nate beans inoculated with *S. faecalis* was successful. A temperature of 88°C for 1 minute was sufficient to obtain a 100 per cent kill.

REFERENCES

Burton, M. O. 1949 Comparison of coliform and enterococcus organisms as indices of pollution in frozen foods. Food Research, **14**, 434–438.


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**Microbiological Process Report**

**Continuous Fermentation**

A Discussion of Its Principles and Applications

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For the purposes of this discussion, fermentation will be defined in its broadest sense. It is a process in which microorganisms catalyze the conversion of a suitable substrate into a desired product. The process may or may not require oxygen, and the microorganisms themselves may be included among the desired products. Thus, the definition in no way limits the process to one of anaerobic dissimilation, as it does in the classic sense. Fermentation processes may be classified according to the manner in which the substrate is added and the product withdrawn. Thus, in a batch fermentation, the substrate is charged initially, and, when the fermentation is complete, the product is withdrawn. Continuous operation involves addition of the substrate in an unbroken stream and withdrawal of fermented medium in the same manner. In semicontinuous processes the substrate is added and the product removed at intervals, and in this way certain characteristics both of batch and continuous operation are combined. The present discussion will be confined to the strictly continuous case, with occasional reference to those batch and semicontinuous processes which act as prototypes or serve as illustrations. Continuous fermentations may be of many types with respect to the equipment used and with respect to the flow of fermenting media through that equipment. In analyzing them one type will be considered as basic, that is, the homogeneous flow fermentor. Here, the fermentation occurs in a single vessel, agitated so that its contents are homogeneous. The nutrient stream is continuously supplied, and the fermentor contents, with the product they contain, are continuously withdrawn in order to establish a steady state. Other types of continuous fermentation will be discussed as modifications of this basic system.

A continuous fermentation has striking advantages over the corresponding batch process. The primary advantage is a marked reduction in processing time, with the same holding capacity of equipment or, alternatively, a marked reduction in equipment size for the same rate of production. Furthermore, as a consequence of steady-state operation, the product can be expected to have greater uniformity. A continuous fermentation is more adaptable to instrumental control, and it is better integrated into such other parts of the over-all process as the preparation of medium and the recovery of product, which may be operated more economically and efficiently in a continuous manner. From an experimental point of view, a continuous fermentation is ideal. At steady state, the time factor is eliminated, and the effect of nutrients, temperature, pH, agitation, aeration, and other factors upon the
fermentation may be established easily without the complication of continually changing conditions. The rate of growth, as will be seen later, becomes an independent variable (to a degree) and may be studied for its effects on yield and other dependent factors. Monod (1950) presented an excellent theoretical discussion of the use of continuous fermentation as an experimental tool. Novick and Szilard (1950, 1951) used this tool effectively for the study of mutation rates in bacteria; Maxon and Johnson (1952, 1953) used it for a study of aeration and agitation in submerged culture.

There are, however, disadvantages in the use of continuous fermentation which have limited its widespread adoption. Perhaps the major drawback is inherent in the nature of the operation. If the fermentation step in a process is operated continuously, it is desirable to have all other steps of preparation and recovery also in continuous operation. In such a process, a failure in one step will force a complete shutdown. Since fermentation, as in the case of the manufacture of antibiotics, may be extremely complex, an occasional failure must be expected. If these failures become frequent, continuous operation will lose its advantages, whereas in batch operation only one batch is lost with each failure. Failure may come from many sources, mechanical or instrumental. There are others, such as contamination and genetic instability, common only to certain fermentations. These will be discussed later.

Although the limitations of continuous fermentation are recognized, they are not in every case insurmountable. In the simpler fermentations, such as the propagation of food and fodder yeast and the production of ethyl alcohol, the method has proved its worth. As our experience increases it is probable that more and more fermentation products will be made in this manner.

This discussion approaches the subject of continuous fermentation first from a theoretical viewpoint. Equations that govern the relationships of throughput, microbial propagation, product formation, substrate utilization, and so forth are presented. However, since a fermentation is a highly complex living system, a strictly mathematical treatment of all the factors is neither possible nor, for the present, desirable. Instead, the practical significance of a few key equations is discussed and an attempt made to define the scope of their application. Combination of theory and experience leads to a description of the effect of operating variables on yield, productivity, and the like in various continuous systems.

For this discussion, fermentation processes will be classified according to their purpose:

I. Propagation of microorganisms

II. Product formation

Several variations of the basic homogeneous flow fermentor will be considered:

A. Single vessel

B. Multivessel

C. Recycling

D. Two phase

For each process type, the theoretical discussion will be followed by a description of actual operations, as recorded in the literature. To assist the reader in following the development of the equations, all symbols and their meanings are listed below:

C The concentration of contaminating organisms in the fermentor or effluent stream.

c The rate of growth of contaminating organisms, concentration per unit time.

F The rate of continuous feed to the fermentor, volume per unit time.

K A constant in the kinetic expression for the effect of substrate concentration on growth rate (equation 7).

M The concentration of mutant organisms in the fermentor or effluent stream.

m The rate of growth of mutant organisms, concentration per unit time.

P The concentration of fermentation product in the fermentor or effluent stream.

p The rate of product formation, concentration per unit time.

r The throughput rate, volume of feed per unit time per unit operating volume.

S The concentration of substrate limiting to growth in the fermentor or effluent stream.

s The rate of utilization of substrate, concentration per unit time.

V The operating volume of liquid in the fermentor.

X The concentration of the desired organism in the fermentor or effluent stream.

x The rate of growth of desired organism, concentration per unit time.

Y The yield of desired organism on the substrate used, x/s.

Yp The yield of fermentation product on the substrate used, p/s.

θ Time.

θ0 The holdup or retention time in a fermentor, 1/r.

θ0' The total holdup time in a series of fermentors.

μ The Naperian growth rate of desired organisms, concentration per unit time per unit concentration.

ϕ The proportion of a cell population that mutates per generation.

Subscripts

a Refers to conditions in the first fermentor in a series.

b Refers to conditions in the second fermentor in a series.

c Refers to contaminating organisms.

i Refers to initial conditions at time, θ, equal to zero.

m Refers to mutant organisms.

o Refers to conditions in feed.

p Refers to fermentation product.

Propagation of Microorganisms

Theoretical considerations—single vessel. Following the derivations of Monod (1950) and Golle (1953) for this type of continuous fermentation, it will be assumed that an agitated and aerated fermentor is being continuously fed fresh nutrient medium at a constant rate, F, and that fermenting medium is being con-
tinuously withdrawn from the fermentor at the same rate. Thus, a constant volume of fermenting medium, \( V \), is maintained in the fermentor. It will be further assumed that the contents of the fermentor are being agitated sufficiently to insure homogeneity and that the entering fresh medium is thoroughly mixed with the fermenting medium in a short time. The amount of agitation necessary for optimum fermentation is usually more than enough for this purpose.

A material balance for one component of the system leads to a generally applicable equation:

1) \[ FX_0 + xV = FX + V \frac{dX}{d\theta} \]

That is, the rate at which a component is being fed to a fermentor plus the rate at which it is being produced is equal to the rate at which it is withdrawn plus the rate at which it accumulates in the fermentor. For the present, let us assume that there is no product, in this case microbial cells, in the entering stream. If equation 1 is applied to this component, then \( X_0 = 0 \) and:

2) \[ xV = FX + V \frac{dX}{d\theta} \]

We shall define several more terms at this point:

- \( r \): The throughput rate \( = F/V \).
- \( \theta \): The holdup or retention time \( = 1/r \).
- \( \mu \): The Naperian growth rate (Monod, 1950) \( = x/X = (\ln 2)/\text{generation time} \).

When \( r \) is incorporated in equation 2, we find:

3) \[ \frac{dX}{d\theta} = x - Xr \]

And, since at steady state the concentration of cells is not changing \( (\frac{dX}{d\theta} = 0) \):

4) \[ x/X = \mu = r \]

By closer consideration of the behavior of this system we can demonstrate what the forces are that bring about this equilibrium condition in which the Naperian growth rate is equal to the throughput rate, and we can also show what the limitations of such an equilibrium are.

If a fermentation is supplied with adequate aeration, the metabolic rates will ordinarily be limited by the availability of one of the required nutrients. When the general material balance for a continuous system, equation 1, is applied to the substrate that is limiting to growth:

\[ Fs_0 - sV = Fs + V \frac{dS}{d\theta} \]

and:

5) \[ \frac{dS}{d\theta} = r (S_0 - S) - s \]

Now, a yield constant, \( Y \), the proportion of substrate used that is incorporated into the cells formed, is defined:

\[ Y = \frac{x}{s} \]

\( Y \) and \( \mu \) are incorporated into equation 3 to give:

6) \[ \frac{dS}{d\theta} = r (S_0 - S) - (X/Y)\mu \]

Using equation 6 we can demonstrate the stability of the steady state, \( r = \mu \). At equilibrium \( \frac{dS}{d\theta} \) is zero, of course, but if the concentration of cells is disturbed so that \( X \) is less than its equilibrium value, then \((X/Y)\mu \) will be less than \( r (S_0 - S) \). Since \( \frac{dS}{d\theta} \) will then be greater than zero, \( S \), the substrate concentration, will increase. Now, the growth of cells is, of course, an enzymatic reaction, or rather the summation of many such reactions, and may be expected to follow the general behavior of such reactions with respect to the effect of substrate concentration on rate (Michaelis and Menten, 1913). Thus, as an approximation at least:

7) \[ \mu = \mu_{\text{max}} \frac{S}{K_S + S} \]

Data from Harris et al. (1948b) demonstrate that this type of relationship exists in the growth of Torula utilis on wood hydrolysate and sulfate waste liquor. Equation 7 indicates that increasing \( S \) causes \( \mu \) to increase, and \( \mu \) becomes greater than \( r \). A rearrangement of equation 3 gives:

8) \[ \frac{dX}{Xd\theta} = \frac{x}{X} - r = \mu - r \]

Thus, with \( \mu \) greater than \( r \), \( X \) will increase in the exponential fashion dictated by equation 8. As \( X \) increases, the growth rate will diminish once again until steady state is reached at \( \mu = r \). Here, \( \frac{dS}{d\theta} \) must be zero, and from equation 6:

9) \[ X = Y (S_0 - S) \]

Similar reasoning will demonstrate the return to steady state which occurs when \( X \) is greater than its equilibrium value. This derivation is valid only when the yield, \( Y \), has one definite and unique value at each growth rate in the given system. This is essentially true for purely aerobic metabolism, but it is not true in certain other cases (see Mixed metabolism).

Consider now what happens to a fermentation of this sort operating at steady state when the throughput rate is changed. If \( r \) is decreased, equation 6 shows that \( \frac{dS}{d\theta} \) is less than zero and \( S \) decreases. Decreased \( S \) causes decreased \( \mu \), according to equation 7. The effect continues until once again \( \mu = r \) and steady state is re-established at the new throughput rate. As before, \( X = Y (S_0 - S) \). A similar re-establishment of equilibrium will occur if \( r \) is increased.

There is a limit, however, imposed theoretically by the fact that the growth rate will not exceed a certain maximum. Under optimum conditions, this maximum is specified by equation 7 at the point where \( S = S_0 \) or essentially, since \( K_S \) is usually small with respect to
CONTINUOUS FERMENTATION

S₀, μ = μ_max. If r exceeds μ_max, then the cells will "wash out," and X will decrease to zero in an exponential manner according to equation 8:

\[
\frac{dX}{X d\theta} = \mu_{max} - r.
\]

These material-balance and kinetic derivations form the theoretical basis for most continuous fermentation processes. We are led to the following major conclusions for the case of continuous addition and withdrawal from a single vessel, in which purely aerobic metabolism is occurring to produce microorganisms as the desired product:

1) The Naperian growth rate, μ, is equal to the throughput rate, r, for steady state. This is a stable equilibrium which is regained despite disturbances, provided the growth rate is less than its maximum.

2) The population at steady state is uniquely determined at each throughput rate by the yield of cells from substrate, the concentration of substrate entering, and the concentration of substrate in the fermentor:

\[
X = Y (S₀ - S).
\]

3) The Naperian growth rate is a function of the substrate concentration in the fermentor, and thus the substrate concentration depends upon the throughput rate.

In a practical process, the desired results are a maximum rate of product formation for a given fermentation capacity and at the same time a small proportion of unconverted raw material in the effluent. That is, we want to maximize x and minimize S. In order to maximize x, it is necessary to consider the factors that contribute to it. From equation 4:

\[
x = rX = r Y (S₀ - S)
\]

As r is increased, x will also increase until decreasing Y and increasing S cause x to reach a maximum and then decline. If the substrate is not highly valuable with relation to the product, then the throughput rate which gives this maximum rate of product formation will be economically optimal. However, if the unused substrate is of relative value, a lower throughput rate will be necessary to minimize this loss. In many cases of microbial propagation, as pointed out by Monod (1950), the yield is nearly independent of the growth rate. Furthermore, it is often the case that the constant, K_s, of equation 7 is small relative to the substrate concentration. This means that r, the throughput rate, may be held at a value only slightly less than μ_max without a significant decrease in product formation rate or an appreciable concentration of substrate in the effluent.

Further increases in product formation can often be achieved by increasing the concentration of substrate in the feed, S₀. By this procedure the cell concentration is increased, while the throughput rate is held constant.

This is possible, however, only if the growth rate is limited by the substrate concentration. It may be that in an aerobic propagation process the availability of oxygen will limit the growth rate. This will almost certainly become the case as the cell concentration is increased by increasing the substrate concentration in the feed. When this state is reached, x can only be further increased by increasing the rate of oxygen transfer to the respiring cells. Otherwise a higher concentration of cells must be accompanied by a lower throughput rate. The experience of the Germans in producing food yeast by the Waldhof Standard Process, as reported by Saeman, Locke, and Dickerman (1945, 1946), was that higher substrate concentrations necessitated lower addition rates. The yeast output remained the same.

Increase of production rate by increasing the available oxygen is possible only within limits. A highly aerated culture contains a large proportion of entrapped air. The actual amount of liquid holding capacity, V, of a given fermentation vessel is thereby proportionately decreased. This, of course, diminishes the total output rate, F, for a given throughput rate, r. The maximum output that can be achieved must be determined experimentally. In selecting the proper degree of aeration, a balance must be struck between the desirable effect of higher cell concentrations and the undesirable effect of decreased operating volume. High degrees of aeration will also necessitate the increased use of defoamers, mechanical or chemical.

Recycling the organism. The productive capacity of a continuous propagation may be further increased over that indicated by equation 10 by the expedient of recycling a portion of the cells produced. If the general material balance equation (equation 1) is used, but X₀, the concentration of cells in the entering stream, is not zero, then:

\[
FX₀ + xV = FX + V \frac{dX}{d\theta}
\]

and by further manipulations in the same manner as before:

\[
\frac{dX}{d\theta} = x - (X - X₀) r
\]

Again at steady state \( \frac{dX}{d\theta} = 0 \), and r no longer is equal to \( \mu \), but:

\[
r = \frac{x}{X - X₀} \text{ or } r = \frac{\mu X}{X - X₀}
\]

Thus, the throughput rate may be increased over the growth rate by the factor \((X - X₀)\). It was shown in the case where there was no recycling that \( X = Y S₀ - S \) (equation 9). Similar reasoning will demonstrate that for the present case \( X = Y S₀ - S + X₀ \).

Let us assume some values for the factors involved in order to demonstrate the magnitude of the advantage obtained by recycling. Suppose that yeast is being
produced continuously on a medium containing 10 grams/liter \( S_0 \) of glucose, the limiting nutrient. The growth rate is 0.3 hr\(^{-1}\), and under these conditions the concentration \( S \) of glucose in the effluent is negligible. The yield is found to be 50 per cent at this growth rate. If there is no recycling, then the production capacity, \( x \), from equation 10 is:

\[
x = r Y (S_0 - S) , \text{ where } r = \mu \\
= 0.3 \times 0.5 \times 10 = 1.5 \text{ grams/liter hour.}
\]

Suppose that recycling is used, that is, cells are separated from the product stream and returned to the fermentor with the feed. Assume that the rate of recycling is such that \( X_0 \) is 10 grams/liter. Then:

\[
x = r Y(S_0 - S) , \text{ where } r = \mu \frac{X}{X - X_0} \\
= \mu [Y(S_0 - S) + X_0] \\
= 0.3 (0.5 \times 10 + 10) = 4.5 \text{ grams/liter hour.}
\]

By increasing the concentration of active growing cells, the catalyst for this reaction, by a factor of 3, as a result of recycling \( \frac{2}{3} \) of the cells produced, a factor of 3 increase is realized in productive capacity. This is the equivalent of increasing the limiting substrate concentration in the feed, as previously discussed. It has the additional advantage of not necessitating a change in \( S_0 \), which might involve an expensive concentration process. In an aerobic process, recycling, as with increasing substrate concentration, will be advantageous only when excess oxygen is available or can be made available without excessive loss of holding capacity.

More than one vessel. It is sometimes desirable to operate a continuous fermentation in several vessels in series. That is, the product of the first fermentor flows continuously as feed into a second fermentor, and so on, the stream passing through as many vessels as desired.

In discussing the theoretical behavior of such a system let us assume that there are two fermentors in series. The first of these will conform to the equations developed for the single vessel; the second behaves as a single vessel with recycling. The capacity of the combination may then be compared to that of a single vessel by use of the appropriate relations already derived. For a single vessel the holdup time is \( \theta_h = 1/\mu \). For two tanks in series the holdup time is the sum of that in each:

\[
\theta'_h = 1/\mu_s + 1/\mu_b \left( \frac{X_b - X_s}{X_b} \right). \quad \text{(See equation 12.)}
\]

In a propagation system where the growth rate is not a marked function of substrate concentration above a certain minimum it may be that: \( \mu = \mu_s = \mu_b \). In this case it may be shown from the above equations that:

\[
\theta'_h = \left( 2 - \frac{X_s}{X_b} \right) \theta_h.
\]

Since the concentration of cells is greater in the second tank \( X_b \) than in the first \( X_s \), it is evident that the holdup time, \( \theta'_h \), for the two tanks in series is up to two times longer than that for a single tank, \( \theta_h \). Thus, there is no advantage in using more than one fermentor if the growth rate is the same in each.

However, if the fermentation is such that a higher growth rate can be maintained in the first tank because of the presence of a higher concentration of limiting substrate, then an advantage can be demonstrated. In this case \( \mu_s \) will be greater than \( \mu_b \), while \( \mu_b \) equal to \( \mu \), the growth rate in a single tank. This situation would also occur if there were two substrates in the medium, one capable of supporting a higher growth rate than the second. In the first tank, the more easily assimilable nutrient would be used, and the harder to assimilate nutrient left as the limiting substrate in the second fermentor. In either case:

\[
\theta'_h = 1/\mu_s + \theta_h \left( 1 - \frac{X_s}{X_b} \right).
\]

If we assume, for example, that \( \mu_s = 2\mu \) and that \( X_s = 0.9 \), then

\[
\theta'_h = 1/2\mu + \theta_h (1 - 0.9) = 0.5 \theta_h + 0.1 \theta_h = 0.6 \theta_h.
\]

The holdup time for the two fermentors in series is reduced significantly over that for a single fermentor doing the same job. This means, of course, a reduction in total tankage for the same rate of product formation.

Mixed metabolism. In the previous considerations, the simplifying assumption was made that in aerobic microbial propagation the substrate is converted by a single definite series of metabolic processes into cellular material, the over-all result being, for example:

\[
\text{glucose (s)} \rightarrow \text{yeast (x)} + \text{CO}_2.
\]

This is approximately true in some cases, such as in the propagation of \textit{Torula utilis}. It is not true, however, in others. In bakers' yeast, \textit{Saccharomyces cerevisiae}:

\[
\text{glucose (s)} \rightarrow \text{yeast (x)} + \text{ethanol (p)} + \text{CO}_2.
\]

Here, there are two processes competing for the available substrate. It has been shown (Maxon and Johnson, 1953) that even under excess aeration both processes may operate in a ratio depending upon the rate of substrate utilization (figure 1), but that below a certain rate only the oxidative metabolism is evident and no ethanol is formed.

In a fermentation of this type, the yield will be highly dependent on the substrate concentration, and thus
on the growth rate and the throughput rate. This is because one of the competing metabolic processes gives a lower yield than the other. If \( r \) is changed in the range of growth rates where both types of metabolism are occurring, the changing yield will cause the steady-state population to change (equation 9). Finn and Wilson (1953) demonstrated this effect in the aerobic propagation of \textit{Bacterium linens}.

It may also be that in a continuous system where two competing types of metabolism are occurring the steady-state population is not a unique function of the throughput rate, as was seen to be the case with purely aerobic metabolism. Suppose, for example, that a portion of a continuous culture is rapidly withdrawn and replaced by fresh medium. This brings about a simultaneous decrease in the cell concentration and an increase in the substrate concentration away from their former values. However, the steady state may not be restored by a return to these values with unmixed metabolism. (See Theoretical Considerations.) Instead, since the changed conditions cause changed metabolism and reduced yield, a new steady state is established at a lower cell concentration. This type of behavior also has been shown to occur with \textit{Bacterium linens} (Finn and Wilson, 1953).

In a practical process, propagation should, of course, be carried out with the highest yielding metabolic process in sole operation. The region of mixed metabolism is to be avoided. Thus, with bakers’ yeast, for example, the growth rate should be maintained at less than that which brings about the formation of ethanol.

\textit{Applications.} The prime practical example of microbial propagation by purely aerobic metabolism in one continuously operated vessel is found in the manufacture of food and feed yeast. A thorough description of this industry has recently become available (Wiley, 1954). Many production installations have been operated, but their general characteristics are similar. Briefly, the fermentation is carried out in a single, large fermentor. For example, the fermentor used at Lake States Yeast Corp., Rhinelander, Wisconsin (Inskeep, \textit{et al.}, 1951) has an operating capacity of 45,000 gallons of air-liquid emulsion or 20,000 gallons of liquor. Such fermentors are provided with agitation and a supply of air. The Walhof system has met with great success for this purpose; it has been used in several installations in Germany (Saeman, Locke and Dickerman, 1945, 1946; Holderby, 1946) and in this country (Inskeep \textit{et al.}, 1951). Agitation and air are both supplied by a single, hollow-bladed impeller. This is located near the bottom of the tank under a central draft tube. Circulation down through this tube caused by the impeller brings about effective mixing and aeration and at the same time beats the foam into the emulsion. Cooling by internal or external heat exchange is required. Fresh medium is continuously fed and product withdrawn. The limiting nutrient is usually the fermentable sugar and may be supplied in molasses, sulfite waste liquor, wood hydrolysate, or agricultural wastes. Supplementation with salts of nitrogen, phosphorus, potassium, and occasionally other elements is usually required. Aqueous ammonia is often added to serve the dual purpose of \( \text{pH} \) control and nitrogen source. A process in which the nitrogen source acts as the limiting nutrient has been patented by Seidel (1943).

The organism most often used in food and feed yeast processes is \textit{Torula utilis}. Other similar organisms have been used with success in Germany, for example, \textit{Candida arborea} and certain poorly identified and mixed cultures (Saeman, Locke and Dickerman, 1945, 1946).

The growth of \textit{S. cerevisiae} in continuous culture has been the subject of investigation for some time. DeBeeze and Rosenblatt (1943) discussed a number of the early processes. Irvin (1954) also mentioned some of the work in this field. A patent assigned to the Fleischmann Company (Bührig, 1929) describes a two-vessel system, a main fermentor and an auxiliary finishing fermentor operated continuously in series. Sak (1928, 1932a, 1932b) holds patents involving continuous yeast propagation in multiple vessel systems with recycling. Other patents on processes of this type are held by Harrison (1930), Olsen (1930), and Daranyi (1936). Unger \textit{et al.} (1942) worked with a pilot plant for the continuous propagation of distillers’ yeast in a single vessel and arrived at design figures for a full-scale yeast plant.

Propagation by these methods is in no way limited to yeast. For example, Gerhardt (1946) found that \textit{Brucella suis} could conveniently be maintained in

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**Fig. 1.** The effect of glucose utilization rate upon the metabolism of bakers’ yeast under excess aeration. Per cent yield is \( g \) dry weight of yeast per 100 \( g \) glucose used. Per cent glucose glycolyzed is \( g \) glucose to ethanol per 100 \( g \) glucose used. Medium B: 1 per cent glucose. Medium C: 10 per cent glucose. (From Maxon, W. D., and Johnson, M. J. Ind. Eng. Chem., 45, Figure 6, page 2559, 1953.)

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<td>Per Cent</td>
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**Fig. 2.** The effect of glucose utilization rate upon the metabolism of bakers’ yeast under excess aeration. Per cent yield is \( g \) dry weight of yeast per 100 \( g \) glucose used. Per cent glucose glycolyzed is \( g \) glucose to ethanol per 100 \( g \) glucose used. Medium B: 1 per cent glucose. Medium C: 10 per cent glucose. (From Maxon, W. D., and Johnson, M. J. Ind. Eng. Chem., 45, Figure 6, page 2559, 1953.)

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**Diagram 3.** The effect of glucose utilization rate upon the metabolism of bakers’ yeast under excess aeration. Per cent yield is \( g \) dry weight of yeast per 100 \( g \) glucose used. Per cent glucose glycolyzed is \( g \) glucose to ethanol per 100 \( g \) glucose used. Medium B: 1 per cent glucose. Medium C: 10 per cent glucose. (From Maxon, W. D., and Johnson, M. J. Ind. Eng. Chem., 45, Figure 6, page 2559, 1953.)

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**Figure 4.** The effect of glucose utilization rate upon the metabolism of bakers’ yeast under excess aeration. Per cent yield is \( g \) dry weight of yeast per 100 \( g \) glucose used. Per cent glucose glycolyzed is \( g \) glucose to ethanol per 100 \( g \) glucose used. Medium B: 1 per cent glucose. Medium C: 10 per cent glucose. (From Maxon, W. D., and Johnson, M. J. Ind. Eng. Chem., 45, Figure 6, page 2559, 1953.)

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**Diagram 5.** The effect of glucose utilization rate upon the metabolism of bakers’ yeast under excess aeration. Per cent yield is \( g \) dry weight of yeast per 100 \( g \) glucose used. Per cent glucose glycolyzed is \( g \) glucose to ethanol per 100 \( g \) glucose used. Medium B: 1 per cent glucose. Medium C: 10 per cent glucose. (From Maxon, W. D., and Johnson, M. J. Ind. Eng. Chem., 45, Figure 6, page 2559, 1953.)

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**Table 3.** The effect of glucose utilization rate upon the metabolism of bakers’ yeast under excess aeration. Per cent yield is \( g \) dry weight of yeast per 100 \( g \) glucose used. Per cent glucose glycolyzed is \( g \) glucose to ethanol per 100 \( g \) glucose used. Medium B: 1 per cent glucose. Medium C: 10 per cent glucose. (From Maxon, W. D., and Johnson, M. J. Ind. Eng. Chem., 45, Figure 6, page 2559, 1953.)
continuous culture on a laboratory scale. Elsworth and Meakin (1954) presented information on the continuous propagation of *Aerobacter aerogenes* in a 20-liter vessel both on glycerol as a limiting substrate and on a casein-yeast extract medium. No successful process of this type for the production of a mycelial organism has been reported, however, except for a brief mention of the continuous propagation of *Aspergillus oryzae* as a medium supplement in a paper by Ruf et al. (1948). Attempts were made in Germany to manufacture *Oidium lactis*, a mycelium-forming organism, the so-called “Biosyn” (Saeman, Locke and Dickerman, 1945, 1946). Difficulties with contamination in continuous fermentation were encountered and forced abandonment of this type of operation.

In table 1, the rate of product formation and other pertinent data are given for several representative continuous fermentations reported in the literature. Data were taken for industrial, pilot, and laboratory operations. Comparison of the productivity with that of batch fermentations demonstrates the marked advantage in this respect of a continuous process.

**Contamination and genetic instability.** Contamination in fermentation may be a serious problem. A continuous fermentation, which is designed to operate for long periods of time, is particularly liable to the occasional introduction of undesired organisms. The mere entry of foreign organisms into a continuous culture does not, however, assure its failure. Golle (1953) developed equations for the theoretical behavior of fermentation under these conditions. These equations are paraphrased here:

Suppose that a single vessel in continuous operation is being contaminated with a foreign organism at a rate \( C_0 r \). Then, by application of the general material balance equation, as in the development of equation 11 for the case of recycling:

\[
\frac{dC}{d\theta} = c - (C - C_0)r
\]

The growth rate of the foreign organism, \( \mu_c \), is defined by: \( \mu_c = c/C \). Use of these quantities in equation 13 gives:

\[
\frac{dC}{d\theta} = C(\mu_c - r) + rC_0
\]

Upon integration:

\[
\ln \frac{C(\mu_c - r) + rC_0}{C(\mu_c - r) + rC_0} = (\mu_c - r)\theta
\]

where \( C \) is the concentration of contaminating organisms at any time, \( \theta \), and \( C_1 \) is the initial concentration at \( \theta = 0 \).

There are three possibilities with respect to the growth rate of the foreign organism in the fermentation that it is contaminating. It can be greater than, equal to, or less than the throughput rate. If \( \mu_c \) is greater than \( r \) (which equals \( \mu \)), then equation 15 shows that \( C \) will increase exponentially with time. This will continue until the steady-state concentration of limiting substrate is reduced to the point where \( \mu_c = r \) according to a relationship such as is expressed by equation 7. Under these conditions, the growth rate of the original organism will be less than \( r \), and its concentration will decrease to zero exponentially according to equation 8. After a period of time the original organism will be entirely replaced by the contaminating organism. An infection by such an organism, once started, will result in complete failure of the fermentation.

If the growth rate of the foreign organism is equal to the throughput rate, then according to equation 14 its concentration will increase linearly at a rate \( rC_0 \). Contamination by an organism with such a growth rate will not be serious if the rate of entry can be kept negligibly small.

If the growth rate of the contaminant is less than the throughput rate, equation 15 shows that its concentration will approach a limit at infinite time:

\[
C = \frac{C_0 r}{r - \mu_c}
\]
A contamination by such an organism will become serious only if its rate of entry is extremely high and its growth rate only slightly less than that of the desired organism.

In the manufacture of food and feed yeast contamination has been no problem. In fact, no great precautions are taken to prevent it. This is because it is possible to maintain a high throughput rate under nutrient conditions that are unfavorable for the growth of undesired organisms. That is, the pH is kept low, and media such as sulfite waste liquor and wood hydrolysate, which contain inhibiting substances, are employed.

The Aerobacter aerogenes culture described by Elsworth and Meakin (1954) was maintained continuously for 2600 hours. Proper precautions prevented entrance of foreign organisms. Only experience with a given continuous fermentation, however, can establish how serious a deterrent to successful operation contamination will be. It is probable that with strict care most microorganisms can be produced free of contamination.

A problem related to contamination is mutation. When mutation of an organism in steady-state culture occurs, the fate of the mutant depends upon relationships similar to those for a foreign organism. Equations for these relationships have been developed by Golle (1953) and by Novick and Szilard (1950, 1951).

When mutation occurs in a single vessel in continuous operation, it is governed by the following material balance, where \( \phi \) is the proportion of the total population that mutates in each generation. The number of generations per unit time can be shown to equal \( \mu / \ln 2 \). Then:

\[
\frac{dM}{d\theta} = m - rM + \phi X \mu / \ln 2
\]

or

\[
16) \quad \frac{dM}{d\theta} = M(\mu_m - r) + \phi X \mu / \ln 2
\]

This equation is of the same form as equation 14 for the case of contamination. In the same line of reasoning then, if the growth rate of the mutant, \( \mu_m \), is greater than the throughput rate, the entire culture will be replaced by the mutant form. If \( \mu_m \) is equal to the throughput rate, the concentration, \( M \), will increase linearly at a rate \( \phi X \mu / \ln 2 \), and if \( \mu_m \) is less than the throughput rate, \( M \) will approach a limiting value:

\[
M = \frac{\phi X}{\ln 2} \left( \frac{r}{r - \mu_m} \right)
\]

From these relationships it is evident that a continuous propagation system acts as a mutant selector. Novick and Szilard (1950, 1951), indeed, made ingenious use of this fact for the study of mutation rates of Escherichia coli. It should be recognized that the organism in a steady-state system will not necessarily be of the same genetic form as that of the organism originally introduced. It will, rather, be the spontaneous mutant with the highest affinity for the limiting nutrient.

This is usually an advantage when the production of microorganisms is the goal, since a higher productivity can be achieved. It may be in a product formation fermentation, however, that a mutation toward higher substrate affinity will be accompanied by a lower rate of product elaboration. The eventual population, resulting from mutant selection, will then have a lower productivity than the original culture. The seriousness of this problem would have to be determined experimentally for each process. It is not one with a ready solution.

By microscopic examination of bacteria in a culture chamber, Powell (1954) showed that the generation time of individual bacteria varies widely from the average (from 6 to 50 minutes for Streptococcus faecalis). This is an erratic variation and is not consistently inherited by later generations. Powell concluded that selection in a continuous culture will not occur as a result of this type of variation, but only as a result of actual mutation.

**Product Formation**

Although many important applications of continuous fermentation lie in the field of microorganism production, there are still more, whether actually in use or merely proposed, in the field of product formation. Antibiotics, enzymes, solvents, organic acids, and so on are all fermentation products that might be advantageously made in a continuous system.

*Theoretical considerations.* As with propagation, the simplest continuous product formation system consists of a single vessel with continuous addition of feed and continuous withdrawal of fermenting medium. Similar equations apply in this case. The growth rate of the organism is again equal to the throughput rate, and the concentration of cells is, as before, determined by

\[
9) \quad X = Y(S_0 - S).
\]

It is no longer desired, however, to maximize \( x \), the rate of cell formation per unit operating capacity, but rather to maximize the rate of product formation, \( p \). This rate is determined by material balance and kinetic considerations, as is the growth rate, and similar equations can be used to express the relationships. The material balance expression is based on the yield of product, \( Y_p \), from the rate-limiting substrate and is analogous to equation 10:

\[
17) \quad p = rP = rY_p(S_0 - S)
\]

The kinetic factors are summarized by:

\[
18) \quad p = \lambda X
\]

The specific rate of product formation per unit of cell concentration, \( \lambda \), is, of course, a function of the
limiting substrate concentration, S, and the concentration of product, P.

Under given conditions of throughput rate, environment, and nutrition, the cell concentration is fixed at a steady-state value according to the previous considerations for microbial propagation. The quantities S, Y, P, and λ will also be fixed at equilibrium levels, as defined by equations 17 and 18. In this way the productivity, p, at steady state is established.

Product formation associated with growth. In certain fermentations, the growth process and the product formation process are closely linked. The same substrate concentration may be limiting to the rate of both processes, and these rates may respond to changes in this concentration in a similar manner. Such a fermentation is the alcoholic fermentation of glucose by yeast. Here, it is the energy resulting from the anaerobic dissimilation of glucose that permits the yeast to proliferate. In cases of this sort, the effect of throughput rate on productivity in a single vessel may be analyzed according to the concepts described for microbial propagation. As r is increased, the limiting substrate concentration, S, must increase according to equation 7, so that μ remains equal to r. This increased S causes the specific rate of product formation, λ, to increase also, since λ and μ are similar functions of S. In this manner, productivity increases with increasing throughput, as specified by equations 17 and 18, until increased residual substrate and decreased population affect the rate of product formation sufficiently to cause a decline. The economically optimum throughput rate will be less than that for maximum product formation rate by an amount depending upon the relative value of the unused substrate.

It is possible to predetermine the effect of throughput rate upon population level in continuous, anaerobic yeast fermentation by the method of Adams and Hungate (1950). Residual glucose, and presumably ethanol concentration, may also be predicted. The procedure is to run a batch fermentation on the desired nutrient medium. Periodic analyses reveal the cell concentration, X, the glucose concentration, S, and the ethanol concentration, P, at various times. The growth rate, μ, at a given time is found by dividing x, the slope of the growth curve, by X. If a continuous fermentation is operated at this growth rate (determined by the throughput rate), the equilibrium levels of X and S will be the same as those in the batch fermentation. That is, the conditions in a continuous fermentation and those in a batch fermentation are the same when the growth rates are equal. After a period of continuous operation on certain media, it may be that a different substrate will become limiting, nitrogen rather than glucose, for example. In this case, the concentrations X, P, and S will change from the values predicted by analysis of the batch fermentation.

It is often advantageous in a continuous product-formation fermentation to operate with several vessels in series. When product formation is associated with growth, the advantage of this type of operation is obtained under the same conditions as for microbial propagation. That is, the advantage exists only when higher growth rates can be maintained in the first tanks in the series, while the lowered growth rates in the final tanks permit a more complete use of the substrate. Such a situation is apt to prevail in alcohol fermentation, for example. In the anaerobic yeast fermentation, it appears that growth rates near the maximum require high concentrations of limiting substrate and low concentrations of the toxic product, ethanol. Thus, in a single vessel, the high throughput rates necessary for maximum productivity are not compatible with the low effluent substrate and high product concentration also necessary. This reasoning explains the wide acceptance of multi-vessel systems for continuous alcoholic fermentation.

As in microbial propagation, the continuous fermentation for product formation may be increased in productivity by raising the concentration of nutrient in the feed, S0. This increases the concentration of both cells and product. Equations 17 and 18 demonstrate the effect on p. This procedure has limitations, since it is known that λ is a function of P. That is, high product concentrations inhibit the product-forming enzymes. They may also inhibit the enzymes of growth. Thus, the point is reached where increasing S0 will decrease Yp while S rises. Rate of product formation will rise no further.

An effect similar to increasing substrate concentration can be achieved by recycling the organism. This has the effect of increasing X without affecting λ (equation 18). In this manner, the productivity is enhanced. A higher throughput rate is possible while Yp and S remain the same (equation 17). The limit is reached when product inhibition of μ and λ causes X and Yp to fall, and S to rise significantly.

It is possible, of course, to return all the cells to the fermentation, that is, total recycling. If this is done, the concentration of cells in the fermenting medium will continue to rise until growth ceases completely (or rather growth rate and autolysis rate are equal) as a result of product toxicity, lack of nutrients, or other undefined factors. In such a case, the throughput rate may be as high as desired without decreasing X. Productivity will still, however, depend on throughput rate, as indicated by equations 17 and 18.

Product formation not associated with growth. In the above paragraphs, the case in which the same substrate is limiting both to growth rate and product formation rate has been under consideration, and both rates reacted similarly to the concentration of this substrate. This is often not the situation. For example, if an aerobic yeast fermentation is fed a medium low in nitrogen, the growth rate may be nitrogen-limited while
glycolysis proceeds at such a rate that it is limited by the concentration of glucose. More important examples occur in the formation of other fermentation products, enzymes, antibiotics, and pigments, for example, which are less directly connected with the growth process. For illustration, in the batch penicillin fermentation, there are two distinct phases. For the first 24 hours growth occurs on the rapidly assimilable carbohydrate sources. The pH is low. The remainder of the fermentation evidences little growth. The pH is high. It is in this period that penicillin production rate reaches its maximum. Thus, the conditions required for growth and those required for productivity are widely different.

When this is the situation, a single-vessel continuous process without recycling can only poorly provide the proper environment both for growth and product formation. High throughput rate, which is necessary for high productivity (equation 17), necessitates high growth rate (since \( \mu = r \)). The equilibrium conditions that permit high growth rate will, on the other hand, be incompatible with a satisfactorily high rate of product formation. The two metabolic processes must be separated for the best results.

There have been few reported attempts to accomplish this separation of functions in a continuous system. The theory predicts that it can be done by recycling the organism, by use of a multivessel system, or by use of a two-phase system.

Two-phase systems. In our previous considerations, the fermenting mixture consisted of a more or less homogeneous suspension of microorganisms in a suitable medium. The product formed was separated from the cells in a separate stage of processing by filtration or centrifugation. This single-phase type of fermentation is usually the most convenient. In certain cases, however, it is possible to separate the system into two phases. The major example is the vinegar or acetic acid fermentation. Here the fermentor or “generator” consists of a mass of beech wood shavings upon which the Acetobacter species which is responsible for the desired oxidative conversion of ethanol to acetic acid is fixed. In this way the organism is maintained in the solid phase while the substrate and product remain in the liquid stream which trickles through.

There are further examples of two-phase continuous fermentations (see Applications). Usually the phases are solid and liquid. There are cases where the organism is in a liquid phase while the substrate is in a second, immiscible liquid or a gaseous phase.

A two-phase system does not comply with the criterion of homogeneity which was important to the theoretical relations developed earlier. Fermentative conversion does not occur in one or more equilibrium stages, but in a continuum. Thus, the concentration of acetic acid in a vinegar generator rises gradually to its maximum in the effluent. Such a situation is not represented by the equations already derived. Similar principles may be used, however, to evolve the relationships for various processes of this sort.

Applications. Many continuous fermentation processes for product formation have been successfully operated. The majority of those reported in the literature have been for the anaerobic production of ethanol by yeast. While the objectives are similar, the manner in which these fermentations are run is widely varied. The differences occur in the microorganism, the medium, the conditions, and in other respects. Examples are available of every type of continuous operation that has been described here. Included are single-vessel systems with and without recycling, multivessel systems with from 2 to 12 vessels in series, two-phase systems and others which display major and minor modifications of these principles.

Bilford et al. (1942) investigated a single-vessel continuous alcoholic fermentation process on a laboratory scale. Their tests were run in a small fermentor agitated either mechanically or with carbon dioxide. The nutrient media were glucose (10 to 12 per cent) in yeast water or molasses (Cuban blackstrap, refined and beet, 12 to 13 per cent reducing sugar), and these could be fermented to completion with holdup times of 4 to 7 hours. The effluent reducing sugar concentration ranged from 0.1 to 1.5 per cent. The Guillaume-Boulanger process (DeBeeze and Rosenblatt, 1943) used in Europe in the large beet sugar and molasses alcohol plants is a modified single-vessel type. The main conversion is carried out in a large continuously operated fermentor. In order to allow complete usage of the sugar a 2½ hour batch fermentation of the effluent is carried on in small auxiliary tanks.

Molasses provides a readily used source of carbohydrate and contains no substances inhibitory to the alcoholic fermentation. It is, therefore, rapidly and completely used in continuous operation. Multivessel operation is undesirable, and recycling is not necessary. The low pH and high throughput rate minimize the chance of contamination.

On certain other substrates, advantage must be taken of the multivessel and recycling principles to permit high, continuous productivity.

The process developed for the conversion of acid-hydrolyzed grain carbohydrates to alcohol by Joseph E. Seagram and Sons, Inc. (Altsheler et al., 1947; Ruf et al., 1948) involves the use of two vessels. In the pilot operations described by Ruf et al. (1948), the holding time is 8 hours in the primary fermentor, 4 to 6 hours in the secondary. Both tanks are mechanically agitated. Fermentations up to 9 days in pilot plant and 6 months in laboratory were obtained without contamination or yeast degeneration. Figure 2 shows some analyses made during the course of a pilot-scale run. In the two runs compared, corn mash alone was used in the first, and
Pilot studies in a six-fermentor system of this type have been made by Harris et al. (1948c). Torula utilis was used to ferment wood hydrolysate containing 5 per cent reducing sugar. At steady state with a total of 24 hours retention time 80–82 per cent of the fermentable sugar was used, and an alcohol yield of 40 per cent on total fermentable sugar was obtained. From tank to tank the equilibrium sugar concentration was: 5.2 per cent (feed), 4.3 per cent (first tank), 3.3 per cent (second tank), 2.3 per cent (third tank), 1.5 per cent (fourth tank), 1.1 per cent (fifth tank), and 0.8 per cent (sixth tank).

The patent literature details some special equipment for fermentations of this sort. Victerero (1948) arranges the equilibrium stages vertically in order to facilitate flow of gas and medium from tank to tank. Scholler and co-workers (1937, 1940) describe a special fermentation apparatus that embodies the principles of a multivessel continuous system and is provided with a final settling chamber where the yeast separates for return with the feed.

Two-phase systems are not commonly used in the alcoholic fermentation. The "Fesselhefe" method (Saeman, Locke and Dickerman, 1945), in which the yeast is bound on twigs, has been used in Germany, however, for a sulfite waste liquor process. This type of operation has been patented by Romer (1924). An Indian patent (Counciel of Scientific and Industrial Research, 1962) has appeared on the use of a battery of columns containing pumice impregnated with yeast. Molasses was the carbohydrate source, and less than 0.5 per cent sugar remained in the effluent.

There are relatively few instances of continuous fermentation being used for the manufacture of products other than ethyl alcohol. There is, of course, the case of acetic acid and vinegar, as mentioned before. A review of this process has been made by Vaughn (1954). In 1931, Whittier and Rogers developed a laboratory-scale single-vessel continuous fermentation for the conversion of whey to lactic acid.

Interesting applications of continuous fermentation in the petroleum industry have been patented by the Standard Oil Development Company (Taggart, 1946) and the Texaco Development Company (Zobell, 1953). The Standard Oil process is a two-phase fermentation carried out in a bubble-cap column. Light, gaseous hydrocarbons are continuously converted to the corresponding fatty acids and their esters through the oxidative action of such organisms as Bacillus parafinnicus n. sp., B. methanics, and B. ethanicius n. sp. The bacteria in a salt solution pass downward over the trays. The gaseous hydrocarbons mixed with air pass upward through the bubble-caps. The contact that results permits oxidation of the substrate and proliferation of the bacteria. The product is recovered from the aqueous stream. The process is one of growth-associated product formation. It involves several equilibrium stages and

![Diagram](http://aem.asm.org/)

**Fig. 2.** A two-vessel continuous alcoholic fermentation of acid-hydrolyzed corn mash. Comparison of data obtained with and without submerged culture mold supplement. (From Ruf, E. W., Stark, W. H., Smith, L. A. and Allen, E. E. Alcoholic Fermentation of Acid-hydrolyzed Grain Mashes, Ind. Eng. Chem., 40, 1154–1158, Figure 4, p. 1157.)

in the second submerged culture Aspergillus oryzae was added as a supplement. The supplementation evidently changed the limiting nutrient. This permitted a slightly higher cell population in the primary fermentor, a lower concentration of reducing sugar in both fermentors, and a higher alcohol concentration in the secondary fermentor. This example demonstrates the advantages of continuous operation for experimentation.

The method of Alzola (1942, 1945) for continuous fermentation also uses the multivessel principle. Here, five fermentors of equal size are connected in series. Agitation in the fourth and fifth is supplied by carbon dioxide pumped from the first and second. The concentration of nutrient in each tank, as measured by specific gravity, declines as follows: Second tank, 7° Bé; third tank, 4° Bé; fourth tank, 2.5° Bé; fifth tank, 2° Bé.

The additional advantages of recycling are made use of in the alcoholic fermentation of wood hydrolysates and of sulfite waste liquor, substrates less readily used by the yeast. For a general reference see McCarthy (1954) and Saeman and Andreassen (1954). Multivessel installations are used, and the effluent yeast is separated by centrifugation as a cream to be added with the feed to the first fermentor in the series. This type of process is quite widely used in Europe (Saeman and Andreassen, 1954; Saeman, Locke and Dickerman, 1945, 1946; DeBeeze and Rosenblatt, 1943) and plants are operated by the Puget Sound Pulp and Timber Company, Bellingham, Washington (Ericsson, 1947; McCarthy, 1954) and by Commercial Alcohols Limited, Gatineau, Quebec (McCarthy, 1954). The fermentors are large, up to 120,000 gallons capacity at Gatineau, and are agitated either mechanically or with carbon dioxide. From 3 to 8 tanks are arranged in series. The total holdup time varies from 6 to 24 hours. Up to 12 per cent of the fermenting medium is yeast (pressed).
may, therefore, be classified as a multivessel system, but it is unique in that the substrate stream and the bacterial catalyst stream are in separate phases flowing countercurrently.

The Texaco process has some similarities. Here, however, the reaction is reductive. The complex sulfur compounds that occur in crude petroleum are often undesirable in the final product. By this method they are converted to easily removed mercaptans and hydrogen sulfide. An organism such as Desulfovibrio desulfuricans is employed. It can use hydrogen for the reduction of sulfur compounds by virtue of the hydrogenase enzyme that it produces. For continuous operation a bubble-cap column is used. The crude hydrocarbon flows upward and the aqueous medium containing the bacteria flows downward. Hydrogen is supplied at the bottom of the column and flows out the top with the gaseous reaction products. Alternatively, the hydrogen may be supplied in situ by the action of microorganisms, for example, Clostridia on a glucose medium. This process, like the Standard Oil process, is a two-phase multistage system. Product formation is growth-associated.

A few attempts have been made to use continuous fermentation for the production of antibiotics. The process of the Joseph E. Seagram and Sons, Inc. (Kolachov and Schneider, 1952) is a notable example. A single aerated and agitated fermentor is employed. It is continuously fed a lactose, glucose, corn steep liquor medium, and the penicillin-containing, fermented medium is continuously withdrawn. A holdup time of 48 hours is maintained. More rapid throughput would necessitate a higher growth rate, incompatible with penicillin production. The yield of 600 units/ml gives a productive capacity of 12.5 units/ml hour.

Other antibiotic fermentations of a continuous nature have been reported. Moor (1945) describes a laboratory-scale two-phase penicillin surface culture process. A mat of Penicillium notatum is grown on the surface of a corn steep medium. Fresh medium, fermented product, and the mycelial matt move continuously through the apparatus. A similar process has been patented (Lilly, 1933). Here, the mycelium grows on the outside of filter cloth tubes where it is aerated. The medium to be fermented passes down inside these tubes. Japanese workers (Wakaki et al., 1952) described a semicontinuous submerged culture process for penicillin fermentation.

The acceptance of continuous methods for the manufacture of food and feed yeast and fermentation alcohol is easily understood. These industries depend upon a large volume and a low profit margin. Thus, equipment of tremendous capacity operating at maximum output is required. High efficiency and low waste are necessities. The advantages gained in productivity for a given equipment capacity by continuous fermentation are great. Furthermore, the time lost in cleanup and preparation necessary to batch fermentation is obviated. The difficulties that could arise from contamination or yeast degeneration are not encountered, and no expensive precautions are required to prevent them. For these reasons the continuous process may be expected to have even wider application in these industries.

As the technology of fermentation develops, it seems inevitable that manufacturers will turn to this type of operation for other products. The advantages to be gained, whether increased output, automatic control, or product uniformity, all become available when the difficulties of contamination, genetic instability, and so forth are solved. Meanwhile, continuous fermentations on a laboratory scale will have great value in the collection of experimental data. This discussion was intended to summarize the information on the subject presently available and to provide a background for new developments and a starting point for new ideas.

REFERENCES


Elsworth, R., and Meakin, L. R. P. 1954 Laboratory and pilot plant equipment for the continuous culture of bacteria with examples of its use. Chemistry & Industry, July 24, 926-927.


