Fermentation Kinetics and Model Processes

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In the conception of this symposium it was intended that fermentation kinetics and continuous fermentation be treated as one subject. After some discussion, principally among Dr. Maxon and the chairmen, it was decided to split the intended subject into two parts, (*1*) fermentation kinetics," particularly as they relate to batch processes, and (*2*) continuous fermentation." One of the objectives of this symposium is the critical review and summary of an area in fermentation engineering. In separating fermentation kinetics from continuous fermentation, an area almost lacking in basic knowledge is extracted from the area in which most of its limited knowledge has been applied. The papers that deal either specifically or primarily with a kinetic analysis and interpretation of batch fermentation processes are very few indeed. In order to make this presentation a more challenging task, occasional resort will be taken to some studies in continuous fermentation. For the most part, however, reference shall be made to the now better developed fields of chemical kinetics and enzyme catalysis.

Previous Batch Kinetic Studies

Studies of batch fermentation processes in nearly all development programs involve periodic observations of growth, carbohydrate utilization, and product formation throughout the course of the fermentation. Kinetic analysis is the interpretation of these observations and the factors which influence them, in order to throw light on proposed reaction schemes or fermentation patterns. Such analyses carried out to date have followed mainly three avenues of approach—phenomenological, thermodynamic, and kinetic.

Phenomenological approach. Gaden (7) has made use of such observations to classify fermentation processes into three broad types based on a comparison of specific reaction rates, i.e., rates per unit weight of cellular tissue, and their relationship to carbohydrate utilization. This classification and the example processes cited are shown in Table I. Maxon (18), at the same time, chose to classify fermentations based on the relationships between growth rate and rate of product formation, as shown in Table II.

TABLE I

EXAMPLE TYPES OF FERMENTATION PROCESSES ACCORDING TO GADEN (7)

Type	Specific rate relationships	Example
I	Product formation directly related to carbohydrate utilization	Ethanol
II	Product formation indirectly related to carbohydrate utilization	Citric Acid
III	Product formation apparently not associated with carbohydrate utilization	Penicillin

TABLE II

EXAMPLE TYPES OF FERMENTATION PROCESSES ACCORDING TO MAXON (18)

Type	Specific rate relationships	Example
I	Product formation and growth are synonymous	Propagation of microorganisms
II	Product formation associated with growth	Ethanol
III	Product formation not associated with growth	Penicillin

These appear to be the first notable suggestions of logical kinetic examinations of fermentation processes. Gaden (8) later divides fermentations into classes as follows: (*1*) cell propagation, (*2*) direct metabolic products, and (*3*) indirect metabolic products. Because of their phenomenological basis all these classifications are somewhat broad. The technique of plotting specific reaction rates, nevertheless, provides a powerful analytical tool. Its application was amply demonstrated recently in the lactic acid fermentation studies of Luedeking (15).

Thermodynamic approach. Thermodynamics was the basis of the approach taken by Calam *et al.* (2) in their study of the penicillin fermentation, carried out to determine if a relationship existed among growth, respiration, and biosynthesis. From experimental measurements of fermentation rates they calculated the activation energies of the rate-determining step in the reaction networks involved in each of these metabolic functions. Based on the three different activation energies found, the three functions were claimed to involve different enzyme systems. A certain amount of care must be exercised in interpreting such thermodynamic data. While they provide strong presumptive and useful evidence, this evidence is not in itself conclusive.

Kinetic approach. Truly kinetic approaches have been suggested and applied in batch fermentation studies only recently. Luedeking and Piret (16) and more recently Deindoerfer and Humphrey (4) showed that cer-

tain fermentations having simple kinetic and stoichiometric relationships were, in theory, suited for predictable continuous multistage operation. In the first actual kinetic approach to a fermentation of more complicated nature, Luedeking (15) examined in detail the homofermentative production of lactic acid by *Lactobacillus delbrueckii*. He showed that the formation of lactic acid was related to both a growth and a nongrowth phase by an expression of the form

$$dC/dt = x\ dM/dt + y\ M \tag{1}$$

where C is product concentration, M is concentration of cell mass, t is time, and x and y are parameters which are functions of pH. Notice that if either x or y is zero, and if the rate of product appearance is directly proportional to the rate of limiting nutrient disappearance, the kinetic requirements of either a nongrowth- or growth-type simple fermentation process defined by Deindoerfer and Humphrey (4) are met, respectively, as shown by Eqs. (2) and (3)

$x = 0$ (nongrowth)

$$dC/dt = y\ M = -z\ dN/dt \tag{2}$$

$y = 0$ (growth)

$$dC/dt = x\ dM/dt = -z\ dN/dt \tag{3}$$

where N is the concentration of substrate or limiting nutrient concentration. Thus, the lactic acid process represents a fermentation which appears to be the combination of two types of simple processes.

The Kinetic Parameter

The crux of any kinetic analysis lies in determining how the rate of product formation and its stoichiometric coefficient vary with respect to the chemical and physical factors influencing them. Meaningful quantitative knowledge here is practically lacking in all fermentations except those involving tissue synthesis alone. Fortunately the physical factors known to affect the kinetics and stoichiometry of a fermentation, to a reasonable degree, can be held constant. Actually, then, the only factor that is of concern under such circumstances is nutrient concentration. A kinetic parameter, i.e., reaction rate for product appearance, can then be defined as follows:

$$k(N) = z\ dN/dt \tag{4}$$

When the product is cell tissue, z/x is the yield constant defined by a number of other workers.

Expressions for the kinetic parameter. For the case of unicellular growth, the rate of growth can be expressed in terms of a limiting nutrient concentration:

$$dM/dt = k(N)\ M \tag{5}$$

In this instance the kinetic parameter is the specific growth rate of the microorganism, i.e.,

$$k(N) = (dM/dt)/M \tag{6}$$

In the development of his theory for bacterial growth limited by a single essential nutrient, Monod (19, 20) relates the kinetic parameter to the limiting metabolite in the following manner:

$$dk/dN = \alpha(1 - k) \tag{7}$$

Monod proposes as an approximate solution to this equation the expression

$$k(N) = k_m N/(K + N) \tag{8}$$

This expression is interesting because of its resemblance to the familiar hyperbolic rate equation for a substrate-limited enzyme reaction.

Another expression which is a more general solution of Eq. (7) was proposed by Teissier (27) and also adopted by Spicer (25):

$$k(N) = k_m\ (1 - e^{-N/K}) \tag{9}$$

When N/K is small, Teissier's expression is approximately equal to that of Monod. Both of these expressions illustrate the general relationship that, up to a certain value of N, the specific growth rate is a linear function of the limiting nutrient concentration, as shown in Fig. 1. When N is large the specific growth rate approaches a maximum value, k_m.

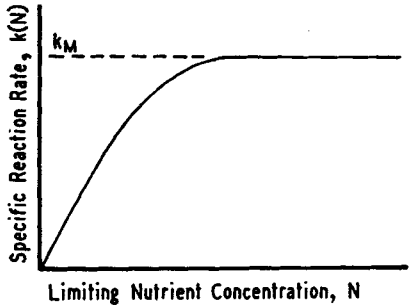

FIG. 1. Dependency of the specific growth rate on the limiting nutrient concentration.

An even more general, but empirical, equation based on the observations just mentioned was suggested by Moser (21):

$$k(N) = k_m/(1 + KN^{-B}) \tag{10}$$

When the exponent, B, is one, this equation also reduces to that of Monod (19, 20). Other expressions which relate the specific growth rate to toxic product accumulation or to space limitations have also been developed, but they will not be considered at this time.

Monod's equation, Eq. (8), has found widespread application in relating population density and limiting nutrient concentration in the continuous culture of unicellular microorganisms in chemostat- and bactogen-like apparatus. It has even been suggested for use in continuous fermentations. It should be remembered, however, that this equation, as well as the others described, relate only the effect of a single limiting nutrient on the specific growth rate. Usually in most industrially important fermentation processes, conditions of a single limiting nutrient are not maintained. Monod's equation applies to these fermentations only under the special circumstances where a hyperbolic rate equation is justified.

Theoretical basis for hyperbolic rate equation. Since the over-all rate of a network of consecutive reactions is controlled by the slowest, or rate-determining step, a formula which reflects in its form a single-step enzymatic rate expression lends some theoretical support to Monod's equation. Indeed, such a formula can be derived for a series of reactions in which binary enzyme-substrate complexes are formed. However, not all enzymatic reactions are as simple as the familiar Henri scheme, popularized by Michaelis and Menten and later expanded by Briggs and Haldane to include the nonequilibrium situation. One might conjecture, by analogy with other rate expressions for more complicated enzymatic reaction schemes, what conditions must be met for the hyperbolic rate expression of Monod to apply.

Laidler and Socquet (14) derived a rate equation for a two-substrate reaction in which each substrate locates independently on its respective neighboring site on the enzyme. Using terms consistent with the nomenclature of this paper, their equation would yield by analogy an expression of the form

$$k(N) = \frac{k'N_1N_2}{(1 + K_aN_1)(1 + K_bN_2)} \tag{11}$$

This equation reduces to the simple hyperbolic form only when one substrate concentration is maintained constant, or when one substrate is sufficiently in excess of the other. Segal *et al.* (24) also consider a similar

ternary complex formation but with an ordered sequence of substrate addition, and their equation is of the form

$$k(N) = \frac{k'N_1/(1 + K_a/N_2)}{\dfrac{K_c(1 + K_bN_2)}{(1 + K_a/N_2) + N_1}} \tag{12}$$

Here, too, the equation reduces to hyperbolic form if the second substrate is maintained constant. The equation of Chance (3) for peroxidase kinetics involves the reaction of an oxygen acceptor with a binary complex followed by immediate dissociation. The resulting expression also reduces to hyperbolic form under certain conditions.

$$k(N) = \frac{k'N_1N_2}{(K_a + K_bN_2) + N_1} \tag{13}$$

These are but a few examples of the types of enzyme reactions which reduce to hyperbolic form. They can be extended to include reactions with hydrogen ions, and with inhibitors and products, as shown by Hearon, *et al.* (10).

Importance of adequate control. One purpose of the remarks dealing with rate expressions has been to emphasize the need for careful control in kinetic studies. To relate the kinetic parameter to the concentration of a limiting nutrient requires that the other nutrient concentrations hidden in the simplified rate expression are adequately controlled or in sufficient excess so as not to affect the specific reaction rate. The correlation of growth rate with a hyperbolic rate expression without proper pH control appears unwise. Also, in aerobic systems, if oxygen is not maintained in excess, the kinetic parameter becomes a function of more than one limiting nutrient, or very likely a function of oxygen concentration alone. Continuous culture experiments whose steady-state behavior is explained on the basis of a hyperbolic rate equation unfortunately do not provide convincing evidence that the restricted experimental conditions necessary are met. An interesting observation here is the phenomenon of cell washout which occurred in the studies of Herbert *et al.* (11) as high throughput rates were used. As the throughput rate approached the maximum allowable as indicated by the logarithmic growth rate, population density decreased drastically. The question must be asked—was this washout due to instability in the minimum generation time, or was it due to a lowering of the growth rate due to an oxygen concentration less than that required to maintain a hyperbolic rate expression?

Fermentation Classification and Model Processes

Returning now to the more general problem of fermentation kinetics, a more refined classification for categorizing fermentation processes will be

TABLE III

CLASS OF FERMENTATION TYPE REACTIONS

Type	Description
Simple	Nutrients converted to products in a fixed stoichiometry without accumulation of intermediates
Simultaneous	Nutrients converted to products in variable stoichiometric proportion without accumulation of intermediates
Consecutive	Nutrients converted to product with accumulation of an intermediate
Stepwise	Nutrients completely converted to intermediate before conversion to product or Nutrients selectively converted to product in preferential order

suggested. The basis for this classification is a series of typed over-all reactions. Using these reactions and the background available in chemical kinetics, several model processes are suggested for studying such formulations. This classification method has the advantage that it can be described by the simple volumetric observations made during the course of most fermentations and is flexible enough to cover all cases. The type reactions and their definitions are listed in Table III.

Simple reactions. The first type reaction is the simple one already used to categorize simple fermentation processes. The kinetics of two subtypes, growth and nongrowth reactions, are shown in Figs. 2 and 3. Model processes for these reactions, are the growth of yeast and bacteria, and the biooxidation of gluconic acid employing mycelial recycle.

Simultaneous reactions. Simultaneous reactions are those in which more than one product is produced and the relative rates of production of these products vary with nutrient concentration. They involve "overflow" or "shunt" metabolisms of the type suggested by Foster (6). Holme (12) has demonstrated such effects in a series of papers in which the formation of glycogen and various nitrogenous compounds was examined during continuous cultivation of *Escherichia coli* in a deficient medium. Ergosterol and fat accumulation in microorganisms are other examples of this type reaction. Figure 4 shows some results of simultaneous cell protein and cell fat synthesis during the growth of *Rhodotorula glutinis.*

Consecutive reactions. Consecutive reactions are those in which an intermediate accumulates to some degree before product is formed, as shown in Fig. 5. Antibiotic formation in a number of fermentations very likely falls into this type, involving the accumulation of one or more nonactive intermediates. However, because of simultaneous growth, antibiotic fermentations as a whole are more complex. The studies of Mateles and Fuld (17)

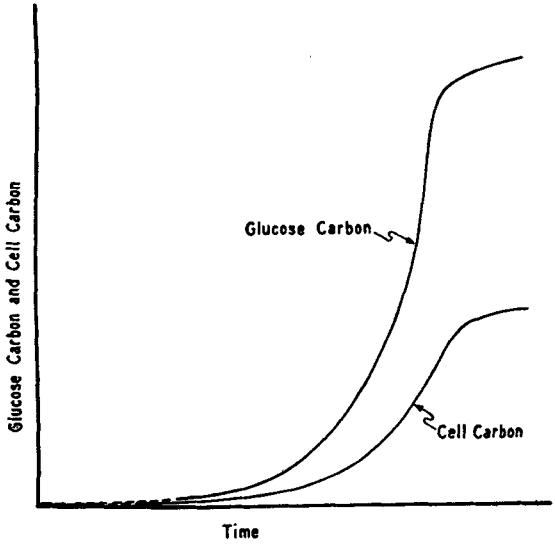

FIG. 2. Simple growth of *Aerobacter cloacae*. [From Pirt (23).]

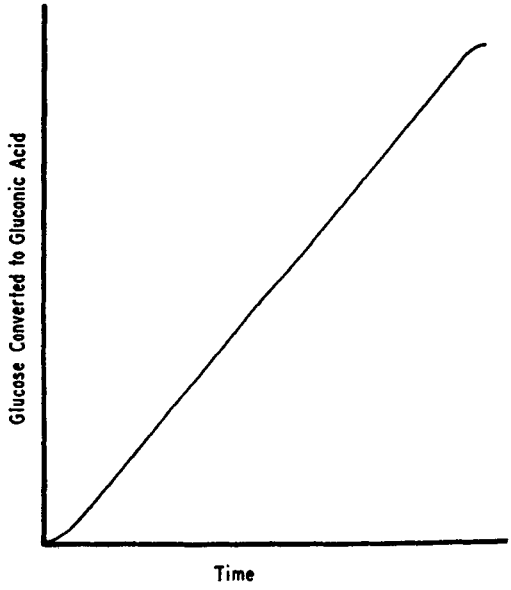

FIG. 3. Simple conversion of glucose to gluconic acid by resuspended *Aspergillus niger* mycelia. [From Moyer *et al.* (22).]

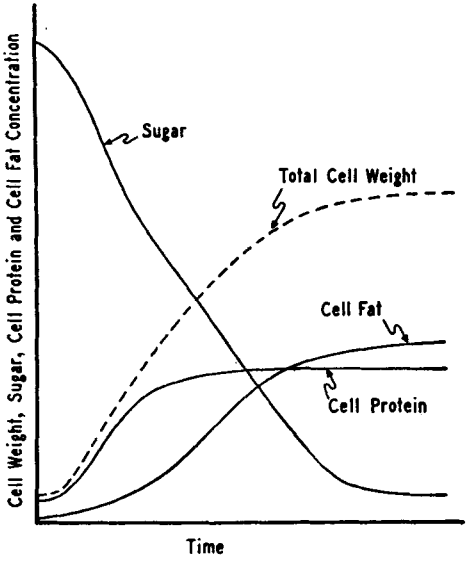

FIG. 4. Simultaneous conversion of sugar into cell protein and cell fat during *Rhodotorula glutinis* growth. [From Enebo *et al.* (5).]

have indicated consecutive reactions in the conversion of progesterone to 11α-hydroxyprogesterone and thence to 6β,11α-dihydroxy progesterone by *Aspergillus ochraceus.*

Stepwise reactions. The simple, simultaneous, and consecutive reactions just mentioned are fully describable by conventional chemical reaction kinetics. Before proceeding to the complex case where a combination of such mechanisms come into play, a type of reaction unique to microbiological processes must first be examined. This is the stepwise reaction which accounts for the phenomenon of enzyme selectivity and adaptation. Examples of this reaction and its characteristics are shown in Figs. 6 and 7. In one case, that of two carbohydrate sources such as glucose and sorbitol, *Escherichia coli* first utilizes completely the sugar before beginning to consume the polyalcohol. Monod (20) calls this type reaction "diauxie." Another example is the biooxidation of glucose to 5-ketogluconic acid by *Acetobacter suboxydans.* Here all the sugar is converted to gluconic acid before ketose formation begins. Similar reactions occur in the multipoint but stepwise attack of the steroid nucleus by some microorganisms. Obviously, these multiphasic reactions are completely different from any occurring in nonbiological systems.

Complex cases—a combination of type reactions. Most fermentation processes involve a combination of the type reactions just described. Their

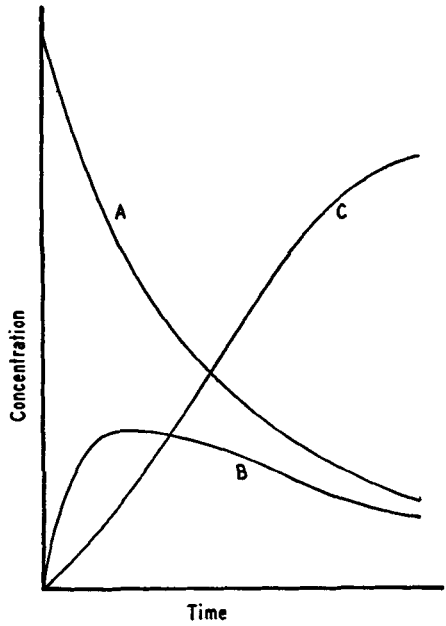

FIG. 5. Consecutive reactions, A → B → C. [From Glasstone (9).]

complexity can obviously vary tremendously. An examination of the fermentation patterns in the pencillin process suggests a number of type reactions. The growth curve in Fig. 8 is typically diphasic. Sugar utilization in this case was probably determined by its rate of addition. Unfortunately, no analyses are available to check whether or not sugar accumulation occurred. The pencillin production curve also exhibits a diphasic character and lags the growth curve. Curves such as this do appear difficult to interpret, but until a thorough and detailed interpretation is earnestly attempted, they should not be dismissed as overly complex. The figure certainly suggests the accumulation of an intermediate product. This, as is now well known in the penicillin fermentation due to the findings of Batchellor *et al.* (1), is 6-amino penicillanic acid and explains the long recognized need for precursor addition during the course of the fermentation.

Future Applications

The last example cited suggests the analytical potential of kinetic analysis. Information such as this, coupled with biochemical evidence, provides the basis for a sound study of fermentation mechanisms. The important implications of an understanding of these mechanisms are many. The possible establishment of a need for a precursor and its optimum point and

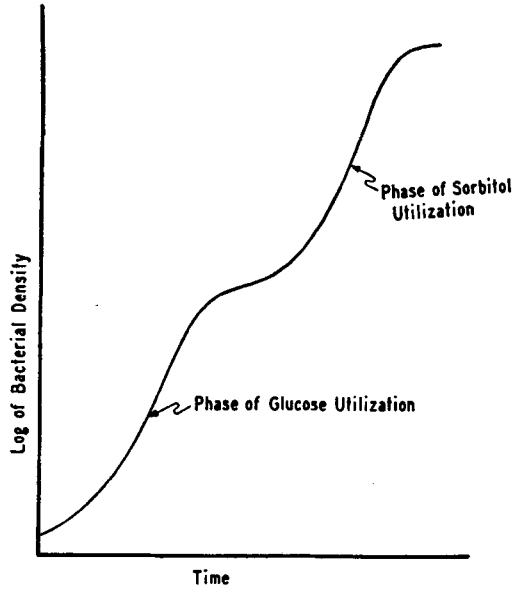

FIG. 6. Diphasic growth of *Escherichia coli*. [From Monod (20).]

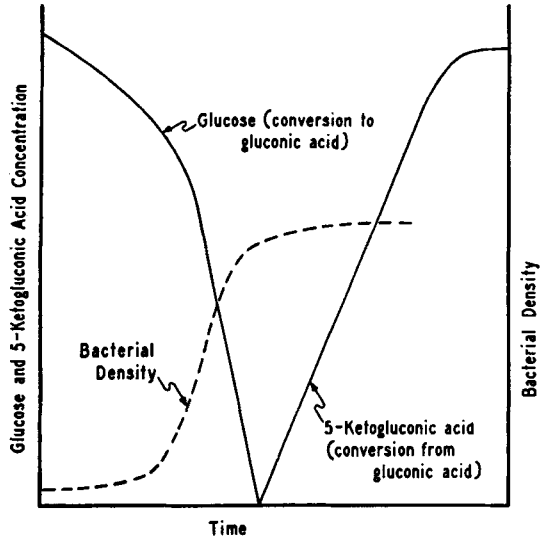

FIG. 7. Diphasic biooxidation of glucose to 5-ketogluconic acid by *Acetobacter suboxydans*. [From Stubbs *et al.* (26).]

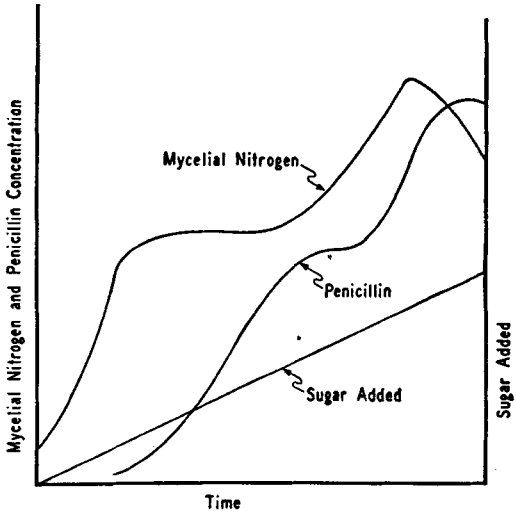

FIG. 8. Penicillin production in synthetic medium with continuous glucose feed (concentrations not to same scale). [From Hosler and Johnson (13).]

rate of addition is just one example. By understanding the appearance and disappearance of important signal components in a fermentation and the factors that influence them, not only can successful multistage systems be designed, but the productivity of the batch fermentation itself can be improved by a program of control which optimizes the individual rate-determining reactions occurring during the course of a fermentation. There is no reason whatsoever why batch fermentations should be run at constant temperature, constant pH, etc., when very likely a controlled variation of these influencing factors might give improved yields. This is an area of fermentation process development which has not received the attention it deserves. But this apparently goes hand in hand with the lack of knowledge of the effect process variables have on kinetic parameters.

Criticism will undoubtedly be raised concerning the amount of analytical work and time for interpretation required for the extensive kinetic undertakings which are suggested. Just a few years ago this criticism would have been justified. However, methods are available today which can cut the analytical work load in such studies to less than that presently expended in fermentation development. These methods include the use of automatically operated instruments, which employ such continuous techniques as vapor phase chromatography for analyzing volatile components in a fermentation, and wet chemical colorimetry and turbidimetry for analyzing nonvolatile constituents. Such commercially available instruments

can greatly increase the amount of information available from fermentations at a very moderate investment.

The interpretation of the large amount of data needed in kinetic studies also is no longer as difficult and tedious a task as might be expected. There are available today electronic analog computers on which reaction schemes can be set up and plotted out for a number of signal components for numerous parametric variations and in a rapid manner. These plots can be compared visually with the observed patterns in a fermentation to see how well the theory fits the actual results obtained.

Summary

Fermentation kinetics has been, until now, a little explored area. Because of this, the comments that have been made are more suggestive than they are conclusive. The tools are on hand, however, to carry out a penetrating analysis on how various factors influence fermentation rates and process yields. A sound and thorough study which combines the fermentation engineer's knowledge of reaction kinetics and their interpretation, the fermentation biochemist's knowledge of the chemistry of the various reactions which occur, and the microbiologist's knowledge in growing and maintaining the active cell tissue which provides the catalytic material for the reactions involved, can easily lead to one of the most substantial technological advances the fermentation industry has ever made.

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