Theoretical Design of Continuous Antibiotic Fermentation Units

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Abstract

Reusser, F. (The Upjohn Company, Kalamazoo, Mich.). Theoretical design of continuous antibiotic fermentation units. Appl. Microbiol. 9:361–366. 1961.—A graphic method of predicting antibiotic yields in continuous flow reactors is presented and discussed using the novobiocin fermentation as a model process. Extension to other antibiotic fermentations and steroid bioconversions is emphasized. In the case of the novobiocin fermentation it was concluded that a combination of one growth stage and one or two antibiotic production stages would be the most economic reactor system to conduct such a fermentation on a continuous basis.

Design theories of continuous chemical reactors and processes have been developed by Denbigh (1951), Schoenemann (1952), and Danckwerts (1953, 1954), but implications of their results and conclusions to fermentation processes may not be generally known.

A brief discussion of their findings is therefore presented in the following:

General Aspects of Continuous Flow Reactors

Continuous flow reactors consist of a reaction vessel which is supplied with a steady flow of reactants while product is withdrawn continuously at the same rate, thus keeping the volume inside the vessel at a constant level.

Types of Reactors

Basically there are two extremes of continuous reactors; the completely mixed tank and the tubular type with complete mixing only in planes perpendicular to the flow direction (piston flow). In an ideal piston flow reactor, all particles will have identical residence times, whereas in a completely mixed tank, they vary around a mean residence time $T'$. The completely mixed (or homogeneous) reactor can be achieved in actual practice. However, the performance of piston flow reactors have been rather far from ideal in most cases.

Residence Time and Spread of Residence Times

The time required for a single particle to pass through the reactor is called its individual residence time. The mean residence time $T'$ (identical with the term "holdup time") is the average of all residence times of a given quantity of particles flowing through a given continuous flow system. The residence times of individual particles may exhibit a wide spread about the mean residence time $T'$ and its extent and particular behavior is used to describe the type of a reactor. The diagrams in Fig. 1 may be obtained depending on the type of flow through the reactor (Schoenemann, 1952; Danckwerts, 1954).

Equations describing the spread of residence times of particles in one or multistage homogeneous tank systems of equal and unequal volume were developed by Mason and Piret (1950). Tubular systems, however, offer more complicated mathematical problems. Some theoretical treatment of these systems is given by Danckwerts (1953). The spread of residence times for any continuous system can also be determined experimentally by injecting a marker into the incoming stream of the reactor and determining the fractions leaving the reactor at different time intervals. We found it convenient to inject a small quantity (1 to 2 $\mu $g) of $\gamma$-ray emitting material of appropriate half-life ($Fe^{59}$) into the incoming stream of reactor systems. The resulting spread of the tracer can then be followed with a scintillation counter along the system and in the outcoming stream without disturbing the equilibrium in the reactor due to multiple sampling. Typical results obtained from a novobiocin1 fermentation in a four-stage completely mixed tank system are shown in Fig. 2.

Efficiency of Different Reactor Types

The spread of residence times of different particles is of importance in determining the performance of a

1 The trade-mark of The Upjohn Company for novobiocin is Albamycin.
given reactor. The spread of residence times among particles will be zero if the flow through the reactor is truly piston-like. In that case the extent of performance will be the same as for a batch reactor operated over the time $T'$. However, if the spread of residence times among particles is significant, a part of the reactor will be occupied by reactants which have been present for a time considerably longer than $T'$, while some reaction mixture left at a time less than $T'$ and the reaction in it was not completed. The effective operating volume of the reactor will therefore be smaller than the corresponding volume of a batch reactor operating over the same period of time $T'$.

Danckwerts (1954) points out that for optimal output, an ideal piston flow reactor with no spread of residence times will be superior in its performance to a homogeneous reactor if the reaction rate of the particular reaction under study decreases with concentration. In a completely mixed reactor, the reaction rate will be equal to the minimal reaction rate in a batch or piston flow reactor operated for the same extent of conversion (see Fig. 3). The average reaction rate of a batch or tubular reactor indicated by the slope of $A$ is greater than the reaction rate for a completely mixed tank as indicated by slope $B$. The concept will reverse if the reaction rate increases with concentration, as in autocatalytic processes (see Fig. 4). The average reaction rate of a batch or tubular reactor indicated by the slope of $A$ is then smaller than the reaction rate for a completely mixed tank as shown by the slope of $B$ for the same extent of completion of reaction. This shows that in the case of an autocatalytic process the output per unit reactor volume of a completely mixed reactor will be superior to that of a batch or tubular type.

Methods of Predicting Yields in Continuous Flow Reactors

The yield to be expected in completely mixed tanks as well as tubular systems is determined primarily by the spread of residence times of particles in the system, the reaction kinetics of the particular process and the holdup time of the system. If the spread of residence times of particles and the reaction kinetics of the batch process can be expressed in mathematical form, then the yield of product can be calculated by substituting these values in the following mass-balance equation:

$$\text{Rate of input} + \text{rate of increase} = \text{rate of formation} - \text{rate of output}.$$ 

If such differential equations offer a solution, yield can be calculated. However, such equations do not always offer a simple mathematical solution but once steady-state conditions have been approached in a continuous system it is evident that the rate of increase of a given component in the system tends to become zero. In that case, the differential equation may be reduced to a simple algebraic expression which has a solution applying to the steady-state, even when the differential equation for the transient state offers no solution. In cases where neither the course of a batch reaction nor the distribution of residence times of particles for a given reactor can be expressed in a closed mathematical form, numerical or graphical solutions are usually available.

The following data have to be procured for a graphical solution (see Fig. 5) (MacMullin and Weber, 1935; Schoenemann, 1952):

1) Course of a batch process for a particular reaction.
2) Establishment of the spread of residence times curve of a tracer flowing through the same reactor. Note that the particular reaction mixture under study has to be used for this experiment.

Some notations:

$T = \text{time required for 100% completion of reaction in a batch}$

$t = \text{sampling time}$

$P = 100\% \text{ completion of reaction at time } T$

$p = \text{per cent completion of reaction at time } t$

$C = \text{total concentration (100%) of tracer added to the system at time } t = 0$

$c = \text{concentration of tracer still in system at time } t$
The course of the batch reaction conducted in the reactor is plotted in relative values as \( p/P \) on the ordinate vs. \( t/T \) on the abscissa as shown in Fig. 5. On the lower side of the same abscissa, the proportion of particles still in the system \( (c/C) \) vs. \( t/T \) is also plotted for the same time period. The proportion of particles still present at \( t_0/T \) correspond to a certain amount of product formation (e.g., in Fig. 5) 40% of the particles are still present at time \( t_0/T \), thus reacting to an extent from 0 to 68%. A number of corresponding values for \( p/P \) and \( c/C \) are then plotted on a second graph as shown in Fig. 6, with \( p/P \) as ordinate and \( c/C \) as abscissa, thus eliminating time. The different points connected together will yield a curve where the area below the curve represents the steady-state yield to be expected in the outlet of the system. Its numerical value is obtained by graphical integration using Simpson's rule or the trapezoidal rule (Granville, Smith, and Longley, 1946).

This method applies only to reactions where the rate decreases with concentration. It does not apply to autocatalytic reactions such as microbial growth since the reaction rate in such processes is proportional to the amount of product present in the system.

All continuous processes may have some particular implications not common to batch reactions: As a consequence of intermixing of different reaction phases, reactants, and transient and end products are present simultaneously in substantial and constant amounts once steady-state conditions are reached. This may have implications due to the fact that competing side reactions may take place within the reactor. The same phenomenon may also have adverse effects on reaction equilibria, exercise inhibitory effects in biological processes, or have other effects on the desired reaction. In such cases, batch data are of limited use at best for predicting yields in continuous reactors.

**Application to Design of Continuous Antibiotic Fermentation Units**

**General Considerations**

The production of most antibiotics includes at least two successive steps: (i) the production of microbial cells and (ii) the biosynthesis of the antibiotic by the cells after a certain time lapse. Since the growth process of microorganisms is autocatalytic, the most effective reactor for cell production will be a completely mixed fermentor. The second step, the formation of the antibiotic by the cells, is an indirect or pseudoautocatalytic reaction since it lags cell formation in respect to time. For most efficient operation, the second step should therefore be conducted in a tubular reactor with no spread of residence times, due to the implication that in completely mixed tanks and tubular reactors with some longitudinal intermixing, a substantial portion of cells will leave the system before they have a chance to contribute to antibiotic formation, whereas a substantial portion of cells remain in the system for a time period much longer than necessary for the elaboration of the antibiotic. This will reduce the actual output of the vessel in comparison to a batch or true piston flow reactor having the same volumes and operating over the same period of time.

The most efficient reactor system to conduct antibiotic fermentations should therefore be a combination of a completely mixed tank (cell generator) and a piston flow reactor (antibiotic formation). This proposed sequence of reactors should yield a higher output per unit reaction volume than either of these types alone. However, the volumes of both reactors have to be kept in proper proportions to maintain the holdup time at an optimum for both reaction phases. Since it is impossible to construct a truly piston flow reactor, the equations developed by Mason and Piret (1950) imply that such flow conditions are conveniently approxi-
mated using a completely mixed, multistage system with a large number of vessels of equal size. If vessels of irregular sizes are used, the spread of residence times of the cells in the system will increase and yields will decrease accordingly.

**Prediction of Yields in Continuous Antibiotic Fermentation Systems**

Due to the complexity of such reactions both reaction steps discussed above are conveniently analyzed by two different graphical systems according to their different kinetics:

Cell growth. Since cell growth is autocatalytic, cell proliferation should be limited chiefly to the first stage of a multistirred tank system to insure most efficient operation of this phase of the process. To achieve this, a point relatively high along the cell growth curve should be chosen for operation as indicated by A in Fig. 7. The holdup time necessary for maintenance of the desired cell density can be determined graphically by the method of Luedeking and Piret (1960).

Antibiotic formation. The antibiotic level in the first stage will be relatively low and the spread of residence times of the cells leaving this stage relatively narrow and can therefore be neglected. The approximate antibiotic titer to be expected in the cell generator is indicated graphically at the point of intersection B (see Fig. 7) where a straight line drawn perpendicular to the abscissa through point A intersects the antibiotic formation curve. This value will be slightly too high due to the negligence of the spread of residence times of the cells in the first stage. Since by operational means growth is limited essentially to stage 1, antibiotic formation in any subsequent stages will be primarily a function of the spread of residence times of the mycelium in the remaining stages.

The yield to be expected in these stages can therefore be determined by applying the graphical method of MacMullin and Weber (1935) and Schoenemann (1952) as indicated in Fig. 7.

**Application to Novobiocin Fermentation**

This method of predicting yields was applied to the novobiocin fermentation. The basic batch data for these calculations are assembled in Fig. 7.

Cell formation. The appropriate holdup time for the maintenance of a cell concentration of 85% of the maximal possible batch value in the first stage as indicated by A in Fig. 7 was determined as outlined in Fig. 8. The slope of the straight line passing through the origin and A had a numerical value of 0.0417 hr⁻¹, thus corresponding to a holdup time of ~24 hr for the first stage.

Novobiocin formation. The novobiocin level to be expected in the first stage should be approximately 29% as indicated by B in Fig. 7. Novobiocin yields to be expected in the subsequent antibiotic production system were calculated graphically as outlined in Fig. 7. It was of interest to compare different antibiotic production systems which have the same total volume (Vₖ) and hence the same total holdup time. Therefore, novobiocin yields for a one-vessel system = Vₖ; a two-vessel system, each with volume Vₖ/2; a nine-vessel system (Vₖ/9 per vessel) and an ∞ vessel system (Vₖ/∞ per vessel) were calculated, assuming completely mixed conditions within each vessel. Note that the cell generator (stage 1) was excluded in these numbers of vessels. The spread of residence times curves were constructed using the equations of Mason and Piret (1950).

![Graphical determination of the holdup time necessary for the maintenance of a cell density of 88% of the maximal batch value.](http://aem.asm.org/)

**FIG. 7.** Batch data of a novobiocin fermentation
Results were assembled in Fig. 9 and 10. Figure 9 shows a plot of the fraction of completion of the reaction to be expected with different reactor systems (antibiotic formation phase) and holdup times. Figure 10 shows a plot of productivities of the different systems vs. holdup times for the over-all process (cell generation and antibiotic formation). For comparison, the corresponding data of a batch reaction were also included. The productivities of all continuous systems were calculated on the basis of steady states. The transient state was not taken into account. The productivities shown in Fig. 10 should, therefore, be slightly lower, depending on the length of the transient state during startups and the total time the operation is expected to run before shutdown. Figure 10 indicates that the productivities of continuous novobiocin fermentation systems vs. batch systems will decrease as the holdup time increases. At short holdup times, productivities will be extremely high, whereas the product concentration in the outlet will be accordingly low, which is uneconomical for subsequent filtration and extraction operations.

Figure 10 indicates that productivity increases as the fixed total volume of the antibiotic formation stage is divided into an increasing number of individual small vessels of equal size in an effort to reduce the spread of residence times of the cells. However, the resulting increase in productivity is progressively diminishing with increasing number of vessels for a fixed holdup time as illustrated in Fig. 11. Therefore, a subdivision of the fixed total volume of the antibiotic formation stage in more than one (short holdup times) or two vessels (longer holdup times) is not rectified economically in the case of the novobiocin fermentation.

**Discussion**

The results indicate that the most economical reactor system to conduct continuous novobiocin fermentations would be a combination of a cell growth stage and one or two antibiotic production stages. Holdup times and hence volumes of cell generator vs. antibiotic production stages will have to be kept in proper relation given by economic considerations to keep productivity and absolute titer in the output at a proper equilibrium to insure maximal efficiency of the unit.

In principle the above described method can also be applied to continuous steroid bioconversions.

We wish to stress the approximate nature of the presented method of predicting yields in continuous flow reactors on the basis of performances of batch reactors. As a consequence of varying degrees of intermixing during continuous operations, reactants, intermediates, and end products will be present in substantial constant amounts once steady-state conditions are reached.
This may lead to the occurrence of unwanted side reactions, affect reaction rates, or exercise inhibitory or toxic effects in biological processes. Under such conditions, batch data will be of very limited use for predicting the performance of continuous reactors.

However, the method described may still prove very helpful for the initial design and operation of pilot units and the preparation of preliminary cost estimates. Experimental verification of the theoretical results will ultimately be required.

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LITERATURE CITED


Continuous Fermentation of Novobiocin

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ABSTRACT

REUSser, F. (The Upjohn Company, Kalamazoo, Mich.). Continuous fermentation of novobiocin. Appl. Microbiol. 9:366-370. 1961.—Continuous fermentation trials with Streptomyces niveus in a nine-stage fermentation system (7-liter reaction volume per stage) indicated that the cultures used gradually lost their ability to produce novobiocin when cultured over periods from 10 to 25 days. It was found that mycelial degeneration could be circumvented by operational means during continuous culture using the following technique: Two interchangeable 24-liter stages were installed at the front end of the nine-stage system and connected in parallel with the latter. Alternately one of these two tanks was then used as first stage of the continuous fermentation system. The holdup time in the first vessel was adjusted to limit cell growth chiefly to this stage so that most of the antibiotic production took place in subsequent stages. The first stages were switched at approximately weekly intervals. Each of the new tanks was prepared as a batch, inoculated with a high-producing cell population, and allowed to grow for 3 days before it was connected to the remaining system for continuous operation. Using this technique no evidence of culture degeneration was encountered in subsequent novobiocin production stages over a period of 33 days. In conventional runs without periodic replacement of the first stage, culture degeneration with the novobiocin fermentation occurred within a period of 10 to 25 days of continuous operation. This observation indicates that the described technique offers a solution to the problem of maintaining high steady-state titers in continuous novobiocin fermentations. Extension of this technique to other continuous fermentations where culture degeneration is a problem is indicated.

An excellent review of the current pertinent literature and status of continuous antibiotic fermentation processes, by Gerhardt and Bartlett (1959), indicates that, although several laboratories are known to have investigated the feasibility of continuous antibiotic fermentations, few data have been published to date.

The main problem in the development of successful continuous antibiotic processes involves culture degeneration. Most industrially important antibiotics are produced by polynucleate microorganisms (Streptomyces, Penicillium) where the maintenance of a high-producing genetical composition within a given population upon repeated transfer has proved very difficult.