THE MINERAL NUTRITION OF PENICILLIUM CHRYSOGENUM Q176
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Steinberg (1939a,b,c, 1946) has conducted a comprehensive series of investigations on the inorganic growth requirements of Aspergillus niger. The specific functions of certain minerals in citric acid formation by this organism have also been studied in some detail (Foster, 1939; Perlman et al., 1946; Shu and Johnson, 1947, 1948). No mineral nutrition studies comparable to these have been reported for Penicillium species with regard to either growth or penicillin production. Several important observations have, however, been made.

Moyer and Coghill (1946) and Foster et al. (1943) found that zinc was required by Penicillium notatum for growth and penicillin production. Foster found also that more phosphorus was required for optimal penicillin production than for optimal growth. Stone and Farrell (1946) stated that P, S, Fe, K, Mg, Zn, and Cu were required for penicillin production by Penicillium chrysogenum but give no quantitative data. Koffler et al. (1947) found that iron and phosphate were required for penicillin production, but not for growth on their basal synthetic medium. No work on the inorganic nutrition of P. chrysogenum Q176 has been reported.

Detailed investigations of the sulfur, phosphorus, potassium, magnesium, and iron requirements of the Q176 strain are reported in this paper. Copper requirements were also studied but in somewhat less detail. The basal salt mixture used was adopted largely from the references listed above.

EXPERIMENTAL METHODS

Fermentation techniques and media. Penicillium chrysogenum Q176 was used throughout the experiments. All fermentations were conducted in triplicate in 500-ml flasks on a rotary type shaker (about 320 rpm) at 23 to 25 C. The penicillin and growth values reported are in each instance average values for three flasks. Vegetative inoculum was used throughout the experiments. Methods for handling and sampling cultures were described in previous papers (Gailey et al., 1946; Jarvis and Johnson, 1947).

The basal salt concentrations used in all experiments (except as otherwise noted) were in grams per liter: KH₂PO₄, 3.0; Na₂SO₄, 0.5; MgSO₄·7H₂O, 0.25; ZnSO₄·7H₂O, 0.02; MnSO₄·H₂O, 0.02; Fe(NH₄)₂(SO₄)₂·6H₂O, 0.10; CuSO₄·5H₂O, 0.005; and CaCl₂·2H₂O, 0.05.

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The inoculum medium used in all experiments (except as otherwise noted) contained, in addition to the salts given above, the following ingredients in grams per liter: glucose, 40.0; (NH₄)₂SO₄, 13.0; and CaCO₃, 13.0. The CaCO₃ was autoclaved separately in suspension in distilled water and added to the remainder of the medium immediately before inoculation.

The basal fermentation medium contained in addition to the salt mixture the following ingredients, in grams per liter: lactose, 30.0; glucose, 10.0; ammonium acetate, 3.5, ammonium lactate, 6.0; and β-phenylethylamine (added as the lactate), 1.0. The sugars were autoclaved separately in distilled water. The pH of the remaining ingredients was adjusted to about 6.2 before autoclaving. All ingredients used were of reagent grade except the lactose, lactic acid, and β-phenylethylamine. Special purification procedures varied with the type of experiment and will be discussed in conjunction with each experiment. A 3 per cent inoculum was used in all fermentations.

Analytical procedures. Mycelial nitrogen and penicillin were determined as described previously (Jarvis and Johnson, 1947). The pH of each sample was determined immediately after removal by means of a glass electrode. Iron was determined by an α,α'-bipyridyl method (Jackson, 1938). Phosphorus was determined by a modified Fiske and SubbaRow method (Koepsell et al., 1944).

RESULTS

Phosphorus requirements. No special purification of the media was required for these experiments. The phosphorus requirements for both growth and penicillin production are shown in figure 1. The phosphorus level of the inoculum medium used for the growth experiment was lowered to 0.15 mg per ml. Potassium (as K₂SO₄) was added in an amount equivalent to that normally contained in the salt mixture. Phosphorus was added as NaH₂PO₄. The phosphorus level of the basal fermentation medium was less than 1 μg per ml. Sodium and sulfate ions were shown by appropriate controls to have no effect on the fermentation at the levels used in these experiments.

Some difficulty was encountered in obtaining suitable pH control at phosphorus levels between 50 and 100 μg per ml. At levels below 50 μg per ml, growth and penicillin production were halted by phosphorus deficiency before the pH became too high. At levels over 100 μg per ml, the medium was sufficiently buffered to give good pH control. In order to obtain suitable pH control in the critical range it was found necessary to lower the ammonium acetate concentration of the medium to about 3.0 g per liter.

The time to maximum yield (about 110 hours) was not affected by the phosphorus level between 100 μg per ml and about 600 μg per ml. At higher levels and at 75 μg per ml the fermentations progressed at slightly slower rates. At phosphorus levels less than 50 μg per ml, penicillin production was halted early in the fermentation.

Growth appears to be a linear function of the phosphorus level between 0 and 65 μg per ml. Penicillin production appears to be a linear function of the phosphorus level between 65 and 200 μg per ml. As may be seen from the figure, phos-
phate is required in considerably greater quantities for normal penicillin production than for normal growth. In all fermentations in which the phosphate level was insufficient to promote normal growth, the organism produced a pink, water-insoluble pigment in place of the normal water-soluble, yellow pigments.

Sulfur requirements. In the study on sulfur requirements, an attempt was first made to substitute NH₄Cl (10.5 g per liter) for (NH₄)₂SO₄ in the inoculum medium. The growth obtained on this medium and the growth phase of subsequent fermentations were normal. Following the growth phase of such fermenta-

\[ \text{Figure 1. Phosphorus requirements for growth and penicillin production.} \]

Ammonium lactate (21 g per liter) was finally used as the nitrogen source in the inoculum medium. In accordance with results obtained in preliminary experiments, 167 µg per ml of sulfur (as sulfate) were added to the inoculum medium. No special purification of the media was required. Na₂SO₄ was omitted from the fermentation medium. All sulfates were replaced by chlorides (at equivalent metal levels) except MgSO₄·7H₂O, which was replaced by the acetate.
The new inoculum medium yielded growth levels at least as high as the standard inoculum medium.

The effect of the sulfur level (added as sulfate) on maximum growth and on penicillin production is shown in figure 2. The curves obtained were very similar to those obtained in the phosphorus study except that the mycelial nitrogen curve was not linear. If the points on this curve, however, are corrected for the sulfur bound in the penicillin produced, the relationship remains linear to near-maximum growth. Sulfur deficiency resulted in premature termination of growth.

Figure 2. Sulfur requirements for growth and penicillin production. The penicillin curve represents values taken at the time of maximum yield. The mycelial nitrogen curve represents values taken at the time of maximum growth.

Between 25 and 60 μg of sulfur per ml, the time to maximum yield was somewhat reduced.

Magnesium requirements. The sugars used were clarified by one Al(OH)₃ coprecipitation at pH 7 (Shu and Johnson, 1948). The inoculum medium employed was identical to that used in the sulfur experiments except that the magnesium level was cut to 10 μg per ml. Magnesium was omitted from the basal fermentation medium.

The results are shown in figure 3. Both growth and penicillin production appear to be linear functions of the magnesium level up to about 6 μg per ml. The response to magnesium differs from that to sulfur and phosphorus in that normal growth and penicillin production are reached at the same level of the metal. As in the previous experiments, magnesium deficiency caused early termination of
growth. Somewhat more time was required to reach maximum yield, however, at levels below 8 μg per ml.

Potassium requirements. No special purification of the media was required. Phosphate was added to the basal medium as NaH₂PO₄ and potassium as K₂SO₄. The inoculum medium was standard except that it contained only 50 μg potassium per ml. No potassium was added to the basal fermentation medium.

The effect of added potassium on growth and penicillin production is shown in figure 4. As fermentation time for maximum penicillin yield also varied significantly with the magnesium level, these data have been included in the figure.

![Figure 3. Magnesium requirements for growth and penicillin production. The penicillin curve represents values taken at the time of maximum yield. The mycelial nitrogen curve represents values taken at the time of maximum growth.](http://jb.asm.org/)

The response to potassium is similar to that to magnesium in that normal growth and penicillin levels are reached at the same metal level. It differs, however, in that the response curves are not linear and the time to maximum yield is a distinct function of the potassium level. The time required to reach maximum growth was also increased by potassium deficiency.

Iron requirements. For these experiments the lactic acid used was purified by distillation under reduced pressure, and the lactose by coprecipitation with aluminum hydroxide at pH 7 (Shu and Johnson, 1948). Two coprecipitations and recrystallization reduced the iron content of the lactose (U.S.P.) from about 40 μg per g to 1.7 μg per g. The iron level in the inoculum medium was lowered to 1 μg per ml for these experiments. The iron salt was omitted from the basal medium.
Figure 4. Potassium requirements for growth and penicillin production. The penicillin curve represents values taken at the time of maximum yield. The mycelial nitrogen curve represents values taken at the time of maximum growth.

Figure 5. Iron requirement for growth. The mycelial nitrogen curve represents values taken at the time of maximum growth.
The effect of iron level on the maximum growth obtained by *P. chrysogenum* Q176 during fermentation is shown in figure 5. The data presented were taken from three separate fermentations. No effort was made completely to eliminate iron from the basal medium and consequently no points below the 0.07 μg Fe per ml level were obtained. Levels of iron higher than those shown in the figure cause only a slight increase in growth. About 0.045 mm per ml of mycelial nitrogen are obtained at 25 μg Fe per ml, which was the optimal iron level for penicillin production. Growth was depressed slightly at iron levels greater than 100μg per ml.

![Figure 6](http://jb.asm.org/)  
*Figure 6.* Iron requirement for penicillin production. The penicillin curve represents values taken at the time of maximum yield.

The effect of iron on carbohydrate utilization appears to be closely correlated with its effect on growth. Thus normal sugar utilization occurs at iron levels as low as 0.3 μg per ml.

The maximum penicillin yield obtained during fermentation appears to be a linear function of the iron level between 0.2 μg and 3.5 μg per ml (figure 6). A further increase in yield is obtained, however, up to about 25 μg per ml. Very little toxicity occurs even at levels as high as 300 μg Fe per ml (the iron ion concentration increases very little in this range as a result of iron phosphate formation). Levels of iron below 5 μg per ml cause a premature termination of penicillin production during fermentation. This effect is also shown in figure 6.

More recent information has indicated that the copper level in the medium has a distinct effect on the amount of iron necessary to promote normal penicillin yields. This observation is in agreement with those of Koffler *et al.* (1947). In the
experiments described in figures 5 and 6, the copper level of the media was about 2 μg per ml. In experiments conducted on media to which no copper was added (about 0.7 μg Cu per ml) only 2 μg Fe per ml was required to produce normal penicillin yields.

**Copper requirements.** In view of the demonstrated relationship between iron and copper on the fermentation, the copper requirements for the organism have also been investigated. This work, however, has not been carried through with the same amount of detail as that for the other metals.

In these experiments a preliminary purification of the sugars was made by aluminum hydroxide coprecipitations at pH 9.0 and pH 7.0. In addition to this, the distilled water and all ingredients except the trace salts (Fe, Mn, Mg, Zn, and Ca) were purified by an 8-hydroxyquinoline-chloroform extraction (Waring and Werkman, 1943) procedure at pH 6.0, followed by a dithizone-CCL4 extraction at the same pH.

During preliminary experiments on the requirements of the mold for this element it became evident that an “adaptation” phenomenon was involved. The results of an experiment designed to show this effect are presented in figure 7. In this experiment the fermentation medium contained 3 μg Cu per ml. The “adapted” inoculum was grown in the presence of 1 μg Cu per ml, and the “unadapted” inoculum was grown on a medium containing no added copper. From the figure it may be seen that a long lag phase in growth results if an “unadapted” inoculum is employed. Penicillin production curves naturally show a similar lag, and somewhat higher final yields are obtained when the “adapted” inoculum is used.

About 46 per cent maximum growth and 54 per cent maximum penicillin production were obtained on the purified basal medium. The addition of 0.1 μg of copper per ml raised the growth and penicillin yields to nearly maximum. Copper at the level of 10 μg per ml was not toxic. At 100 μg per ml, the growth was not appreciably affected but the penicillin yield was reduced considerably. The max-
mineral yield obtained upon the addition of various levels of copper to the basal medium was only about 80 per cent of that obtained in the other metal experiments.

Other metal requirements. The zinc requirement for growth and penicillin production was confirmed. Manganese was also found to be required for both growth and penicillin formation. Neither requirement was quantitatively investigated. No attempt was made to determine whether sodium or calcium was required.

DISCUSSION

About 500 units of penicillin per ml were normally obtained on the basal synthetic medium used in the experiments. This compares with 650 to 700 units per ml obtained on corn steep liquor media under the same conditions (Jarvis and Johnson, 1947). The addition of corn steep liquor ash to the basal synthetic medium caused no increase in penicillin yields. The basal synthetic medium used was found to contain each of the investigated metals in amounts falling within the optimal range for growth and penicillin production. The experiments did not, therefore, result in any increases in total yields obtained.

The actual amounts of the minerals found necessary for growth or penicillin production are probably not particularly significant, as one would expect these to vary with the media and conditions used. However, the observations that greater amounts of phosphorus, sulfur, and iron are required for penicillin production than for growth are more important. In each instance the data indicate that the mineral concerned could be in some way specifically involved in penicillin formation. The shapes of the penicillin response curves for the phosphorus and sulfur experiments are in agreement with such a concept. The specific relationship between sulfur and penicillin formation is, of course, obvious. The penicillin yields at iron levels below that required for optimal growth were so low that dependable data were not obtained for that range.

It also seems probable that differences in the type of function represented by the growth response curves would remain distinct under different fermentation conditions. Thus Steinberg (1946), working with A. niger, also found that the growth response to magnesium was linear and that to potassium nonlinear. The latter curve is not superimposable on our curve for P. chrysogenum; but this is readily accounted for by the fact that Steinberg’s data were taken at a set time, while ours were taken at the time of maximum yield.

SUMMARY

Quantitative studies were made on the phosphorus, sulfur, potassium, magnesium, and iron requirements of Penicillium chrysogenum Q176 for growth and penicillin production. Copper requirements were studied, but in less detail. These minerals were required for normal growth at the following levels in μg per ml: phosphorus 100, sulfur 70, potassium 40, magnesium 8, iron 0.2, and copper 0.1.

The iron, phosphorus, and sulfur requirements for normal penicillin production were, respectively, 20 times, 2 times, and about 1.5 times those for normal growth. For potassium and magnesium, normal penicillin production was obtained at the same metal level as that required for normal growth.
All curves representing penicillin production and growth responses to added minerals were essentially linear for considerable ranges, except those for potassium.

The fermentation time was markedly increased by potassium deficiencies and decreased by iron deficiencies. Other mineral deficiencies caused irregular and not nearly so significant changes in the fermentation time.

Seven mg per ml of chloride in the inoculum medium was found to be detrimental to penicillin production in the subsequent fermentation.

It was found necessary to adapt the culture to levels of copper above about 0.5 μg per ml.

REFERENCES


