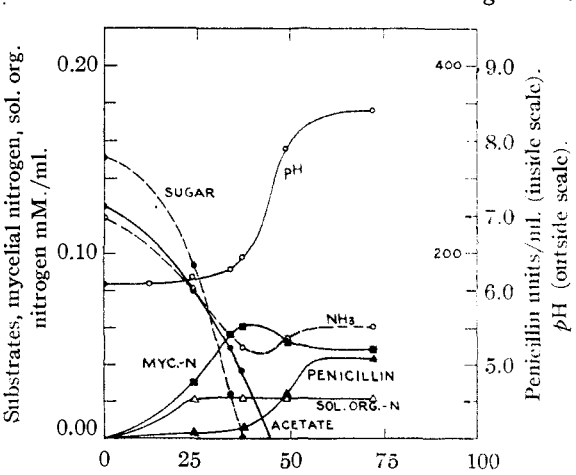

Fig. 2.—Glucose–ammonium acetate fermentation. Medium contained in grams per liter: glucose 30, ammonium acetate 10 and the standard salt mixture.

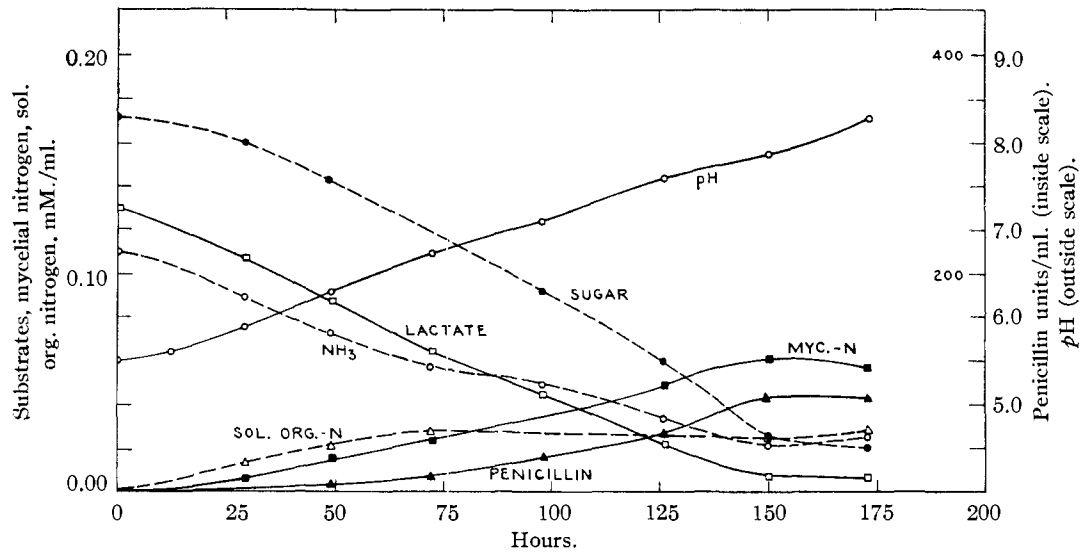

Fig. 3.—Lactose–ammonium lactate fermentation. Medium contained in grams per liter: lactose 30, ammonium lactate 13, and the standard salt mixture. Lactose is reported as mM. of monosaccharide per ml.

As the rate of mycelium production varies directly as the rate of sugar utilization during the growth phase, the use of a mixture of glucose and lactose in the medium will produce suitable rate conditions for the fermentation.

Under the conditions obtaining in our experiments, a lactose-glucose ratio of about 3:1 produced optimal results as shown in Fig. 4.

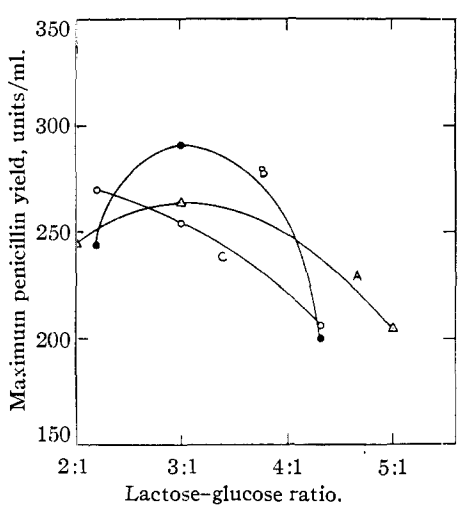

Fig. 4.—Effect of lactose/glucose ratio on penicillin yields. (A) Medium contained 3% total sugar and 0.3 g. of ammonium acetate per g. of glucose; ammonium lactate was added to make about 0.15% total nitrogen; (B) 4% total sugar, 0.35 g. ammonium acetate per g. of glucose, ammonium lactate to make about 0.2% total nitrogen; (C) 4% total sugar, 0.25 g. ammonium acetate per g. of glucose, ammonium lactate to make about 0.2% total nitrogen.

pH Requirements.—The results of two typical experiments designed to determine the optimal *p*H for the growth of the organism are shown in Fig. 5. The differences in maximum growth obtained in these experiments may be attributed to differences in the media used. The optimum *p*H was 6.8 to 6.9 for the media employed, and was independent of total growth obtained. Drifts in *p*H during these fermentations were small and were taken into account in the preparation of the data. Certain observations which will be discussed later in this paper indicate that the *p*H optimum obtained holds true only for media containing acetate.

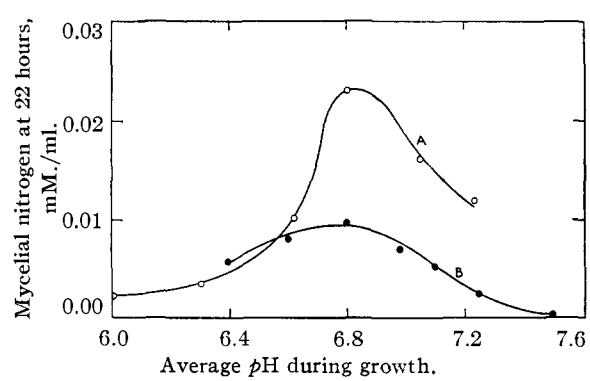

Fig. 5.—Optimum *p*H for growth on media containing acetate. Initial *p*H adjustments were made with sodium hydroxide or sulfuric acid. (A) Medium contained in grams/liter: glucose 30, ammonium acetate 10, potassium dihydrogen phosphate 6, and other inorganic salts as in the standard salt mixture. (B) Medium contained in grams/liter: lactose 22.5, glucose 7.5, ammonium acetate 3, ammonium lactate 7, potassium dihydrogen phosphate 20, and other inorganic salts as in the standard salt mixture.

The *p*H for optimal penicillin production on a lactose-glucose–ammonium acetate-ammonium lactate medium was found to be about 7.3 as

shown in Fig. 6. The fermentations conducted at the lower *p*H levels required frequent *p*H adjustment starting at thirty-six hours. The fermentations conducted at *p*H 7.3 and 7.8 required little adjustment.

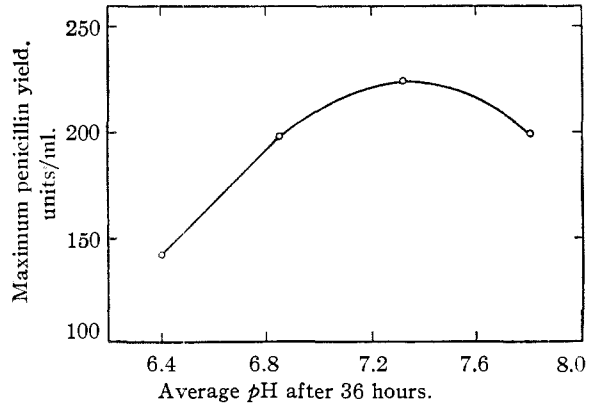

Fig. 6.—Optimum *p*H for penicillin production. Medium contained in grams/liter: lactose 22.5, glucose 7.5, ammonium acetate 3, ammonium lactate 7, and the standard salt mixture. Fermentations were maintained at relatively constant *p*H levels by frequent adjustment with sodium hydroxide or sulfuric acid.

pH **Control.**—From the foregoing data it is apparent that the growth phase of an ideal fermentation on media containing acetate should progress at a *p*H of about 6.8 and the subsequent penicillin formation phase at a *p*H of about 7.3. Investigations designed to obtain an understanding of the factors involved in *p*H control during fermentation, and to obtain a medium approximating the ideal are described below.

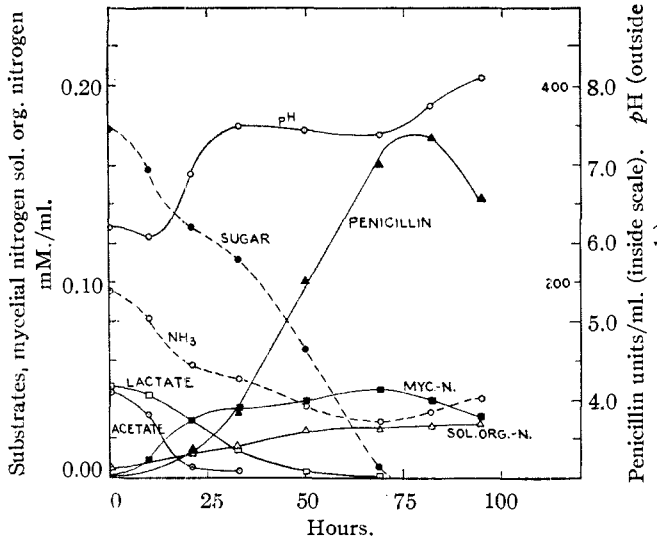

Fig. 7.—Fermentation on standard basal medium. Medium contained in grams per liter: lactose 22.5, glucose 7.5, ammonium acetate 3, ammonium lactate 5, and the standard salt mixture. Sugar is reported as mM. monosaccharide per ml. Sugars were autoclaved separately in distilled water.

The rate of utilization of ammonium ion varies directly with the rate of utilization of the carbohydrate present. Thus it is utilized rapidly during the fermentation of glucose or sucrose (Fig. 2), and very slowly during the fermentation of lactose (Fig. 3). In the lactose–glucose type of medium this results in a rapid utilization of ammonium ion during the growth phase, followed by a slow utilization during the subsequent penicillin formation phase (Fig. 7).

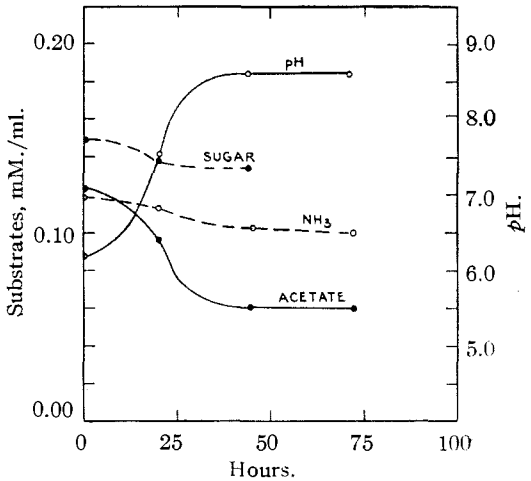

Fig. 8.—Lactose-ammonium acetate fermentation. Medium contained in grams per liter: lactose 30, ammonium acetate 10, and the standard salt mixture. Lactose is reported as mM. of monosaccharide per ml.

Since utilization of ammonium ion by the mold will cause a drop in *p*H, it is necessary that some fermentable anion be present which will be utilized at approximately the same rate as the ammonium ion. As was shown in Fig. 2, acetate is utilized at very nearly the same rate as ammonia during the fermentation of glucose and the *p*H remains nearly constant throughout this period. Upon exhaustion of the glucose, the uptake of ammonia was terminated and the *p*H rose rapidly because of the continued utilization of acetate. Following the exhaustion of acetate, the *p*H continues to rise as a result of ammonia liberation during autolysis of the mold mycelium. During the fermentation of lactose, acetate is utilized much more rapidly than ammonia, causing a rapid increase in *p*H to a level where all fermentation ceases. Such a fermentation is shown in Fig. 8. Hence, it would be expected that in a medium where glucose was used as a carbon source for growth, and lactose as a carbon source for the penicillin-forming phase, that acetate would function as a *p*H controlling agent during the growth phase, but that its utilization would be too rapid during the second phase. That this is true may be seen from Fig. 9.

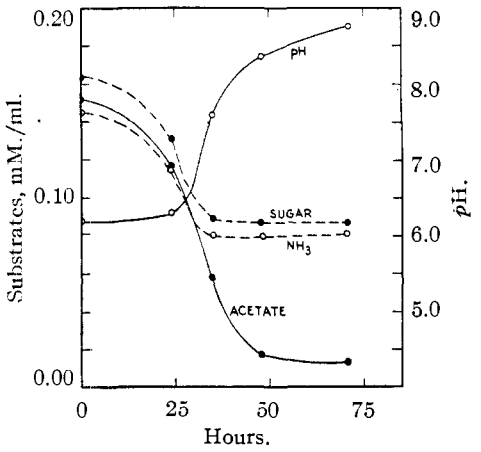

Fig. 9.—Lactose-glucose-ammonium acetate fermentation. Medium contained in grams/liter: lactose 15, glucose 15, ammonium acetate 10, and the standard salt mixture. Sugar is reported as mM. of monosaccharide per ml.

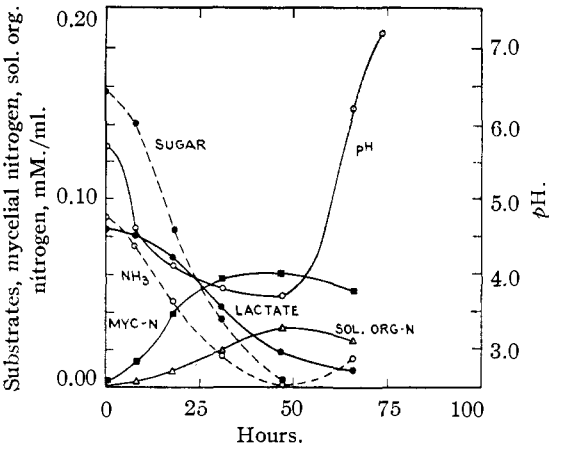

Fig. 10.—Glucose-ammonium lactate fermentation. Medium contained in grams per liter: glucose 30, ammonium lactate 9, and the standard salt mixture. Glucose was autoclaved separately in distilled water.

One method available for the control of *p*H during the penicillin formation phase of the fermentation involves the use of a slowly utilized anion. The application of this method has been successfully used in this Laboratory by the employment of lactate as the second fermentable anion. Two fermentations have been studied which demonstrate that lactate is used at a much slower rate than acetate by *P. chrysogenum* Q176. When lactate is used in conjunction with glucose (Fig. 10), the rate of ammonia utilization is much greater than that of the lactate causing a rapid drop in *p*H. When lactate is used in conjunction with lactose, it is used at a rate only slightly greater than that of ammonia as may be seen from Fig. 3.

In each of the fermentations discussed up to this point (except for the one shown in Fig. 7), penicillin yields have been either low or practically absent because of the lack of time during which conditions favorable for penicillin production were present. In Fig. 7 the chemical changes occurring during fermentation on a medium containing 2.25% lactose, 0.75% glucose, 0.3% ammonium acetate and 0.6% ammonium lactate are shown. The concentrations of acetate and lactate used in this medium are adjusted experimentally such that the acetate controls the *p*H during the primary growth phase and the lactate controls it during the subsequent penicillin-forming phase. The sharp *p*H rise which occurs at the end of the growth phase results from a combination of factors. The amount of acetate employed is somewhat in excess of that required to balance the ammonia utilized during the fermentation of the glucose, a portion of the lactate is used, and a short lag phase accompanied by a slow utilization of ammonia occurs upon exhaustion of the glucose. The *p*H rise at the end of the fermentation is the result of ammonia liberation accompanying the autolysis of mycelium subsequent to the exhaustion of carbohydrate. Growth rate and *p*H conditions favorable for the production of penicillin were present from the twentieth to the seventieth hour in this fermentation, and about 90% of the penicillin formation took place during this period. The fact that cessation of penicillin production and depletion of sugar occurred at about the same time would indicate that the amount of carbohydrate used in the medium was a limiting factor in the yield obtained.

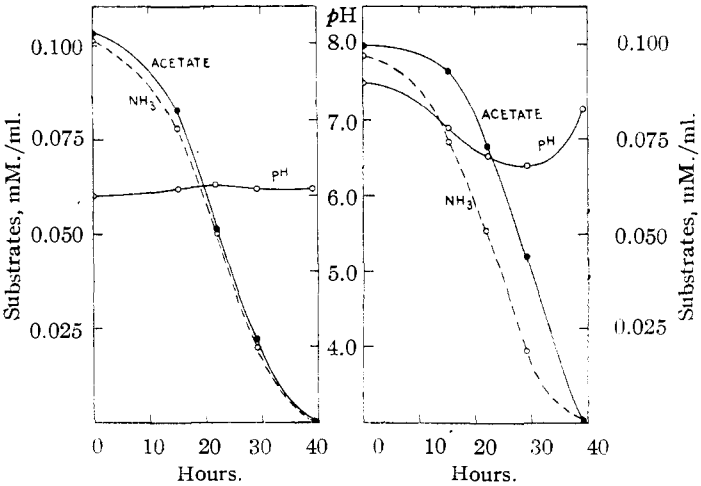

Fig. 11.—Relationship of ammonia and acetate utilization to *p*H. Medium contained in grams per liter: sucrose 30, ammonium acetate 8, and the standard salt mixture. Initial *p*H adjustments were made with sodium hydroxide.

The *p*H during the growth phase of this fermentation is somewhat below the optimum value. Presumably the growth phase would have been completed more rapidly had the *p*H been higher.

Subsequent work has shown that a relationship exists between the *p*H and the relative rates of utilization of ammonia and acetate. In Fig. 11 data on this relationship are given. It will be seen that at *p*H 6, acetate and ammonia are utilized at the same rate and the *p*H remains nearly constant. When the fermentation is started at *p*H 7.5, however, ammonia is utilized more slowly than acetate and the *p*H drops. After exhaustion of the ammonia, the continued utilization of acetate causes a final *p*H rise. When the effects of *p*H on the substrate utilization rates per millimole of mycelial nitrogen present are compared, as shown in Fig. 12, it is apparent that only acetate utilization is appreciably affected by *p*H.

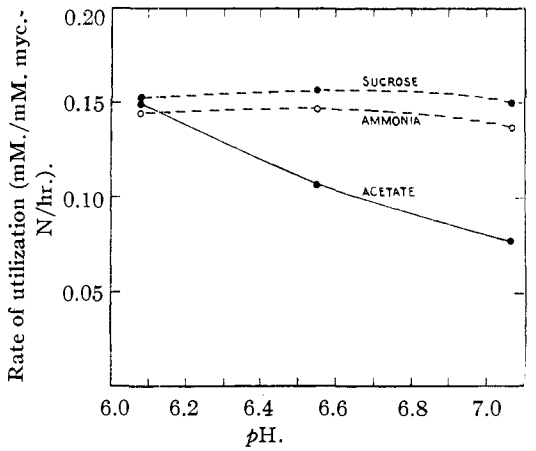

Fig. 12.—Effect of *p*H on substrate utilization rates. Medium contained in grams/liter: sucrose 30, ammonium acetate 8, and the standard salt mixture. Sucrose is reported as mM. of monosaccharide.

The effect of *p*H on the utilization rates of lactic acid and lactose have not yet been thoroughly investigated. In preliminary experiments on lactose-ammonium lactate media, however, the observation has been made that the *p*H tends to rise when the starting *p*H is low, and to fall when the starting *p*H is high. This would indicate that a similar relationship exists among the utilization rates of ammonia, lactate and lactose as was found for ammonia, acetate, and sucrose or glucose. The effect of the starting *p*H on the subsequent *p*H during fermentation on our "complete" medium (Fig. 13) can be explained on the basis of these relationships between *p*H and substrate utilization rates. During the first phase of the fermentation, while glucose and acetate are present, the *p*H tends to approach a value of about 6.1, which is the *p*H at which ammonia and acetate are utilized at equal rates in the presence of glucose or sucrose. The fact that the starting *p*H has very little effect on the *p*H obtained later in the fermentation is presumably due to the establishment of a similar equilibrium between the utilization rates of ammonia and lactate under the conditions present during this phase of the fermentation. This is borne out by the chemical changes occurring during fermentation. The *p*H obtained during this phase of fermentation is also relatively insensitive to the amount of ammonium lactate used in the medium.

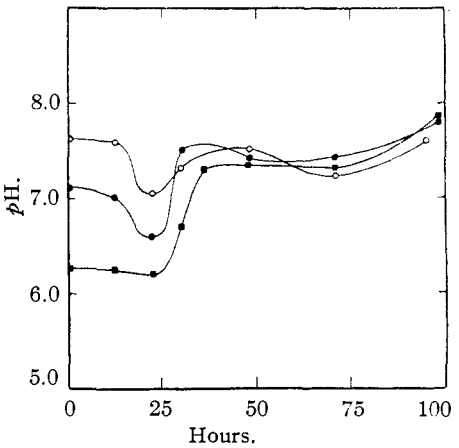

Fig. 13.—Effect of initial *p*H on subsequent *p*H. Medium contained in grams per liter: lactose 22.5, glucose 7.5, ammonium acetate 3, ammonium lactate 7, and the standard salt mixture. Initial *p*H adjustments were made with sodium hydroxide.

R-Group Precursors.—It has been known for some time that phenylacetic acid and certain of its derivatives increase the over-all yields of penicillin during fermentation.[15] In addition, these compounds have been shown to specifically increase the yield of the type of penicillin whose R-group is structurally related to the adjuvant used. A comparison of the effect of the penicillin G precursors: phenylacetic acid, phenylacetamide and β-phenylethylamine are given in Table I The latter compound, added as the acetate, was used in our subsequent experiments.

TABLE I

EFFECT OF CERTAIN PHENYLACETIC DERIVATIVES

Medium[a]	*p*H at hr. 42	66	75	90	Penicillin, μ/ml. at hr. 42	66	75	90
Basal	7.8	7.6	7.8	8.3	94	222	263	264
Basal + 0.05% β-phenylethylamine	7.8	7.8	7.7	7.9	117	272	299	435
Basal + 0.05% phenylacetic acid	7.8	7.9	7.7	8.3	169	333	387	374
Basal + 0.05% phenylacetamide	7.8	7.8	7.4	7.5	119	194	243	384

[a] Basal medium contained in grams per liter: lactose 22.5, glucose 7.5, ammonium acetate 3, ammonium lactate 7 and the basal salt mixture. The initial *p*H was adjusted to 6.3 with sodium hydroxide.

Data on the effect of different levels of β-phenylethylamine acetate in our basal medium on penicillin production and length of time to reach maximum yield are presented in Fig. 14. Levels up to 0.1% may be used without affecting the fermentation time. At this level a nearly optimal

(15) A. J. Moyer and R. D. Coghill, *J. Bact.*, **53**, 329 (1947).

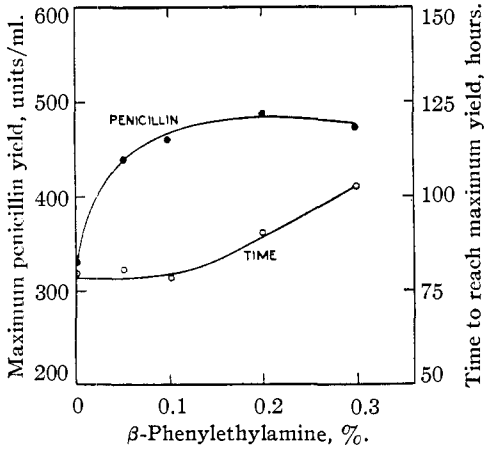

Fig. 14.—Effect of β-phenylethylamine level on penicillin yield and fermentation time. Basal medium contained in grams/liter: lactose 22.5, glucose 7.5, ammonium acetate 3, ammonium lactate 6, and the standard salt mixture. β-Phenylethylamine was added as the acetate. Sugar was autoclaved separately in distilled water.

production of penicillin is obtained. Higher levels of the precursor produce somewhat higher concentrations of penicillin, but limit the growth rate of the organism and consequently extend the fermentation period. The chemical changes occurring during a typical fermentation using 0.1% β-phenylethylamine as the acetate are shown in Fig. 15. By comparison with Fig. 7, it may be seen that the only significant change effected by the addition of the amine was to greatly increase the rate of production of penicillin. Penicillin yields as high as 500 units per ml. have been obtained on the supplemented medium.

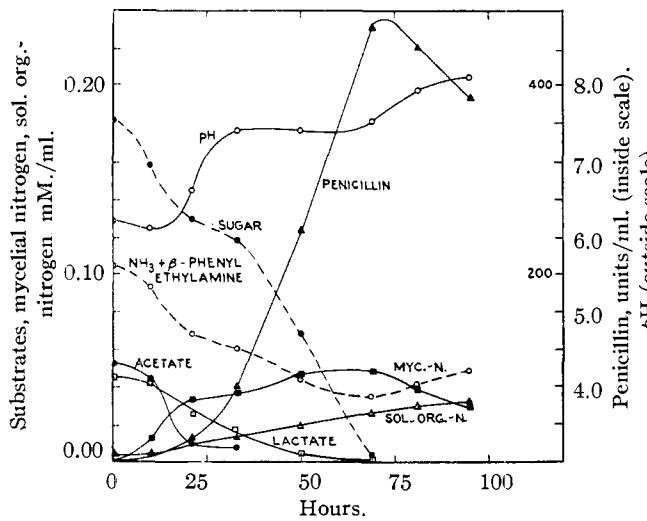

Fig. 15.—Fermentation on standard basal medium plus precursor. Medium contained in grams/liter: lactose 22.5, glucose 7.5, ammonium acetate 3, ammonium lactate 5, β-phenylethylamine 1 (added as the acetate), and the standard salt mixture. Sugar is reported as mM. of monosaccharide per ml. Sugars were autoclaved separately in distilled water.

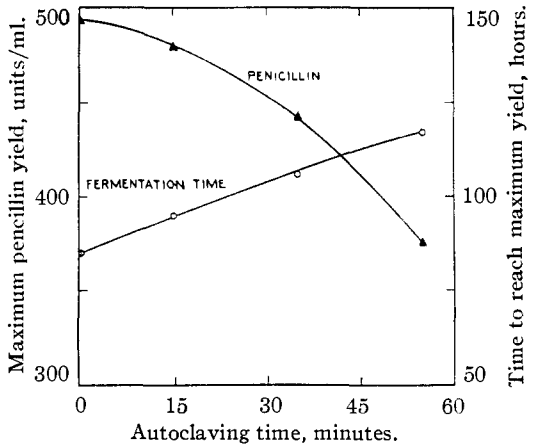

Fig. 16.—Effect of autoclaving on penicillin yield and fermentation time. Medium contained in grams per liter: lactose 22.5, glucose 7.5, ammonium acetate 3, ammonium lactate 6, β-phenylethylamine 1 (added as the acetate), and the standard salt mixture. Zero hours autoclaving time refers to autoclaving sugars separately in distilled water.

Effect of Autoclaving.—During these investigations it was observed that over-autoclaving at *p*H 6.0 appeared to be detrimental to the fermentation. The results of a typical experiment designed to check this observation are shown in Fig. 16. Zero hours autoclaving time actually refers to separate sterilization of the carbohydrates in distilled water, which produces a water-white medium. Autoclaving obviously increases the fermentation time and decreases the final yield of penicillin. The elimination of the autoclaving effect by separate sterilization of the sugars also increases the reproducibility of results obtained over a period of weeks. In the four runs which have been made under these conditions on the medium given under Fig. 16, the yields were, in units per ml., 461, 465, 494 and 503.

Comparison with Corn Steep Liquor Media.—When compared under the conditions described in this paper, somewhat higher penicillin yields have been obtained on corn steep liquor media than on our synthetic medium. The corn steep liquor medium which has proven most satisfactory for our conditions contains in grams per liter: corn steep liquors solids 30, lactose 30, calcium carbonate 10 and β-phenylethylamine 1 (added as the acetate). A 3% solution of octadecanol in lard oil was used as required to control foaming. This medium supports penicillin yields of 650 to 700 units per ml. The higher yield of penicillin obtained on the complex medium is accompanied by a correspondingly higher yield of mycelium (0.07 to 0.08 mM. of mycelial nitrogen per ml. as compared to about 0.05 mM. of mycelial nitrogen per ml. obtained on the syn-

thetic medium). When the two fermentations are compared on the basis of penicillin production per mM. of mycelial nitrogen present, it becomes apparent that at least part of the superiority of the corn steep liquor medium lies in its ability to support the higher yield of mycelium.

In connection with this difference in mycelium production, it should be pointed out that the synthetic medium contains considerably less available carbon for growth than does the more complex corn steep liquor medium. In addition to this, we have recently obtained evidence that the un-ionized acetic acid molecule, which is present in the synthetic medium during the growth phase, is toxic to the growth of the mold. Although this toxicity has not been investigated on a quantitative basis, it is clearly shown by the data presented in Fig. 17. In this experiment, the effect of *p*H on the growth obtained in twenty-one hours is compared on two media, one containing glucose and ammonium acetate and the other glucose and urea. Obviously growth at the lower *p*H levels (where much of the acetic acid exists as the un-ionized molecule) was only inhibited on the medium containing acetic acid. That this effect is not caused by the presence of the ammonium ion in the one medium is demonstrated by the excellent growth obtained at low *p*H levels in the glucose-ammonium lactate fermentation shown in Fig. 11. Un-ionized acetic acid has been previously reported to be toxic to certain other fungi. The literature on this subject was recently discussed by Moyer and Coghill[15] in connection with their work on the toxicity of phenylacetic acid to strains of *P. notatum* and *P. chrysogenum*.

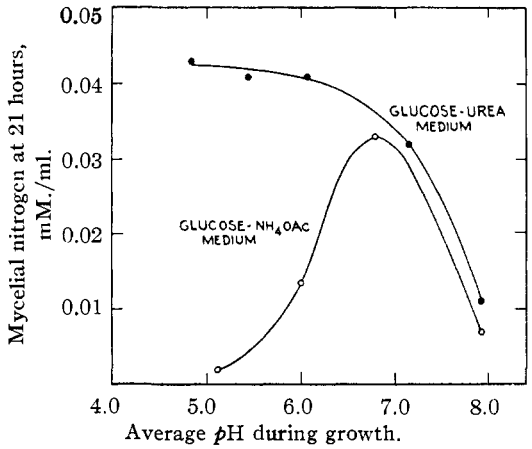

Fig. 17.—Effect of *p*H on growth on two media. Initial *p*H adjustments were made with sodium hydroxide or sulfuric acid. Glucose-urea medium contained in grams/liter: glucose 30, urea 3.5, potassium dihydrogen phosphate 10, and other inorganic salts as in the standard salt mixture. Glucose-ammonium acetate medium contained in grams/liter: glucose 30, ammonium acetate 8, potassium dihydrogen phosphate 10, and other inorganic salts as in the standard salt mixture.

Summary

1. The rate of formation of penicillin was shown to be maximum under conditions which support only a very slow growth rate.

2. On synthetic media of the ammonia–carbohydrate-acetate–lactate type, the optimal *p*H for growth was shown to be about 6.8, and that for penicillin production to be about 7.3.

3. A synthetic medium containing lactose, glucose, ammonia, acetate, lactate and certain inorganic salts was developed which approximated the fermentation rate and *p*H requirements found to be optimal for penicillin production on this type of medium. Penicillin yields of over 300 units per ml. were obtained on this medium.

4. The effect of addition of β-phenylethylamine acetate to this basal medium was studied. Penicillin yields of 500 units per ml. were obtained on this supplemented medium.

5. Autoclaving the carbohydrates together with the other constituents of the medium at *p*H 6.0 was shown to be detrimental to the fermentation.

6. The *p*H during fermentation was shown to be primarily dependent on the relative rates of utilization of the ammonia and fermentable anions used as substrates in the media.

7. The utilization rate of acetic acid was shown to vary inversely with the *p*H. The utilization rates of glucose and ammonia per mM. of mycelial nitrogen present are not appreciably affected by changes in *p*H over the range tested.

8. Acetic acid (un-ionized molecule) was shown to be toxic to the growth of the mold, and the *p*H optimum for growth was shown to be much lower on acetate-free media than on media containing acetate.

9. Results as to penicillin yields and growth obtained on synthetic media were compared to those obtained on corn steep liquor media.

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