DISCHARGE OF SACCHARASE FROM MYCELIUM OF
*Penicillium glaucum*

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(with two figures and one plate)

Introduction

The relation between the composition of the media of fungus cultures and the enzymes produced has been the subject of many investigations. There is very little known about the discharge of the enzymes secreted by the mold into the culture medium. This phenomenon bears the most intimate relationship to the rôle played by the enzymes and to the life-cycle of the molds and it therefore merits more attention.

The first recorded data in the literature that deal with the diffusion of mold enzymes into the medium are found in the study of Fernbach (7) on the saccharase of *Aspergillus niger*. His method can not be regarded as free from objections, (5) but he established the first enzyme unit and he was the first to point out the great importance of the degree of acidity of the reaction mixtures. He measured the distribution of the saccharase between the mold and medium. The main result of his investigations was the discovery of the fact that the total amount of saccharase (activity) expressed in his enzyme unit was fairly constant, decreasing only about 20 per cent. from the second to the fifth day. The distribution ratio of the enzyme between the mold and medium changed during growth. On the second day 3 per cent., and on the fifth day 37 per cent. of the total enzyme content was found in the medium.

The conclusion to be derived from Fernbach's experiment is that the maximum amount of enzyme is formed during the first two days, and later this enzyme simply passes slowly from the mold to the medium. He obtained practically the same results with a yeast, *Saccharomyces pastoria-nus*. Since Fernbach's experiments no work has been done on the distribution of the enzyme between microorganism and medium.

In the experiments of Doby and Kertesz (2) the saccharase content of *Penicillium glaucum* cultures was determined and the enzyme content of the medium was found to be very low. These authors were studying the changes in the saccharase content of the mold when grown with and without potassium, and therefore did not pay further attention to the enzyme content of the medium.

Bridel and Aagaard (1) have shown that when the mold is kept on distilled water, the enzyme diffuses out of the mycelium into the water.

Iwanoff and Kudrjawzewa (8) published a paper in 1929 entitled,
"Ausscheidung der Saccharase aus der Zellen." The main conclusions drawn by these authors were that the discharge of the saccharase from the mycelium depends on the pH of the medium; very little saccharase was discharged if the medium was acid but as it became more alkaline more and more enzyme was discharged into the medium. In this connection it should be pointed out that one essential weakness of the method used by the Russian authors is that the saccharase content of the medium only has been studied and never that of the mold. For this reason the data that these workers presented can not be considered sufficient to show completely the extent of the discharge of the saccharase. Although the enzyme content (capacity) of the medium of two different cultures may be found to be the same, this part of the enzyme content may be only 10 per cent. in one case but 90 per cent. of the total enzyme content produced by the mold in the other case.

It was for the purpose of securing more information concerning the factors influencing the discharge (diffusion) of saccharase from the cells of Penicillium glaucum that the studies reported in this paper were undertaken.

Experimental

Method

The strain of Penicillium glaucum Link used in these experiments was the same as used in earlier experiments, the culture originating from the Royal Hungarian Institution for Industrial Fermentations in Budapest, Hungary.

The salt supplement of the cultures was always the same as given in earlier papers (10, 11, 2) and contained sufficient amounts of P, K, Mg, Ca, Na, Cl, SO₄, N, (NO₃ and H, N) and traces of Fe³⁺ and Zn. After inoculation the mold has been grown in 100-cc. Erlenmeyer flasks containing 50 cc. of medium and at a temperature of 24.0° C.

The procedure used in the enzyme determination was as follows: The mold was taken off of the medium and washed with a quantity of distilled water such that the volume of the medium was restored to 50 cc. as at the beginning of the growth. The dry matter was determined on a small sample of mycelium, drying to constant weight at 95° C. The rest of the mold was ground in a porcelain mortar and suspended in water. A sample of this suspension was taken for the determination of the dry matter content of the suspension and the remainder was used for the determination of the enzyme activity.

The medium was filtered before being used for the determination of the enzyme activity. The reaction mixtures contained in all cases 5 per cent. of sucrose. The amount of sucrose contained in the part of the medium
KERTESZ: DISCHARGE OF SACCHARASE

used has always been determined and correction for this has been made in the calculations. The pH of the reaction mixture was always 4.5 which has been shown to be the optimum for this enzyme (2). The pH was determined by the use of indicators. Phosphate buffer was used throughout and 3 per cent. of toluol was always added. The reaction temperature was 38.0° C. in all experiments.

At the beginning of the experiments and at the indicated intervals, samples were taken from the reaction mixtures, clarified by neutral lead acetate and sodium carbonate, centrifuged, and in the clear solution the reducing sugars were determined. In one series the optical rotation was determined in a 200 mm. tube. For the determination of the reducing sugars Bertrand’s method was used and for the calculation reference was made to the author’s recalculated tables (12). The values reported in the tables have been corrected for the blank found at the beginning of the experiment.

The monomolecular reaction constants were calculated by the use of the familiar formulae:

\[
k = \frac{1}{t \times 0.4343} \log \frac{A}{A - X}
\]

(1)

and

\[
k = \frac{1}{t \times 0.4343} \log \frac{a_o - a_\infty}{\alpha - a_\infty}
\]

(2)

in which “t” is reaction time (min.), \( A \) is the amount of substrate (sucrose) present at the beginning of the reaction, \( X \) is the amount of substrate changed in time “t,” \( a_o \) is rotation at the beginning, \( \alpha \) is rotation after the time “t.” The value of \( a_\infty \) is calculated from the formula

\[
a_\infty = a_o \left(0.417 - 0.05 t\right).
\]

(3)

The \( If \) is calculated from v. EULER’S formula (6)

\[
If = \frac{k \times \text{gm. sucrose in the reaction mixture}}{\text{gm. of dry matter of the enzyme preparation in the reaction mixture}}
\]

(4)

In the case of the enzyme determinations in the medium “cc. of medium used in the reaction mixture” has been substituted for “gm. dry matter.”

The enzyme content of the whole culture of the mold and medium respectively has been expressed in the formula proposed by the author (13):

Total enzyme content of the mold \( = E_1 = (If_1) \times \text{(dry matter yield in gm.)} \)

Total enzyme content of the medium \( = E_2 = (If_2) \times \text{(total volume of the medium in cc.)} \)

(5)

(6)

Before presenting the data obtained, it is necessary to explain why the above method of determination has been used in preference to the method described by IWANOFF and KUDRIJAWZEN. As mentioned before, the Russian authors determined the enzyme content in the medium only. Further, the method used for their determinations was not free from objections. Their method as described in their paper was as follows (loc.

It is to be seen that no determinations of the reducing power were made at the beginning of the reactions. The media used by Iwanoff and Kudrjavzewa contained sugars in most cases and perhaps other substances which have a reducing power; therefore their single determination of the reducing power could scarcely be expected to define the rate of reaction. If the mold was grown, for instance, on sucrose solution the original reducing power of the medium was changed considerably by the inversion of the sucrose. These two opposing reactions (1) the inversion of the original sucrose, and (2) the disappearance of the invert sugar from the solution, make the results obtained by the Russian authors so uncertain, that from their work no more than qualitative conclusions can be drawn.

**Results**

On the basis of the experiments of Iwanoff and Kudrjavzewa attention was first paid to the influence of the reaction of the medium on the discharge of the saccharase to the medium.

In these experiments the *Penicillum glauca* was grown on 50 cc. of medium containing 5 per cent. sucrose and inorganic salts. By the use of N/10 NaOH or N/10 H₂SO₄ the reaction of the medium was brought to the desired pH. A few drops of solutions of suitable indicators were added and the pH corrected daily throughout the experiment by the addition of base from a sterile burette. Of course, during one day the production of acid by the living mold caused a certain shifting toward the acid reaction, therefore the pH values given are approximately 0.5 pH higher than the lowest value actually reached during growth. The cultures were harvested on the fourth day after inoculation. In this single case the determination of the saccharase effect in the mold was done by the polarimetric method.

The results obtained show that contrary to the conclusions stated by Iwanoff and Kudrjavzewa, saccharase has been found in the media of cultures grown in acid reaction. On the whole the total enzyme content (E₁ + E₂) decreased with increasing pH. The pH of the first culture was 3.0 at the beginning, and it was necessary during growth to correct it, because it had decreased to 2.7–2.8. From this experiment it is to be seen that the saccharase of *Penicillum glauca* passes into the medium in quite acid reaction.
**TABLE I**

Saccharase content of the mycelium and medium of *Penicillium glaucum* cultures, grown at different pH's on 5 per cent. sucrose for 4 days

<table>
<thead>
<tr>
<th>pH of the media</th>
<th>Dry matter yield</th>
<th>Saccharase in the mold</th>
<th>Saccharase in the medium</th>
<th>Total saccharase in the medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td>gm.</td>
<td>k x 10^4</td>
<td>If x 10^3</td>
</tr>
<tr>
<td>3.0</td>
<td>0.115</td>
<td>0.1319</td>
<td>10.61</td>
<td>24.13</td>
</tr>
<tr>
<td>4.1</td>
<td>0.111</td>
<td>0.0968</td>
<td>9.19</td>
<td>27.57</td>
</tr>
<tr>
<td>5.1</td>
<td>0.067</td>
<td>0.0549</td>
<td>4.03</td>
<td>22.02</td>
</tr>
<tr>
<td>6.3</td>
<td>0.043</td>
<td>0.0383</td>
<td>6.79</td>
<td>53.20</td>
</tr>
<tr>
<td>7.0</td>
<td>0.026</td>
<td>0.0214</td>
<td>2.11</td>
<td>29.57</td>
</tr>
<tr>
<td>7.9</td>
<td>0.028</td>
<td>0.0236</td>
<td>2.83</td>
<td>35.94</td>
</tr>
<tr>
<td>8.3</td>
<td>0.025</td>
<td>Not determined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.8</td>
<td>Fragments of mycelium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (days)</td>
<td>pH</td>
<td>gm.</td>
<td>mg.</td>
<td>k x 10³</td>
</tr>
<tr>
<td>------------</td>
<td>----</td>
<td>------</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>2</td>
<td>2.9</td>
<td>0.0406</td>
<td>7.5</td>
<td>2.06</td>
</tr>
<tr>
<td>4</td>
<td>2.8</td>
<td>0.1746</td>
<td>58.1</td>
<td>22.40</td>
</tr>
<tr>
<td>7</td>
<td>2.8</td>
<td>0.4340</td>
<td>20.1</td>
<td>5.91</td>
</tr>
<tr>
<td>11</td>
<td>2.6</td>
<td>0.3187</td>
<td>2.3</td>
<td>2.58</td>
</tr>
<tr>
<td>16</td>
<td>2.6</td>
<td>0.3000</td>
<td>8.0</td>
<td>2.20</td>
</tr>
</tbody>
</table>

**Saccharase Content of the Mycelium and Medium of *Penicillium glaucum* grown on 50 cc. of 5 per cent. Sucrose Solution and Inorganic Salts**
The rate of growth, as is to be seen from the dry matter yield, varied materially in the cultures grown at different pH's. All the cultures were therefore in different stages of development, and presumably some of them near to and some far from having their highest saccharase content. Doby and Kertesz found that the mycelium of Penicillium glaucum grown on 5 per cent. sucrose had the highest saccharase content on the fifth day of growth. This work showed the effect of pH on the growth of the mold, but no attempt was made to arrive at conclusions in regard to the distribution of the enzyme between mycelium and medium. For this reason the changes of the saccharase content of growing cultures were studied as will be shown later in this paper.

In the following three experimental series given in tables II, III and IV the mold was grown on 50 cc. of a medium which contained the inorganic salt supplement and 5 per cent. of sucrose (II), the salt supplement and 5 per cent. of sucrose and 0.25 per cent. of asparagin (III), and the salt supplement and 1.5 per cent. solution of Witte peptone (IV).

As can be seen from table II, the dry matter yield increased even after the eleventh day, showing that the supply of nutrients had not been used up during that time. The enzyme content of both mycelium and medium was the highest on the fourth day. The highest enzyme content of the medium was observed on the fourth day and amounted to 17.7 per cent. of the total enzyme content.

As was seen from the pH values of the growing cultures, the reaction of the medium was shifted toward the acid side very quickly, i.e., the original reaction of pH 5.4 was changed to 2.9.

The results presented in table III are similar to those presented in table II. The growth is more rapid, and because of the larger amount of nitrogenous and carbohydrate nutrients the dry matter yield is higher also. The total enzyme (or enzyme activity) produced by the culture at the fourth day is double that of the culture having no asparagin. The rate of increase of the actual acidity in the medium is a little slower in the presence of asparagin, possibly because of the buffer action of this compound.

The maximum enzyme activity per unit of dry matter (If,.) is only 10 per cent. higher than in the culture without asparagin, but the enzyme content of the medium is much higher. But even in this case only on the fourth day was 25 per cent. of the total enzyme content found in the medium; later this value decreased to 5 per cent., only one twentieth of the total enzyme contained in the whole culture.

The results obtained with cultures grown on Witte peptone are presented in table IV. In no case has a definite saccharase activity been found.
<table>
<thead>
<tr>
<th>Age (days)</th>
<th>pH</th>
<th>Acidity of the medium</th>
<th>Dry matter in the determination</th>
<th>Invert sugar in 6 hours $k \times 10^4$</th>
<th>Dry matter yield $E_t \times 10^3$</th>
<th>Used for the determination</th>
<th>Invert sugar in 6 hours $k \times 10^4$</th>
<th>Sucrose in the medium $E_t \times 10^3$</th>
<th>$(E_t + E_s) \times 10^3$</th>
<th>Total saccharase in the medium per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.4</td>
<td>0.1010</td>
<td>15.0</td>
<td>4.29</td>
<td>12.74</td>
<td>0.141</td>
<td>1.33</td>
<td>0.06</td>
<td>3.41</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>2.9</td>
<td>0.2950</td>
<td>75.4</td>
<td>41.62</td>
<td>42.40</td>
<td>0.703</td>
<td>29.3</td>
<td>0.3</td>
<td>18.64</td>
<td>0.44</td>
</tr>
<tr>
<td>7</td>
<td>2.7</td>
<td>0.9048</td>
<td>36.9</td>
<td>11.71</td>
<td>38.91</td>
<td>1.279</td>
<td>4.97</td>
<td>0.04</td>
<td>5.04</td>
<td>0.29</td>
</tr>
<tr>
<td>11</td>
<td>2.7</td>
<td>0.3658</td>
<td>21.4</td>
<td>6.34</td>
<td>4.80</td>
<td>1.268</td>
<td>6.08</td>
<td>0.29</td>
<td>0.29</td>
<td>0.26</td>
</tr>
<tr>
<td>16</td>
<td>2.7</td>
<td>0.3010</td>
<td>15.6</td>
<td>4.47</td>
<td>4.46</td>
<td>1.498</td>
<td>6.68</td>
<td>0.04</td>
<td>0.44</td>
<td>0.42</td>
</tr>
</tbody>
</table>

**TABLE III**

Saccharase content of the mycelium and medium of *Penicillium glaucum* grown on 50 cc. of 5 per cent. sucrose, 0.25 per cent. asparagin, and inorganic salts.
### Table IV

Saccharase content of the mycelium and medium of *Penicillium glaucum* grown on white peptone and inorganic salts

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>pH</th>
<th>Acidity of Medium</th>
<th>Saccharase in the Mold</th>
<th>Saccharase in the Medium</th>
<th>Presence of enzyme determined by the rotation method</th>
<th>Reducing method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dry matter in experiment</td>
<td>Rotation</td>
<td>Invert Sugar</td>
<td>Rotation</td>
<td>Invert Sugar</td>
</tr>
<tr>
<td>2</td>
<td>6.9</td>
<td></td>
<td>0</td>
<td>6</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>7.4</td>
<td>0.037</td>
<td>0.552</td>
<td>2.6</td>
<td>0.540</td>
<td>2.6</td>
</tr>
<tr>
<td>7</td>
<td>7.6</td>
<td>0.084</td>
<td>0.474</td>
<td>2.9</td>
<td>0.510</td>
<td>3.1</td>
</tr>
<tr>
<td>11</td>
<td>7.4</td>
<td>0.062</td>
<td>0.324</td>
<td>1.9</td>
<td>1.9</td>
<td>0.550</td>
</tr>
</tbody>
</table>

Note: The presence of enzyme is indicated by (+) for rotation and reducing methods.
It is to be seen from the data presented that the question of the diffusion of saccharase to the medium in mold cultures is much more complicated than it has been supposed by earlier authors. With changes in the medium, changes occur in the whole life-cycle of the mold. On account of the different substrates, the molds produce different amounts (activity) of enzyme. Furthermore the enzyme content changes with the age of the culture.

In the following experiment mold grown on 5 per cent. sucrose for five days was kept immersed in buffer solutions of various pH in the presence and also in the absence of toluol and the enzyme diffused to the buffer solutions after two days was determined. The mold had been grown in five 100-cc. Erlenmeyer flasks each containing 50 cc. of medium. The weight of mycelium harvested on the fifth day was 13.75 gm. The dry matter content was determined to be 19.15 per cent. The total dry matter yield of the five cultures was therefore 2.63 gm. After washing and drying with filter paper it was divided into 1-gm. portions each representing 0.19 gm. dry matter. Ten of these portions were put in small bottles having glass stoppers and containing 40 cc. of a mixture of 4 cc. N/10 phosphate buffer and 36 cc. of sterilized distilled water making a final buffer solution N/100 with respect to phosphate. The mold was immersed in the solutions. At the beginning of the experiment the enzyme content (activity) of the mold was determined to be $If = 31.6 \times 10^{-3}$. For the determination of the enzyme diffused to the solutions, after filtration 25 cc. was mixed with 20 cc. of a 12.5 per cent. sucrose solution and 5 cc. of phosphate buffer, pH = 4.5. In several cases the solutions were too far removed from pH 4.5 to use directly and in these the pH was corrected by the addition of N/20 NaOH or N/20 H$_2$SO$_4$. From these reaction mixtures 10 cc. was taken out at the intervals indicated, clarified by neutral lead acetate and sodium carbonate, and made up to 100 cc. This solution was used for the determination of the reducing sugars produced by the action of the saccharase. In figure 1 are shown the results of these experiments.

In all these last experiments containing buffer solutions the enzyme activity was higher than in the blank in which the mold had been kept on sterile distilled water. The shape of the curves representing the relations between pH of the solutions in which the mold was kept and the enzyme found in it after two days are very remarkable. In the presence of toluol the highest enzyme content was found in the neutral solution, while in the absence of toluol the lowest enzyme content was found at the same reaction. The highest enzyme content recorded was found in the solution without toluol at pH 8.9. In this case the solution contained 2.8 times as much enzyme as the blank on distilled water.
**Discussion**

A great deal of work has been done on the permeability of the plant cells, and on the factors influencing the diffusion of different plant materials, but among the many theories presented no one theory can be found to explain this phenomenon satisfactorily. It has been supposed that in the diffusion of different compounds through the cell wall, the Van't Hoff rule is not applicable. It was supposed furthermore, that besides the osmotic permeability there exists "selective permeability" (15), which would permit the diffusion of compounds like sugars and some amino-acids. It has been observed that toxic materials of presumably high molecular weight are put out from cells which contain concentrated solutions of compounds of low molecular weight, but which do not pass out of the cell at all. The passage of enzymes through cell walls is no doubt a complicated matter. A few papers dealing with the diffusion of enzymes through collodion and similar membranes have been published but very little has been done on the diffusion through cell walls.

In an earlier paper the author pointed out that the formation of enzymes bore a most intimate relationship with the necessity of the organism for them (10). In the case of a sucrose medium the saccharase was presumably...
produced by the mold for the purpose of splitting the sucrose into simpler compounds which possibly could be more easily utilized by the mold than sucrose. It is entirely possible that the mold is not able to use sucrose at all, since in the case of a sucrose medium saccharase is produced quite generally. If the saccharase is produced by the cell for the purpose of splitting sucrose it is easy to understand that the amount of enzyme required should decrease after most of the sucrose of the medium has been converted into invert sugar (11).

Fig. 2. The relation between sucrose and saccharase content of growing *Penicillium glaucum* cultures. (Grown on 50 cc. of 5 per cent. sucrose solution, containing 0.25 per cent. asparagin and inorganic salts.)
It is to be seen from fig. 2 that the greater part of the sucrose of the medium was inverted in the interval between the second and fourth day. In fact 63 per cent. of the total sucrose content of the medium had disappeared during this period. It is also to be seen that the saccharase content of the cultures was the highest at the fourth day of growth. After the greater part of the sucrose was converted into invert sugar the saccharase content decreased abruptly. It is interesting to note that a small amount of sucrose (or another polysaccharide?) is present even after sixteen days. A very low saccharase activity is to be found at this same time.

The results of the present experiments are not in harmony with those of the earlier investigators. Of course, the subject of their experiments has not been Penicillium but Aspergillus niger, but the author is convinced that the difference is caused more by the method of investigation than by the organism used.

The pH of the media in the experimental series in table II has been determined to be 2.9 on the second day. The discharge of the enzyme however does not start until later, since on the second day no enzyme was found in the medium. This fact is in very good harmony with the results presented in table I, where with the cultures grown on acid media, saccharase has been found in the nutrient solution. Iwanoff and Kudrjawzewa (p. 246) state that if cultures have been grown on a medium containing sucrose for four days, and to the medium oxalic acid is then added, no saccharase effect could be found after two or eight days.

The writer, in an attempt to show this directly, tried to grow cultures (Penicillium and Aspergillus niger) in the presence of oxalic acid in the amounts used by the Russian authors but no growth could be obtained. The reaction of these solutions has been determined to be around pH 1.25–1.70. Whatever saccharase is discharged to such a solution would lose its activity entirely in a very short time, because this high acid reaction has been shown to be very toxic to saccharase (4). Nelson and Palmer (14) observed that yeast saccharase is affected by a reaction of pH 4.6. Furthermore, oxalic acid itself is known to be one of the most toxic of organic acids for molds (9). With these observations of others in mind, it is obviously impossible to say from the experiments of Iwanoff and Kudrjawzewa that the acidity alone of the medium used has prevented the saccharase from passing out of the mycelium into the medium. Even if any enzyme should pass out, it would shortly become inactivated due to the high acidity, aggravated in this case by the toxic oxalate ion.

In the experiments with growing cultures presented in this paper the reaction of the medium always turned acid with the exception of the experiments with the peptone medium, where no definite saccharase effect could be obtained. It is to be seen from the first series, that the medium
of cultures grown at a pH of 6.3, 7.0 and 7.9 contained as much as 30 per cent. of the total saccharase content of the whole culture. The question whether this saccharase is discharged by the normal diffusion of uninjured cells or whether it is coming from the cells killed by the alkaline medium can not be decided from these experiments.

To be able to make a study of the discharge at different pH reactions, without being disturbed by the different rates of growth of the mold, an experimental series has been carried out, the results of which are presented graphically in fig. 1. The results obtained are no doubt due to the operation of a great many factors. The different parts of the curves should be explained in quite different ways, since the influence of the acid and alkaline medium is not identical.

From the first experimental series as well as from these later experiments it can be seen that the mold without toluol discharges a great deal of its enzyme content to an alkaline medium. This observation is in harmony with Iwanoff and Kudriawzewa's results, who found that Aspergillus niger always put out more enzyme on an alkaline than on a neutral medium. On the acid side of this curve enzyme activity could be observed also. This is no doubt the result of a certain growth continued by the mold on the buffer solution. At the neutral reaction, a lower enzyme activity has been found. This reaction is certainly not favorable for the growth of the mold neither does it cause an increase of the enzyme by discharge nor by the plasmolysis of the cells.

Quite different are the results of the experiments in the presence of toluol. If the mycelium of the mold is placed into buffer solutions on which there is some toluol, the toluol is in much more intimate contact with the mold than with a suspension of the mycelium in the autolytic experiments of Doby and Kertesz. Von Euler and B. von Euler-Af Uggglas (3) established the fact, that the action of different protoplasmic poisons is quite different on living and non-living cells. They found that toluol killed the saccharase of Monilia, if living cells were exposed to it, but had only a very little effect, if dead (dried) cells were treated. The experiments here described show further that toluol is more toxic to the saccharase at either acid or alkaline reaction than at neutral reaction.

To make a further study of the effect of different treatments on the cells of Penicillium, microscopic sections have been prepared.1 Photographs (x 80) of some of these are presented on the accompanying plate VIII. No. I is taken from a section prepared from the mold which had stood on an acid buffer solution for two days without toluol. It can be seen that there is a secondary growth quite distinct from the mycelium which had been

1 The writer's sincere thanks are due to Dr. Mabel Nebel and to Dr. Bernard R. Nebel for preparing these sections and photomicrographs.
formed on the original medium. There is also a rich spore formation to be seen. No. II is prepared from the mold from the alkaline solution without toluol. The mycelium and the layer of spores are wrinkled and the hyphae are partly empty as a result of the effect of the alkalinity. In this sample the greatest discharge of the saccharase had been found. Plate VIII no. III is taken from the section prepared from the mold from acid solution with toluol. The structure in general is very badly affected by the toluol. No secondary growth can be seen and no plasma can be observed in the sporangia. The mold in alkaline solution and in the presence of toluol is even more drastically affected (no. IV).

No doubt the present state of our knowledge is not sufficient to draw general conclusions in regard to the discharge or diffusion of enzymes. This can be done only after collecting more data and making further studies of this interesting as well as important phenomenon.

Summary

1. The discharge of saccharase in growing cultures of Penicillium glaucum has been studied. The highest enzyme content of the mycelium and medium has been found to occur on the fourth day of growth. Never more than one third of the total enzyme content of the culture has been found in the medium.

2. The saccharase can pass into the culture medium even at acid reaction, but the rate of discharge is lower in an acid than in an alkaline medium.

3. A marked decrease of the enzyme content of both mold and medium has been observed after most of the sucrose present in the medium was inverted.

4. When the mycelium of five-day-old mold containing saccharase (\(I_f = 31.6 \times 10^{-3}\)) had been kept on a series of buffer solutions of pH's 4.0–8.7, saccharase was detected in solution after two days in all cases.

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EXPLANATION OF PLATE VIII

No. I. Section from five days old Penicillium glaucum, which stood in acid buffer
solution (pH = 4.1) for two days.
No. II. Section from five days old Penicillium glaucum which stood in alkaline
buffer solution (pH = 8.9) for two days.
No. III. Section from five days old Penicillium glaucum which stood in acid buffer
solution (pH = 4.0) in the presence of toluol for two days.
No. IV. Section from five days old Penicillium glaucum which stood in alkaline
buffer solution (pH = 8.7) in the presence of toluol for two days.
KERTESZ—DISCHARGE OF SACCHARASE