The Effect of Phosphate Supply on the Rate of Growth and Fat Formation in Yeasts

K. L. SCHULZE

Civil and Sanitary Engineering Department, Michigan State University, East Lansing, Michigan

Received for publication March 16, 1956

During the years 1942 through 1945 the author was given the assignment of directing a research program on fat formation in yeast. The work resulted in the first full-scale production of a fat-enriched yeast from sulfite liquor in 1944, and a summary of the data obtained was published (Schulze, 1951). The yeast was being produced at one of the largest paper mills in Germany. The process used was aerobic fermentation on a continuous flow basis, very similar to the process now used at several factories in the United States.

Recently, methods of mathematical treatment of continuous flow operation have been developed. We have applied the mathematical approach to the data obtained on the production of high-fat yeast from sulfite liquor. The results are presented in this paper.

MATERIALS AND METHODS

The sulfite liquor was prepared by removing most of the free SO₂, adjusting the pH by mixing with lime, and adding nutrient salts, mainly liquid ammonia, ammonium biphosphate, and potassium chloride. The nitrogen level was kept at approximately 900 ppm and the phosphate level at 700 to 800 ppm as P₂O₅. The reducing sugar, as measured by the Bertrand method, ranged from 3 to 4 per cent.

In the production phase, the prepared sulfite liquor is fed at a continuous rate into the aeration tank where the yeast is propagated. The liquid level and, therefore, the volume of the mash are kept constant by an overflow line. The mash containing yeast leaves the aeration tank at the same rate as the sulfite liquor enters. The feed rate is adjusted so that a constant concentration of yeast is maintained. The process is mainly controlled by checking the residual sugar and the yeast concentration in the effluent. Normally the residual sugar is kept at 0.4 to 0.8 per cent, so that approximately 80 to 90 per cent of the incoming sugar is oxidized or converted into yeast cells. An increase in the residual sugar is usually the first sign that the process is not working normally. In most cases this condition can be cured by reducing the feed rate (Q) which means increasing the detention time \( \frac{V}{Q} \). With the process in balance, the detention time is inversely proportional to the growth rate \( K \) of the organisms. Thus a continuous flow type process provides a very convenient way to evaluate \( K \) under various conditions. The situation may be expressed graphically as follows.

\[
\begin{align*}
\text{AERATION TANK} & \\
C_oQ & \rightarrow MC_o & \rightarrow QMC_o \\
\text{feed} & & \text{effluent}
\end{align*}
\]

The following mathematical formulation which has been used by Garrett and Sawyer (1952) may serve to illustrate this point:

\[
\begin{align*}
C_o &= \text{Concentration of nutrient in feed} \\
C_e &= \text{Concentration of nutrient in feed and effluent} \\
Q &= \text{Rate of flow} \\
V &= \text{Volume of unit} \\
V &= \text{Detention time} \\
Q &= R = \text{Inverse detention time} \\
M &= \text{Concentration of organisms in the tank and in the effluent} \\
K &= \text{Rate of growth}
\end{align*}
\]

It then follows:

\[
\begin{align*}
V \frac{dM}{dt} &= KM - QM \\
\frac{dM}{dt} &= KM - QM \\
\frac{dM}{dt} &= KM - RM
\end{align*}
\]

The rate of change of the concentration of organisms in the tank \( \frac{dM}{dt} \) is equal to the gain in organisms \( (KM) \) minus the loss of organisms in the effluent \( (RM) \). To keep the process balanced it is necessary to maintain a steady concentration of organisms in the tank, \( \frac{dM}{dt} = 0 \). The equation shows that, to fulfill this condition, \( R \) has to be equal to \( K \); therefore, \( KM - RM = 0 \). \( M \), the concentration of organisms, is undetermined in this equation. It depends above all on the difference between \( C_o \), the sugar concentra-
tion in the feed and \( C_s \), the residual sugar concentration in the effluent. As long as these are kept constant \( M \) will be constant. Thus \( R \), the inverse detention time, gives us a direct measure of \( K \), the growth rate, provided the process is in balance. If \( Q \), the feed rate, is low we also have a low value of \( R = K \), the growth rate is low and vice versa.

The yeast normally produced under the conditions outlined above is rich in protein but low in fat. On the average, it contains 55 per cent protein, 4.5 per cent fat, 4 to 5 per cent total phosphorus as \( P_2O_5 \), and 8 to 9 per cent ash on a dry-weight basis. Preliminary experiments had shown that by reducing the phosphate concentration in the nutrient solution, it was possible to increase the amount of fat in the cells 6 to 7 times. At the same time, it was found that cells which were rich in fat had a low reproduction rate. In order to get more detailed data on the growth rate and the fat formation under various phosphate supply rates, a modified continuous flow process was used.

Sulfite liquor lends itself very readily for such an experiment because it originally contains very small amounts of phosphorus, in the order of 30 to 50 ppm \( P_2O_5 \). As has been mentioned, for normal yeast production the phosphate concentration was stepped up to about 800 ppm by adding ammonium biphosphate. In the experiment, a liquor was used which had been prepared as normal except that no phosphate was added. The phosphate was introduced by a separate feed line in form of a 2 per cent ammonium biphosphate solution. This arrangement made it possible to vary the phosphate supply at will. The sulfite liquor contained about 900 ppm nitrogen as liquid ammonia so that the slight variation in nitrogen supply with varying doses of the phosphate solution should be insignificant.

The aeration tank containing 60 to 80 L liquid was started with a normal yeast and a normal phosphate supply which corresponded in this case to 18 ml per min of a 2 per cent ammonium biphosphate solution. This is later designated as 100 per cent phosphate supply and the supply rates are given as a percentage of the full amount. Under these conditions, the process was in equilibrium at a yeast concentration of 14 to 18 g per L and a detention time of 7.1 hr, which corresponds to a growth rate of \( K = 0.141 \). The residual sugar in the effluent ranged from 0.4 to 0.6 per cent and the yeast showed a normal average composition as mentioned above. At this stage, the phosphate supply was shut off completely. As a result, the residual sugar curve showed a continuous upward tendency. In order to keep the process in balance, the feed rate had to be reduced step by step. The total phosphorus content of the cells decreased gradually to 0.5 per cent. The detention time finally reached 165 hr, which means that the growth rate \( K \) decreased to 0.006. In other words, the carbohydrate assimilation and the reproduction of the cells had ceased almost completely. From this point on the phosphate supply was started at increasing rates from 1 per cent to 100 per cent and for each supply rate equilibrium conditions were checked and analyzed. Temperature, \( \text{pH} \), aeration, concentration of the yeast cells and residual sugar in the effluent were kept constant as much as possible. The yeast used in starting several of these experiments was Candida tropicalis, but no pure cultures were maintained. During the course of the experiments a mixed population established itself, of which Candida was the major constituent. But there were always 2 to 4 other types of yeast present which were not identified.

RESULTS

The results of these experiments are presented in the accompanying tables and graphs. First, it is interesting to follow the residual phosphate in the effluent. Table 1, column 2, shows that in the beginning it remains between 8 and 20 ppm, regardless of the increasing phosphate supply. This indicates that all the phosphate which is continually flowing into the aeration tank is being taken up by the yeast cells and converted into an indiffusible form. At 56 per cent ammonium biphosphate supply the first slight increase

<table>
<thead>
<tr>
<th>Relative Phosphate Dose in Per Cent of Maximum Supply</th>
<th>Residual Phosphate PPM P2O5 in Effluent</th>
<th>Phosphorus in Yeast Per Cent P2O5</th>
<th>Detention Time hr</th>
<th>Reprod. Rate ( K \times 10^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8-20</td>
<td>0.48-0.6</td>
<td>42-165</td>
<td>0.6</td>
</tr>
<tr>
<td>2.5</td>
<td>8-20</td>
<td>0.5 - 0.7</td>
<td>20</td>
<td>5.0</td>
</tr>
<tr>
<td>10</td>
<td>8-20</td>
<td>-</td>
<td>9.3</td>
<td>10.75</td>
</tr>
<tr>
<td>12</td>
<td>8-20</td>
<td>-</td>
<td>13.3</td>
<td>7.52</td>
</tr>
<tr>
<td>15</td>
<td>8-20</td>
<td>-</td>
<td>9.4</td>
<td>10.64</td>
</tr>
<tr>
<td>25</td>
<td>8-20</td>
<td>2.3</td>
<td>8.9</td>
<td>11.20</td>
</tr>
<tr>
<td>31</td>
<td>8-20</td>
<td>-</td>
<td>7.9</td>
<td>12.65</td>
</tr>
<tr>
<td>40</td>
<td>8-20</td>
<td>-</td>
<td>7.5</td>
<td>13.33</td>
</tr>
<tr>
<td>56</td>
<td>20</td>
<td>4.8</td>
<td>7.1</td>
<td>14.10</td>
</tr>
<tr>
<td>78</td>
<td>25-80</td>
<td>5.2</td>
<td>7.1</td>
<td>14.10</td>
</tr>
<tr>
<td>100</td>
<td>152-480</td>
<td>5.2</td>
<td>7.1</td>
<td>14.10</td>
</tr>
</tbody>
</table>

Experimental conditions: Total phosphorus in yeast cells determined gravimetrically; residual phosphate in effluent determined colorimetrically.

Maximum dose of phosphate supply: 18 ml 2 per cent ammonium biphosphate solution per min.

Feed: sulfite liquor from beech wood, reducing sugar 3 to 3.5 per cent.

Nutrient salts: 28 g N as ammonia, 15 g KCl per kg reducing sugar.

\( \text{pH} \) in feed, 4.2 to 4.3; \( \text{pH} \) in aeration tank; 5.0 to 5.5.

Volume of aeration tank (mash): 60 to 80 L.

Residual sugar in effluent: 0.4 to 0.6 per cent.

The detention times are average values of several days run in equilibrium.

Aeration: Approximately 1800 L air per hr.
EFFECT OF PHOSPHATE ON YEAST GROWTH

Fig. 1. Relationship between phosphate dose, residual phosphate, and phosphorus concentration in yeast.

Fig. 2. Relationship between the rate of phosphate supply and the reproduction rate $K$.

in the residual phosphate is noticeable, and at 78 per cent it goes up to 80 ppm. At 100 per cent supply, the data indicate that most of the phosphate is not used but leaves the tank in the effluent (132 to 480 ppm residual phosphate). The explanation is given by the analysis of the total phosphorus in the cells. Column 3 shows that this level increased according to the supply from 0.5 per cent to a maximum of 5.2 per cent on a dry weight basis. This limit is reached somewhere between 56 and 78 per cent ammonium biphosphate supply. In figure 1, these data are plotted together. The abscissa indicates the relative phosphate supply in per cent of the maximum dose and the ordinates represent the residual phosphate in the effluent or the total phosphorus of the yeast cells measured as $P_{2}O_{5}$ on a dry weight basis.

In general, the following conclusions can be made: Aerobically growing yeast cells accumulate phosphate as long as the phosphate concentration in the medium is above 8 to 20 ppm and as long as the total phosphorus concentration in the cells has not reached a saturation value, in this case 5.2 per cent. At a concentration below 8 ppm, the phosphate is not available to the cells under the conditions studied, even if they are phosphate deficient. Therefore, the phosphate concentration of the effluent remains between 8 and 20 ppm regardless of the increasing phosphate supply. All the phosphate above 8 to 20 ppm is accumulated by the cells and converted into cell phosphorus. As soon as the phosphorus demand of the cells has been satisfied, any additional phosphate supply appears as residual phosphate in the effluent.

Furthermore, it can be concluded that, up to at least 56 per cent relative phosphate supply, the increase of the supply rate actually means an increase in the phosphorus content of the cells.

It has already been mentioned that the phosphate supply has a deciding influence on the rate of carbohydrate metabolism and the growth rate $K$. In columns 4 and 5, table 1, the values for the detention time and the reproduction rate $K$ are given. In figure 2, these data are plotted to show the relationship between the rate of phosphate supply and the reproduction rate $K$.

Even though the experimental values are scattered due to the many factors which had to be controlled simultaneously, it can be seen that no direct proportionality exists. The curve is similar to an adsorption or saturation curve. The increase in $K$, the reproduction rate, is determined by the phosphorus concentration gradient, in other words by the difference between a saturation value and the concentration $X$ at a given point. When the concentration gradient is great, a small increase in the phosphate supply produces a large increase in the growth rate. But from 56 per cent relative phosphate supply on, $K$ becomes practically independent and stays at the same level. Bearing in mind, that up to this rate of phosphate supply we are actually dealing with increasing phosphorus concentrations in the cells, it can be concluded that this curve

<table>
<thead>
<tr>
<th>Table 2. Relationship between phosphate dose and fat content of the yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Phosphate Dose in Per Cent of Maximum</td>
</tr>
<tr>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>56</td>
</tr>
<tr>
<td>2.3</td>
</tr>
<tr>
<td>2.3</td>
</tr>
<tr>
<td>1.0</td>
</tr>
<tr>
<td>1.0</td>
</tr>
</tbody>
</table>

* Fat—total ether extract.
† Protein—total N $\times$ 6.25.
illustrates the relationship between the rate of cell growth and the phosphorus concentration in the cell.

Table 2 shows the influence of the phosphate supply on the fat formation. It is quite apparent that a high phosphate supply results in a yeast which is rich in protein and low in fat. With decreasing phosphate supply, the balance between these two cell constituents tends to become reversed: The fat increases and the protein decreases. That a balance between protein and fat exists is shown in column 4. The sum of the two components remains at an average of about 54 per cent throughout the experiment.

**Discussion**

In summing up, it can be said that by a controlled phosphate dosage in an aerobic continuous flow process it is possible to control the phosphorus concentration in the yeast cells. Apparently, there exists a definite lower and upper limit as to the amount of phosphorus yeast cells are able to accumulate. In the case investigated, the lower limit was 0.5 per cent and the upper limit 5.2 per cent, measured as P2O5 on a dry-weight basis. The concentration of phosphorus in the cells has a deciding influence on the rate of carbohydrate assimilation, the rate of reproduction, and the protein and fat metabolism. At high phosphorus levels (4 to 5 per cent P2O5), the cells reproduce fast and are rich in protein and low in fat. At low phosphorus levels the rate of carbohydrate assimilation and the growth rate are also low. Simultaneously the balance between protein and fat synthesis is shifted towards the synthesis of fat.

That a decrease in phosphate supply stimulates fat synthesis has been confirmed for several other microorganisms. Thus *Fusarium* sp. increased its fat content from 7.6 to 46.8 per cent in correlation with a decrease from 1.5 g KH2PO4 per L nutrient solution to 0.15 g KH2PO4 (K. L. Schulze, unpublished data). Pan et al. (1949) found the same type of relationship in *Rhodotorula gracilis*. The same organism was studied by Nielsen and Nilsson (1953) and Nilsson and Nielsen (1953) with similar results. In addition, their data indicate that nitrogen deficiency produced a higher fat content than lack of phosphate. Lundin (1950) reported that with a combination of low phosphate and nitrogen supply 50 to 64 per cent of fat could be obtained in *Rhodotorula gracilis*. Lack of nitrogen has been known since the early work of Lindner in 1922 to stimulate fat synthesis in *Endomycopsis vernalis*. Recently, Maas-Forster (1955) showed that by decreasing the phosphate supply, 50 to 60 per cent fat was formed in *Endomycopsis*. She also observed that the uptake of phosphorus and nitrogen are correlated. Lack of phosphorus resulted in a marked decrease of nitrogen assimilation. On the other hand, if the nitrogen in the nutrient solution was depleted, phosphorus assimilation stopped completely. Apparently, both factors control the amount of protein synthesis. When either one of them is in deficiency, protein synthesis decreases and the excess intermediary products of the carbohydrate metabolism are channelled into fat synthesis.

The relationship between phosphate supply and reproduction rate is actually a relationship between phosphorus concentration in the cells and the reproduction rate K. In general, it has the form of a saturation or adsorption curve. K is proportional to the concentration gradient or the difference between the saturation concentration and the existing concentration of phosphorus in the cells.

**Summary**

A mathematical treatment has been employed to evaluate the effect of phosphate supply on the rate of growth and fat formation in yeast when grown on sulfite liquor. The phosphorus concentration in the yeast cells is established by controlling the phosphate concentration of the medium in an aerobic continuous flow process.

At high phosphorus levels, the cells reproduced fast and were rich in protein and low in fat. At low phosphorus levels, the rate of carbohydrate assimilation and the growth rate were also low. Simultaneously the balance between protein and fat synthesis was shifted toward the synthesis of fat.

**References**


