

Fermentation Process Kinetics*

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Abstract: Information on fermentation process kinetics is potentially valuable for the improvement of batch process performance; it is essential for continuous process design. An empirical examination of rate patterns in various fermentations discloses three basic types: (1) 'growth associated' products arising directly from the energy metabolism of carbohydrates supplied, (2) indirect products of carbohydrate metabolism and (3) products apparently unrelated to carbohydrate oxidation. Effects of operating variables on the primary kinetic processes, growth, sugar utilization and antibiotic formation, in the penicillin process, illustrate the special nature of this type.

INTRODUCTION

In the design of any chemical, or biochemical, process one must consider two more or less distinct aspects. First, there are the chemical reactions themselves and secondly, the numerous physical processes which precede, accompany and follow them. Some of these physical processes are quite clearly separate, like the purification of raw materials and products. Others, like the transport of materials to and from the surface of a solid catalyst, are intimately bound up with the reactions themselves.

For a long time, methods available for dealing with the physical aspects of chemical processes were better developed than those for handling the chemical changes themselves. This was largely the result of empirical simplifications offered by the 'unit operations' concept in chemical engineering. With the rapid development of chemical kinetics and, equally important, methods for applying kinetic relationships to process design, this disparity has been overcome.

Kinetics is concerned with reaction rates in general; 'process kinetics' simply suggests a primary concern with the rates of commercially practised reactions and, particularly, with the effects of process variables on them.

Since fermentation is only another type of chemical process, albeit a special and complex one, possibilities for applying ideas and techniques developed for more conventional chemical systems should always be sought. This is

especially true for kinetics. Although the study of fermentation rates is relatively new, it promises much for the fuller and more efficient exploitation of biochemical reaction systems.

Development of Fermentation Kinetics

Final product yields and substrate conversions were the only criteria of performance in early commercial fermentations. As the technology developed, however, greater attention was paid to time factors; 'productivity', the average rate of product formation (Fig. 1), soon became popular as a basis for comparison. On the other hand, instantaneous rates were largely ignored until the studies of gluconic acid production by Wells, Moyer, Gastrock et al.^{12,19,20,26} in the late 1930's. They were among the first to report rates of sugar utilization and acid formation in detail.

The introduction of antibiotic fermentations greatly stimulated interest in fermentation rates. It was recognized from the first that these processes were markedly different from most earlier fermentations. Studies of the chemical changes in penicillin biosynthesis required frequent analysis of carbohydrate and nitrogen levels, cell weight and antibiotic titre. From these, general rate patterns could be discerned and it was soon noted that the process comprised two more or less distinct phases; growth and antibiotic production.

Dulaney et al.,⁸ noticed the same general behaviour in streptomycin fermentations. They defined an initial 'growth phase' in which mycelium was rapidly generated, accompanied by a reduction in soluble medium constituents (carbon, nitrogen, phosphorous), rapid sugar utilization and high oxygen demand. Virtually no streptomycin was produced. Following this was an 'autolytic phase', characterized by a marked drop in mycelial weight, release of nitrogen and inorganic phosphorous to the medium, low oxygen demand and rapid antibiotic synthesis. All strains examined exhibited the same basic pattern and gross medium changes had little effect on it.

Calam, Driver and Bowers⁶ were among the first to support these general observations with specific experiments. Penicillin fermentations were carried out at several temperatures between 12° and 32°C and average rates of growth, respiration and penicillin synthesis noted. By plotting the observed rates in the Arrhenius manner (logarithm of rate versus reciprocal absolute temperature) it was possible to

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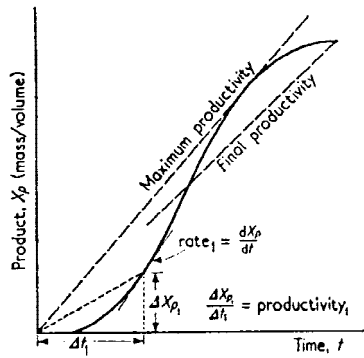


Figure 1. Fermentation rates and productivity.

characterize each process by the slope of the line obtained, the 'thermal increment'. Since these three rates all exhibited significantly different thermal increments, the authors concluded that the 'pace-setting enzyme-systems' involved are different.

Any survey of the literature on fermentation rates underscores the dearth of direct kinetic studies of this type. One cardinal reason for this is the matter of experimental procedure itself. Rate information can best be obtained in steady-state (continuous) systems with automatic control of process variables.

In an excellent example of this approach, Kempe, Gillies and West¹⁵ studied rates of acid production by *Lactobacillus delbrueckii* at controlled pH. Rates were determined by differentiating the automatically recorded curve of alkali addition. Steady-state operation at various temperatures provided values for an Arrhenius-type plot which gave an activation energy of 17 kcal/g mole, a value in the range characteristic of many chemical reactions.

For one reason or another satisfactory methods for automatic regulation and control in fermentation studies have only recently been introduced and most experiments so far reported involve the classical batch technique. Data which permit the computation of rates are rare—and often inadequate because of the absence of key values. Of course the aim of these experiments was yield improvement in batch processes, not the gathering of kinetic data. Still, despite the inherent limitations of the unsteady-state, batch technique, a surprising amount of information has been accumulated and a great deal has been learned about the general kinetic aspects of various fermentation processes.

From an analysis of the rate patterns in batch alcohol, citric acid and penicillin fermentations, for example, Gaden⁹ distinguished between three broad kinetic groups.

- (1) Processes in which the desired products (ethanol, gluconic and lactic acids, for example) arise directly from oxidation of the primary carbohydrate.
- (2) Processes in which the products (citric acid, for example), though also resulting from carbohydrate dis-

simulation, do so indirectly and accumulate only under conditions of restricted or abnormal metabolism.

- (3) Processes in which product formation has no apparent association with carbohydrate oxidation (penicillin and many other antibiotics are examples of this type).

It must be recognized that a classification of this sort is based on purely empirical examination of batch fermentation results, not on a full and complete understanding of the individual mechanisms involved and their relationships to one another. Still, until such understanding has been achieved, empirical analysis is a powerful and useful tool—so long as its limitations are kept constantly in mind.

More recently Luedeking¹⁶ investigated the kinetics of the lactic acid fermentation using a batch process at controlled pH. He showed that rate of product formation is indeed proportional to the rate of substrate utilization as expected. Furthermore, rates of acid production could be related to rates of growth by a simple expression involving two constants dependent on the pH of the fermentation.

Subsequently, the performance of single or multi-stage continuous lactic acid processes were predicted from these batch results by analytical and graphical methods.¹⁷ Equations for both transient and steady-state operations of the continuous-system have been developed.

Kinetic Phenomena in Fermentation

The first problems in studying fermentation kinetics are (1) the establishment of consistent rate expressions, and (2) the selection of meaningful rate processes to be measured.

Rates and Productivity

To avoid confusion, the term 'productivity' has been recommended for the time-average output of a process.⁹ The expression 'fermentation rate' can then be reserved for the instantaneous rate of change of any concentration factor—sugar, product, cell weight, etc. These distinctions are shown graphically in Fig. 1.

Productivity is defined as the final product concentration divided by the time from inoculation to delivery of the batch. It might seem more reasonable to divide by the total process time from delivery of one batch to delivery of the next. This would include many operational factors involved in turnover of a tank, like cleaning, batching and filling, which have little or nothing to do with the actual fermentation system. While it is essential for proper economic analysis of the plant, such an overall productivity has little use in analyzing the fermentation process itself.

Two bases for expressing fermentation rates have been proposed:⁹

- (1) The *volumetric* rate, or the rate of change of concen-

tration with time; its units are mass/unit time (unit volume).

- (2) The *specific* rate, or the volumetric rate divided by cell concentration; its units are mass/unit time (unit cell mass).

The first is the preferred form for process design, especially for continuous systems, because it includes a volume term. The second is best for kinetic analysis because it puts everything on a comparable basis—unit mass of tissue. It does not follow, of course, that this unit tissue mass is physiologically identical throughout the fermentation process.

Rate process in fermentation

Rate measurements may be applied to an almost infinite number of factors in a fermentation system. Three of these however, have been consistently singled out for study—growth, sugar utilization and product formation.

Growth is taken as a rough expression of the total catalytic activity in the system. Admittedly, tissue accumulation is only the crudest expression of the true levels of activity of the various enzyme systems involved. Until these can actually be determined, however, it is the best measure we have.

Synthetic processes require the metabolic energy released by oxidation of primary carbon sources and sugar utilization is generally taken as an indication of the rate of energy release to the system. While it is true that proteins and fats are similarly degraded, with accompanying energy release, carbohydrate sources are ordinarily the major energy suppliers. At the same time these materials are frequently the substrates from which specific products are formed. The key rate, product formation needs no further elaboration.

Perhaps the greatest difficulty encountered in the examination of any complex fermentation process is the lack of any stoichiometric relationship between reactants and products. Lacking this, measurements of the three basic rates defined above may still be made. They offer the singular advantage of being determined directly from the measurements most commonly made in fermentation process studies, tissue mass, sugar and product concentrations.

Complete rate patterns, on both volumetric and specific bases for a typical complex fermentation (streptomycin biosynthesis) are shown in Fig. 2. They were calculated from data of Sikyta et al., in the manner previously described.⁹

Fermentation process variables

Primary process variables in fermentation are: (1) temperature, (2) pH, and (3) nutrient (or reactant) concentration (including oxygen). In addition, certain conditions of the physical environment, like fluid turbulence and equipment design features which effect mass transfer in the reaction zone, must be considered.

Note that the fundamental composition of the nutrient

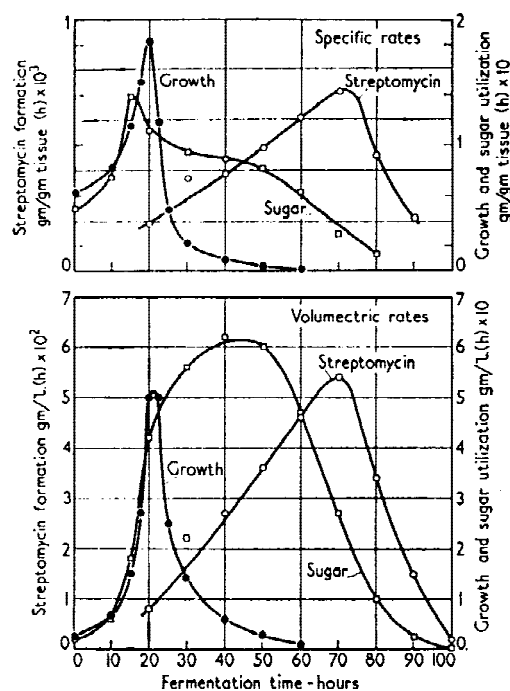


Figure 2. Streptomycin fermentation rates.

environment, as opposed to the concentration of specific components (sugar, nitrogen sources, etc.), is not included. This is considered an inherent feature of the process system and not a 'process variable' in the usual sense. While this view is reasonable for most other chemical reaction systems, it may not be so for fermentation. One cannot synthesize ammonia unless the reaction mixture contains both nitrogen and hydrogen (the mole ratio of these reactants is the 'process variable') but tetracycline can be produced in a wide variety of nutrient media.

Fermentation Process Types

Fermentation processes may be classified in a number of different ways. The first systematic approach was proposed by Gale¹¹ who grouped microbiological processes in a series of type groups, oxidation, reduction, hydrolysis, etc. Such an arrangement though fundamentally attractive, is only suitable for specific reactions operating on specific substrates to yield specific products. Unfortunately, many commercially important fermentation processes cannot be so neatly described.

Gale's classification scheme has recently been extended by Stodola²⁴ and others,²⁵ who have proposed a more detailed breakdown of 'type reactions'. In this scheme, microorganisms, or more specifically their enzyme complements, are looked at as added means for controlled organic synthesis. Again, this concept is not applicable to most of the fermentation processes now practised commercially—at

least at the present level of knowledge regarding mechanisms.

A different approach was proposed by Gaden.¹⁰ It is summarized in modified form in Table I. Here fermentation processes rather than specific reactions are grouped together and the overall free energy change involved is the basis for classification.

The primary advantage of this scheme is technological; it coincides with the general classification of fermentation rate patterns suggested earlier.⁹ Experience has shown that fermentation processes fall more or less into three kinetic groups, which may be designated 'types I to III' for convenience. Their relationship to the general reaction types is shown in Table I and summarized below:

Type I: processes in which the main product appears as a result of primary energy metabolism. Examples of this type of system are most common in the older branches of fermentation technology, for instance: (1) aerobic yeast propagation (mass propagation of cells in general), (2) alcoholic fermentation, (3) oxidation of glucose to gluconic acid, and (4) dissimilation of sugar to lactic acid.

Type II: processes in which the main product arises indirectly from reactions of energy metabolism. In systems of this type the product is not a direct residue of oxidation of the carbon source but the result of some side-reaction or subsequent interaction between these direct metabolic products. Examples are: (1) formation of citric and itaconic acids, and (2) formation of certain amino acids.

Type III: processes in which the main product does not arise from energy metabolism at all but is independently elaborated or accumulated by the cells. It is perfectly true that carbon, nitrogen, etc., provided in essential metabolites appear in product molecules but the major products of energy metabolism are CO₂ and water. Antibiotic synthesis (Fig. 2) is a prime example of this type.

Each of these types demonstrates a fairly distinctive rate pattern. These are shown schematically in Fig. 3. The Type I processes show only one maximum for each of the rate processes and these are virtually coincident, hence the term 'growth-associated' often used for products of this process type.

In the Type II process two rate maxima are distinguishable. In the first phase tissue is produced with little product formation; in the second product formation rate is maximized. Rapid carbohydrate utilization is common to both. Unfortunately, very few kinetic data are available for this group. In fact, until the recent development of microbiological processes for amino acids (probably Type II), the citric acid fermentation was the only example for which rate information had been published.

Type III processes again show two distinct phases. In the first tissue accumulation and all aspects of energy metabolism are maximized with virtually no accumulation of the desired product, in the second oxidative metabolism is prac-

Table I. Fermentation process types.

Dissimilation reactions $\Delta F = -$	Biosynthesis $\Delta F = +$
Type I. Simple: $A \rightarrow \text{products}$ $A \rightarrow B \rightarrow C \rightarrow \text{products}$	Type III. Biosynthesis of complex molecules: Polymerizations—carbohydrates, proteins Antibiotics, vitamins, etc. Fats
Type II. Complex: $A \rightarrow B \rightarrow C \rightarrow$ \downarrow \downarrow D E \swarrow \searrow products	

tically over and product accumulation is maximum. Both penicillin and streptomycin (Fig. 2) fermentations are excellent examples of the Type III kinetic pattern.

It must be emphasized that these are only generalizations for technological convenience. They are neither perfect nor comprehensive and great variations may occur. A particular fermentation type may exhibit widely different behaviour with major changes in medium composition and process conditions. Strain variations, on the other hand, seem to have little effect on the general rate patterns.

Exceptions are found in all groups, especially Type III. In fact it may prove necessary to subdivide this group further as more kinetic information on complex processes becomes available.

One apparent exception is the production of oxytetracycline. Doskočil et al.⁷ have presented a very complete study

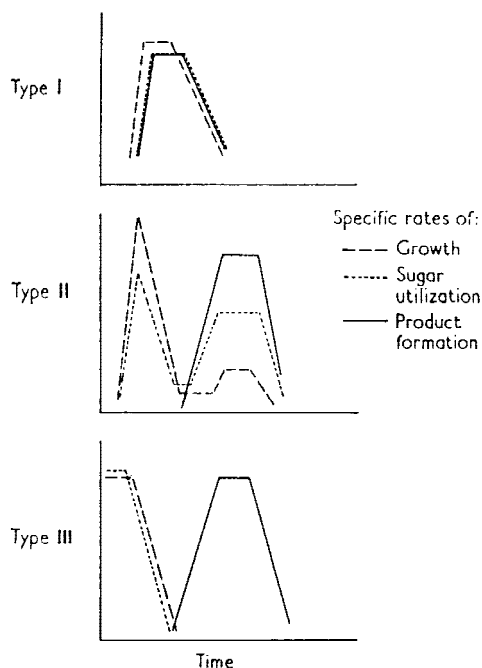


Figure 3. Fermentation rate patterns.

of metabolic changes observed during this fermentation. Rate curves calculated from these are shown in Fig. 4.

These authors⁷ did not attempt any detailed analysis of rates, but they did suggest a multiphase nature for this process. Specifically they proposed five periods as follows:

- (1) Lag: virtually no metabolic activity.
- (2) Growth of primary mycelium: very high level of metabolism (respiration, nucleic acid synthesis, etc.), no antibiotic formation.
- (3) Fragmentation of primary mycelium: respiration and nucleic acid synthesis fall, antibiotic synthesis is just starting.
- (4) Growth of secondary mycelium: rapid antibiotic production, renewal of nucleic acid synthesis, further decrease in respiration.
- (5) Stationary phase: no further growth, metabolic activity low but antibiotic synthesis continues.

Another process which one would expect to fall in Type III is the chloramphenicol (chloromycetin) fermentation. On the basis of very scanty data, however, it too appears to be an exception to the general pattern. If it is in fact, then the two processes which give a typical behaviour both involve organisms which normally fragment during growth. This may well lead to a characteristic kinetic behaviour different from that for streptomycin; unfortunately, the information available is not sufficiently complete to permit any firm conclusion.

A generous amount of sub-classification would undoubtedly remove most discrepancies. At the same time, however, it would make void the primary purpose of this approach—the establishment of certain reasonably reliable generalizations about fermentation rate patterns which can serve as a basis for further kinetic studies.

Fermentation Kinetics and Continuous Processes

Many reasons, both practically useful and intellectually satisfying, can be offered to justify more intensive study of fermentation kinetics but one outweighs all others: we cannot hope to operate continuous processes at a predictable steady state unless the relationships between major rate processes and the effects of process variables on them are known.

The reactor system which is apparently best adapted to continuous fermentation is the homogeneous, overflow type, with virtually complete backmixing. To establish an overflow reactor at steady state all rate processes must be in balance. It is possible to achieve this by simply letting the system hunt for such a point, but no one can predict in advance where this point will be. Such a procedure is hardly an adequate basis for plant operation.

For the 'kinetically simple' Type I fermentations, prediction of continuous steady state operating conditions from

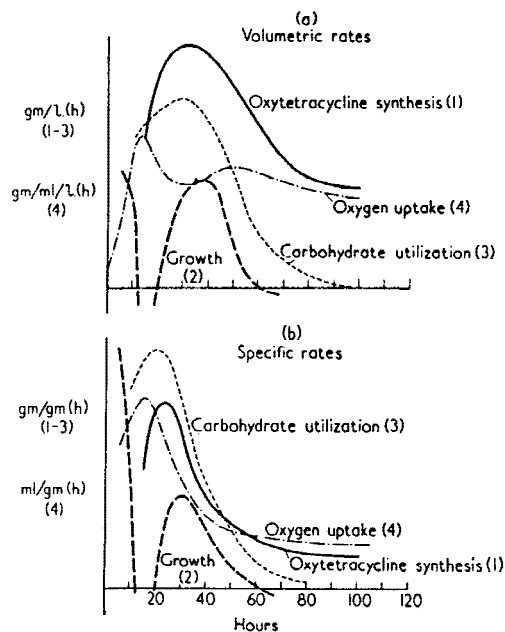


Figure 4. Oxytetracycline fermentation rates.

batch data is theoretically possible.^{13,17,18} Furthermore, this type of process can be operated satisfactorily in a single stage system, although additional stages may be added to ensure economical utilization of nutrients supplied. Both these points have been demonstrated experimentally in a number of cases.^{5,13,17}

On the other hand, kinetic considerations alone demand at least two stages for satisfactory operation of the more complex process types (II and III). In the first, conditions will be adjusted to provide maximum rates of growth, and energy metabolism, in the second, for maximum product formation. Kinetic studies for continuous process design should therefore be aimed primarily at elucidating the relationships between these various rates and the major, controllable process variables.

The only complex fermentation process for which studies of this sort have been made is the biosynthesis of penicillin. In the final section of this paper, that information will be collected and related to illustrate the kinetic nature of the Type III process.

Penicillin Process Kinetics

Early attempts to clarify the effects of process variables on the two phases of the penicillin fermentation were seriously handicapped by the inadequacies of available experimental techniques. Even so a general picture was obtained. With improved procedures this has been greatly amplified over the last decade until the effects of major process variables on growth and antibiotic formation are reasonably understood. Temperature and pH are the best examples.

Temperature

Stefaniak et al.²³ found no effect on overall penicillin yields between 20° and 29°C with an early culture (X-1612). At 32°C, however, antibiotic yields fell while oxidative metabolic processes (sugar utilization, etc.) were more rapid.

In the work previously cited, Calam, Driver and Bowers⁶ set the optimum temperatures for growth and penicillin formation at 30° and 25°C, respectively. These conclusions were arrived at rather indirectly because they did not, in fact, separate the two phases of the process experimentally.

This was done by Owens and Johnson²¹ who showed that growth rates were highest around 30°C while penicillin synthesis proceeded most rapidly near 20°C. A two-stage fermentation with the temperature reduced from 30° to 20°C after 40 h gave the highest penicillin titre.

pH

The importance of pH in the penicillin fermentation was early recognized. Lacking reliable means for external control, most processes employed medium formulations which provided a degree of internal buffering. A number of laboratory studies with externally controlled pH have been reported,^{2,3,14} however, and the results are plotted on a common basis in Fig. 5. Note that the rates indicated are average rather than instantaneous. This does not alter the fundamental relationships shown.

From these experiments it is clear that the growth phase of the penicillin fermentation should be operated at a pH value around 4.5–5 while antibiotic formation will be maximized around 7–7.5.

It is also interesting to note the effect of external pH control on rate patterns in a penicillin fermentation. Brown and Peterson⁴ have reported batch fermentations employing

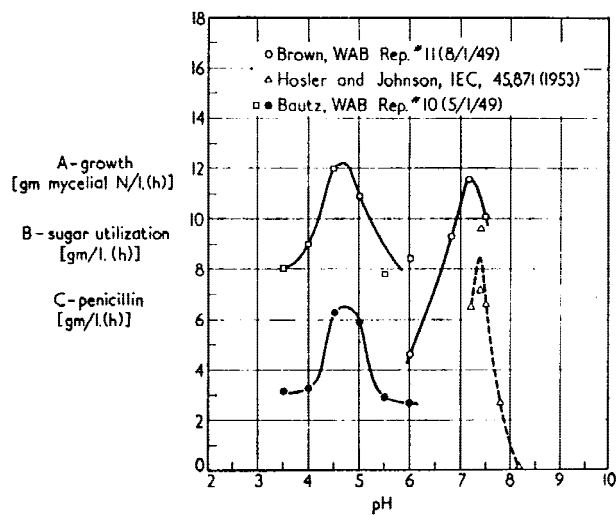


Figure 5. pH effects in penicillin biosynthesis.

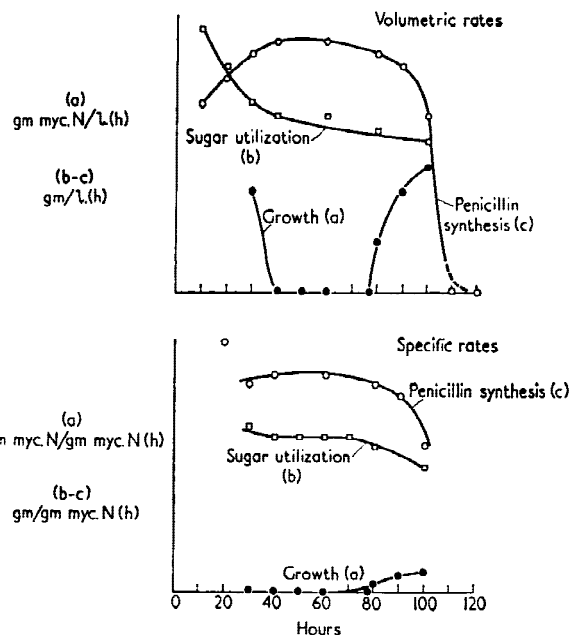


Figure 6. Penicillin fermentation rates with pH control.

a medium which tended to become alkaline. After 30 h, the pH was adjusted to 7.0 with acid and held there (approximately) by controlled acid addition. Volumetric and specific rate patterns calculated from their results are shown in Fig. 6. Since no determinations of mycelial nitrogen were made before the 30-h point, specific rates (based on mycelial nitrogen, not dry tissue in this case) cannot be computed for the early hours.

With pH control, constant rates of metabolism and product formation may be sustained for a long time, even in the unsteady-state batch process. Extended batch processes of this type may very well be practical competitors of continuous operations, particularly if the operating problems which have often been encountered in continuous systems prove difficult to overcome. The limit on such a process, assuming continuous nutrient addition as well as pH control, will presumably be imposed by the accumulation of products toxic to the organism or inhibitory to its enzyme systems.

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