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MIXING AND SEGREGATION IN PURE AND HETEROGENEOUS BACTERIAL CULTURES

by

Donald J. Schaezler

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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I. INTRODUCTION

The rational study of reacting systems, whether for theoretical concepts, design, or operation, usually involves the formulation of mathematical models relating dependent variables to independent variables and system parameters. Bypassing atomic level phenomena, the most basic approach is to assume that a continuum exists, at least in each phase, and to insert kinetic expressions along with phenomenological laws into transport phenomena equations. If convective fluid movement occurs, in principle the momentum balance equation must be solved for a velocity distribution which can be inserted into the mass balance equation. This is easy only for laminar flow; for turbulent flow the easiest approach is the use of "effective" transport coefficients and assumptions regarding velocity profile. Such simplification often leads to "ideal flow" mass balance equations of the "plug flow" or "perfectly stirred tank" type. Deviations from ideal flow are handled with models assuming more spatial variation within the system or by population-balance models. Whether any such models adequately represent steady state and transient system behavior over a range of situations depends on the validity of the kinetic
expressions and simplified flow models. In particular, the role of macrokinetics and physical transport in kinetic expressions should be recognized, and mixing and segregation models must be distinguished.71

This research was concerned with the mathematical modeling of bacterial reactions in batch and continuous stirred tank reactor (CSTR) systems. Above all else the purpose of this research was to demonstrate the basic quantitative rationality of microbial growth systems. The phenomena associated with such systems should be rational events requiring only sufficient system knowledge and insight for quantitative understanding. In particular the research was concerned with the following aspects of prediction of microbial process behavior:

(1) the effect of substrate concentration on growth rate for dispersed batch cultures of bacteria and the effect of mixing on such dependence;

(2) the question of segregation effects in CSTR's with dispersed bacterial cultures;

(3) the prediction of steady states in CSTR's using the results from aspects (1) and (2);
(4) the prediction of transient responses in CSTR's using data from batch systems seeded with bacteria from the corresponding steady state CSTR.
II. BACKGROUND

A. Microbial Growth Rate Expressions

For microbial systems the desired kinetic expressions involve formulations for cell growth, cell-component increase, substrate removal, or product formation. Almost universally kinetic expressions are based on the sigmoid growth curve characteristic of events occurring when a small inoculum of cells is placed in a nutritionally adequate medium, and the system is allowed to react in a batch manner\textsuperscript{73,128,93,129,24,121}. The formulation is usually in terms of the expression

\[
\frac{db}{dt} = \mu b
\]

(1)

where \( b \) represents cell mass, and \( \mu \) is the growth rate of the organisms. \( \mu \) is constant and maximum during the logarithmic growth phase but decreases to zero during the declining growth phase and maximum stationary phase. The declining phase is explained either by toxic product inhibition, substrate limitation, or both. The particular type of limitation seems to shift from substrate limitation to product limitation as the initial substrate concentration increases. In general, for a given medium, \( \mu \) can be written

\[
\mu = \mu_m(T, pH)f(s_1, p_1)
\]

(2)

where \( \mu_m = \) maximum growth rate as a function of temperature, \( T \), and pH, and \( f \) is a fractional factor as a
function of all substrates, $s_i$, and all products, $p_i$. The effect of $T$ on $\mu_m$ has been studied by Barber$^3$, Monod$^{92}$, Johnson and Lewin$^{60}$, and Ingraham$^{58}$. The effect of pH has been studied by Gale and Epps$^{37}$. The form of $f(s)$ was first quantified by Monod$^{92}$ who presented the following empirical expression:

$$\mu = \frac{1}{b} \frac{db}{dt} = \frac{\mu_m s}{K_m + s} \tag{3}$$

for dilute cultures in defined media. Models for toxic product limitation are often of the form$^{24,111,125}$

$$\mu = \mu_m (1 - ap) \tag{4}$$

Toxic product effects in concentrated cultures have been observed by many workers$^{99,20,114,109,75,36,35,83,85}$. Monod's formulation, equation (3), and other simple formulations giving similar form of $\mu(s)$$^{98}$ have often been used successfully to fit experimental data for batch and continuous systems. However, there seems to be a good deal of controversy over the magnitude of $K_m$ values or critical concentration values (concentration below which $\mu$ depends on $s$). Some cases where high $K_m$ values are used involve fairly concentrated cultures. Results from careful dilute culture studies seem to provide values of $K_m$ in the order of 1-10 mg/l or less for a variety of limiting substrates$^{93,92,103,51,53,4,57,39,68,61}$, and such conclusions can be inferred from
many experiments with simple substrates\textsuperscript{20,119,100,14,9,124,46,139,140,141,131,132}. If kinetic expressions such as equation (2) are to be meaningful they should be fairly independent of cell concentration, so that results indicating large $K_m$ values for simple substrates should be questioned as contrary to established results.

Some possible explanations for results indicating a great dependence of $\mu$ on $s$ are given below.

(1) Many studies on $\mu(s,p)$ have been concerned with the transition of log phase cells to stationary phase cells. Herbert\textsuperscript{52} pointed out that the apparent decline of $\mu$ was first noted and extensively considered for complex, undefined media. In simple, defined media a declining phase was almost entirely absent. He attributed this to effects of sequential exhaustion of non-essential, but useful, nutrients as proposed by Monod\textsuperscript{93}. Monod also attributed the effect to sequential depletion of certain reserves of metabolites in some cases. The same complications could arise during polyauxic growth on several substrates or even diauxic growth on a single substrate and its product. It may be more correct to assume $\mu = \mu$(substrate nature) = constant until one nonessential nutrient, metabolite reserve, or substrate is exhausted. $\mu$ then decreases but remains independent of any substrate concentration. This
process continues until finally the stoichiometric limiting nutrient is exhausted. What appears at first to be a long declining growth phase is actually a series of log phases of decreasing growth rates. A similar explanation has been used in the water pollution field\textsuperscript{139,140,138,141} to explain why zero order removal of single substrates is often observed in mass cultures whereas first order removal of BOD, a measure of total substrate, is observed in multisubstrate or complex media. Actually, quite complicated forms of \( \mu(s) \) could occur, using a total substrate measure for \( s \), depending on whether concurrent or sequential removal of individual substrates occurs, quantities of each substrate present, log rates for each substrate, required adaption times, etc.

(2) The form of equation (1) may be incorrect under certain circumstances. In some cases linear growth and linear substrate removal have been observed\textsuperscript{90, 55,112,140,141,132,93,107,72,134}. In these cases

\[
\frac{db}{dt} = k_L b(0) \tag{5}
\]

should be used. Modeling according to equation (1) leads to an apparently strong dependence of growth rate on \( s \):

apparent growth rate = \( \frac{1}{b} \frac{db}{dt} = \frac{k_1}{k_2 - s} \tag{6} \)
where $k_1$ and $k_2$ are constants dependent on initial conditions $b(0)$ and $s(0)$ (see APPENDIX, section A). Figure 1 illustrates equation (6). Correct modeling according to equation (5) leads to critical values of $s$ in the order of 1-10 mg/l or less. Such cases may be due to utilization of substrate mainly for storage products without an increase in cell numbers or synthesizing ability. Such cases may be similar to division lag phenomena as reviewed by Herbert even though logarithmic increase of cell mass is usually reported for division lag cases.

Linear growth can also occur if a fixed amount of growth factor is present or if interphase mass transfer limitations cause a constant flux of a growth limiting material such as oxygen.

(3) Product inhibition has been mentioned above. Ramkrishna et al. studied the theoretical consequences of various product inhibition models, but the effect is generally not important in dilute bacterial cultures. However, even in dilute cultures inadequate buffering can lead to pH effects which act like product inhibition. A reasonable model including pH effects would be

$$
\mu = \mu_m(T, pH) = \mu_m(T) \left(1-a(pH-pH')^2\right), \text{ analogous to }
\mu_m(T) f(p)
$$

(7)
Such a formulation can be readily inferred from the data of Gale and Epps\textsuperscript{37}. pH can in turn be related to $s(0) - s$ by a titration curve for the buffered medium. Figure 2 illustrates the resulting $\mu(s)$ for several cases, and Figure 3 illustrates results in a CSTR. The calculations are shown in the APPENDIX, section B. Similar results would hold for more general product inhibition where $\mu = \mu_m(T, \text{pH})(1-\text{ap})$ is a common form\textsuperscript{24,111}.

(4) Diffusion or mass transfer could also interfere with proper kinetic expressions. Diffusion interferences are mentioned by Noyes\textsuperscript{105} and Denbigh\textsuperscript{29} for chemical reactions and by Mueller, et al.\textsuperscript{99}, Swilley et al.\textsuperscript{133}, and Kehrberger et al.\textsuperscript{64} for bacterial growth. Rashevsky\textsuperscript{113} considers many aspects of diffusion effects in biological systems. Swilley et al.\textsuperscript{133} apparently made a mistake in their calculations, from which they concluded that diffusion in the liquid bulk should affect growth rates for dispersed bacterial cultures. A slightly different approach is now considered. Consider a single spherical bacterium of radius $\frac{1}{\mu}$, dry weight of $10^{-12}$ gm, and yield factor of 0.5. If this bacterium is in the log phase, the required flux of material while it is growing is
\[ N = \frac{2 \times 10^{-12} \text{g m substrate}}{12.5 \times 10^{-8} \text{cm}^2 \cdot g_t} \]  

(8)

where \( g_t \) = generation time, and the growth of a single bacterium is assumed to be linear with time. For \( g_t = 1 \) hour, \[ \frac{N}{g_t} = \frac{0.16 \times 10^{-4} \text{g m}}{\text{cm}^2 \cdot \text{hr}} = \frac{4.45 \times 10^{-9} \text{g m}}{\text{cm}^2 \cdot \text{sec}} \]  

(9)

As long as cells do not aggregate, and the culture is dilute enough so that bacteria are fairly far apart, diffusion to only one bacterium need be considered regardless of the bacterial concentration. Then with the flux calculated above a particular model such as the existence of a stagnant boundary layer surrounded by a well mixed fluid or diffusion in an infinite fluid medium with an "effective diffusivity" can be used to investigate possible diffusion limitations. For example assume a \( 1\mu \) thick stagnant boundary layer exists around the bacterium, with the concentration of substrate outside this layer equal to \( c_b \), and the concentration of substrate at the bacterial surface equal to \( c_s \). Then it can be shown (APPENDIX, section D) that \( c_b - c_s = 2.23 \times 10^{-2} \text{mg/l} \). This is a negligible drop. However, if diffusion must take place through thicker layers or through layers of viscous material or to aggregates of cells, the concentration drop could be considerable. A
simple case considered by Rashevsky\textsuperscript{113} considers diffusion in an infinite medium, transfer across a membrane, and diffusion with reaction within a spherical cell. The use of the flux and parameters above leads to a concentration difference of only $6.68 \times 10^{-2}$ mg/l between the center of the cell and the fluid at an infinite distance (APPENDIX, section D). Both of these examples consider steady state diffusion which is directly applicable to steady state CSTR's and approximately applicable to non-steady state systems with moderate reaction rates.

Interphase mass transfer of oxygen can easily become limiting as mentioned above with respect to linear removal. Abson and Clark\textsuperscript{1} and Wilson\textsuperscript{138} have discussed necessary changes in Warburg type instruments to prevent such limitation. The theory of interphase mass transfer with reaction in one phase is too complex to go into here. But as a suggestive example consider a bacterial culture of 100 mg/l dry weight in the log phase with a generation time of 1 hour and a stoichiometric requirement of 1 mg oxygen per mg bacteria formed. The instantaneous rate of oxygen utilization is thus 69 mg/l/hr. If the dissolved oxygen deficit is 8 mg/l this requires
a mass transfer coefficient, \( K_{L}a \), of 8.6 hr\(^{-1}\).

This is higher than coefficients observed for many bubble aeration cases\(^{16,31}\). \( K_{L}a \)'s for vigorously shaken systems may be only about 60 hr\(^{-1}\) so logarithmic growth may be maintainable only up to about 700 mg/l dry weight of bacteria depending on growth rate and stoichiometry. Aeration deficiency has been suggested by Deindoerfer\(^{25}\) as a possible explanation for anomalous continuous culture results.

It is important that kinetic expressions used in mass balance equations represent actual dependencies on s, p, T, pH, b, or whatever. Fitting a particular expression to a set of data is not enough, for the aim must be to have predictive capability over a range of conditions. Though mass transfer or diffusion limitations may mask the real reaction kinetics, as long as expressions properly reflect concentration dependencies correct predictions will result.

B. Continuous Culture and Transient Phenomena

Steady and transient states in continuous processes are not only important from a practical point of view, but offer excellent opportunities for testing growth models.

The continuous culture of microorganisms has been practiced since at least the 1910's in the laboratory,
the fermentation industry, and in biological waste treatment systems$^{104,87,59,89}$. However, the careful theory and practice of continuous culture which has led to its use as an experimental tool and as a well controlled and designable system in many areas had their origins in about 1950 when Monod$^{94}$ and Novick and Szilard$^{103}$ published papers dealing with continuous culture apparatus, theory, and results. The initial systems of study were well-mixed vessels (CSTR's) with growth and growth rate limited by exhaustion of an essential nutrient. Thus the rate expression developed by Monod$^{93,92}$ and at least partially verified by others was inserted into mass balance equations for cells and substrate, and steady state levels were predicted by setting time derivatives equal to zero. Theoretical and experimental studies were quickly extended to multiple reactors$^{45,51}$, product inhibition$^{125,35,75,36}$, complicated metabolism such as endogenous metabolism$^{98}$ and polyauxia$^{87,78}$, and chemical composition and metabolic capabilities (physiological state) of micro-organisms as a function of growth rate and growth medium$^{79,52,51,56,104,18}$. By 1962 several reviews had appeared$^{104,87,52,51,59,56,42}$, symposia concerned with theory, general concepts,
and practical applications had been convened in Prague, Stockholm, London, and Chicago, and dozens of other papers published.

Steady state results in CSTR's were generally in good agreement with theory, however yield coefficients were not always constant and washout did not always occur when predicted. Some problems were attributed to equipment inadequacies, and some improvement was made by slight model adjustments. Also many experiments were performed at high influent substrate concentration where Monod's model, verified for more dilute cultures, might not hold (see discussion under section A above).

In 1954 Finn and Wilson reported steady oscillations about average values in a nominally steady state culture of yeast. Their observations primed the pump of investigation into transient behavior and stability. Spicer investigated the local stability of steady states with small perturbations for \( \mu = ks, \mu = (1-e^{-ks}) \), and for a particular case of toxic product rate limitation. He also provided general criteria for local stability to be applied to any functions for rate of formation of cells and substrate.
Moser\textsuperscript{98} investigated the course of culture variables from time of inoculation to achievement of steady state using the linear approximation to Monod's model and concluded cell and substrate concentrations tended to the easily derived steady state values as time tended to infinity. Northam\textsuperscript{102} discussed the same problem as Moser without particular conclusion, but did conclude that Luedeking and Piret's\textsuperscript{75} model for toxic product limitation was stable, using Spicer's\textsuperscript{125} criteria. Luedeking and Piret\textsuperscript{75}, not surprisingly, made this same conclusion. They also presented limited experimental data confirming their batch culture predictions of some special cases of transient response. Fuld et al.\textsuperscript{36} indicated the possibility of frequency response methods for investigation of continuous culture systems. Their experimental results and conclusions were sketchy and met with some criticism\textsuperscript{108}. Bungay\textsuperscript{7,8} has also discussed frequency response methods for microbial cultures. Mateles and his coworkers\textsuperscript{86,83,84,85,120,142} have made several investigations into transient responses to step changes in dilution rate and temperature for carbon limited, nitrogen limited, and product limited cultures. They could find no evidence of steady oscillations or overshoot in transition for nutrient limited
or product limited cultures. Unfortunately they present neither models nor data for batch growth. They do not predict responses but merely describe them. They found that responses depend on initial dilution rate or \( T \), initial substrate level, and the magnitude of the step change. \( \mu \) could increase a certain amount instantaneously after a "shift up", but a lag was necessary before \( \mu \) reached the new growth rate if the shift was too large. Mateles et al.\(^{86}\) concluded that Monod's model was not adequate for transient responses, nor was the Arrhenius relationship for \( \mu(T) \). Ramkrishna et al.\(^{111}\) made an important contribution with respect to the modeling of batch cultures and steady and transient states in continuous cultures. They reasoned that a model should be able to predict all phases of batch growth including the lag, stationary, and death phases before it is used for continuous culture predictions, transient responses in particular. The Monod model obviously cannot do this since it is based only on the transition from the log to the stationary phase. They presented mechanistic models which coupled substrate and product rate limitations. Some of their models described two components of cell mass, one roughly equivalent to proteins and the other to nucleic acids.
They argued that such structured models were necessary to give a measure of physiological state of the organisms, a property which affects lag phase growth. Their models not only predict the proper effects of physiological age and inoculum size on the lag phase, but qualitatively predict the proper nucleic acid composition as a function of growth phase (see Herbert's\textsuperscript{52} review). Insertion of their models into CSTR mass balances leads to interesting results. One model always has stable steady states, while for another steady states are stable foci which become unstable foci at low dilution rates. This is related to the problem of continuous cultures going into a lag phase at low growth rates\textsuperscript{98,104}. Their models predict that nontrivial steady states are not always achieved from arbitrary initial conditions, that achievement depending on inoculum size and physiological age, as well as dilution rate. Koga and Humphrey\textsuperscript{69} investigated stability of a Monod model with variable yield due to maintenance energy requirements and found that damped oscillations could occur during transients. This situation is very similar to that of Ramkrishna et al.\textsuperscript{111} who, in effect, have a variable yield due to toxic product effects. Mor and Feichter\textsuperscript{96}, using dense cultures of \textit{S. cerevisiae}, found damped
oscillations after a step change in dilution rate if the original dilution rate was in the region where yield was significantly lower than normal due to endogenous effects, but not if the original dilution rate was above this region.

Another special area of interest is continuous culture using multisubstrate media. Maxon discussed design of a two vessel system for a two component substrate which Malek questioned because of lag and adaption phenomena. Baidya et al. investigated the use of a glucose-lactose mixture which exhibited a long diauxic lag in batch culture, and Harte and Webb did the same for a glucose-maltose mixture which exhibited a short diauxic lag in batch culture. Their results included the observations that once adaption to the second sugar occurred in the continuous culture it lasted for a long time; repression was not due to glucose itself because it was always present at almost nondetectable levels ($K_m$ for glucose was about 1 mg/l for both systems); the response to the addition of the second sugar depended on dilution rate; lag times were much longer in the continuous system and were related to the dilution rate; the second sugar could be almost completely removed at dilution rates greater than the $\mu_m$ for the second sugar. A simple explanation for the last observation
is offered in the APPENDIX, section C. For diauxic growth with substrates $s_1$ and $s_2$, having influent concentrations $s_{1,in}$ and $s_{2,in}$, providing growth rates $\mu_1$ and $\mu_2$, with $K_{m,1}$ and $K_{m,2}$ approximately zero, and with equal yield factors, the explanation says that substrate $s_2$ can be almost completely removed for

$$D \leq \left[ \frac{s_{1,in} + s_{2,in}}{s_{2,in}} \right]/\mu_2 = D_2^*$$

(10)

provided this $D$ is less than $\mu_1$ and $\mu_1 \geq \mu_2$. For $D_2^* < D < \mu_1$, $s_2$ increases with $D$, and there is an apparent dependence of $\mu$ on $s$ even though both $K_m$'s are approximately zero. This is pertinent to the discussion in section A(1).

Although continuous cultures of microorganisms had been used in the waste treatment field for several decades, the basic concepts of completely mixed vessels and growth regulated by substrate concentration were not recognized for mixed cultures until the work of Garrett and Sawyer in 1952 for chemostat type cultures and the work of Busch and Kalinske in 1956 for flocculent cultures with partial cell return after settling. Both of these works supported the idea of growth rate as a linear function of substrate concentration. However, substrate concentrations were measured by BOD, and complex substrates were used (see section A(1)).
Mathematical models of activated sludge systems and kinetic approaches to design of well mixed treatment plants have flourished. McLellan\textsuperscript{90} reviewed many aspects of this work. Several papers by Busch\textsuperscript{10,11,12} exemplify the rational approach of using kinetic data from batch systems to predict continuous system performance. He stresses the use of continuous cultures to hydraulically select the proper organisms for batch culture tests\textsuperscript{15}.

The use of well defined, repeatable mixed culture systems for basic research has lagged. Komolrit and Gaudy\textsuperscript{70} used continuous, dispersed (i.e. nonflocculent) cultures to study diauxic phenomena for catabolite inhibition and repression with sorbitol-glucose and glycerol-glucose pairs. Chian and Mateles\textsuperscript{17} and Mateles and Chian\textsuperscript{82} studied similar phenomena in pure and mixed cultures as a function of dilution rate for glucose-lactose, glucose-fructose, and glucose-butyrate pairs. Grady and Gaudy\textsuperscript{47} investigated diauxic type effects in continuous mixed cultures with a lysine-glucose substrate. Unfortunately little or no batch data is given for any of these systems, so that comparison of continuous culture steady and transient states with batch data predictions cannot be made. Gaudy, Ramanathan, and Rao\textsuperscript{41} made a comprehensive series of batch and continuous experiments
to test the validity of various expressions for $\mu(s)$, to see if steady states in dispersed mixed cultures could be attained, and to test predictions of these steady states from batch data. This work was extended to higher influent substrate concentrations and to cell concentration and return systems\textsuperscript{110}. Inspection of the media for both works indicates that buffering capacity was inadequate to maintain constant pH. In fact, the results for the chemostat-type systems are strikingly similar to the hypothetical cases in Figure 3 which demonstrate pH effects. Figure 8 of Gaudy et al.\textsuperscript{41} shows that at $D$ approximately equal to $0.5\mu_m$, carbohydrate COD in the filtrate is approximately zero, implying a $K_m$ of approximately zero for glucose. In contrast, $K_m$ values are concluded to be 75-125 mg/l. This conclusion seems to be at odds with their results and with established results for pure and mixed cultures utilizing glucose and other simple substrates (see section A). Quite possibly incorrect procedure has led to erroneous conclusions. In addition "steady states" showed fairly variable solids and influent COD concentrations.

Transient phenomena in mixed cultures have been studied by Eckhoff and Jenkins\textsuperscript{32} for activated sludge systems, by Komolrit and Gaudy\textsuperscript{70}, Storer and Gaudy\textsuperscript{130}.
and Grady and Gaudy\textsuperscript{47} for dispersed mixed cultures, and by McLellan\textsuperscript{90} for a flocculent mixed culture with repeatable characteristics. Storer and Gaudy\textsuperscript{130} viewed transient responses as depending on hysteresis effects on $\mu(s)$. Their procedure was one of fitting curves to results rather than prediction. As in the works of Komolrit and Gaudy\textsuperscript{70}, Gaudy et al.\textsuperscript{41}, and Ramanathan and Gaudy\textsuperscript{110}, glucose was measured by a test specific only for carbohydrates. Comparison of results by this method and by the specific glucostat test by Gaudy\textsuperscript{40} for a steady state continuous culture indicated that glucose concentration was much closer to zero than carbohydrate concentration. This may in part explain the postulate of a high $K_m$ for glucose by many of these authors. The first successful attempt at prediction of transient responses to changes in dilution rate or influent substrate concentration was apparently that of McLellan\textsuperscript{91,90}, who used a flocculent culture with internal sludge return and controlled sludge wastage. He used batch systems, seeded with organisms from a steady state continuous culture to predict transient responses. Apparent expressions for $\mu(s)$ from batch studies predicted neither steady nor transient states in continuous culture, but McLellan developed a theory using the concepts of available reaction potential and batch
reaction time vs. hydraulic retention time to predict when he would observe an increase in soluble carbon in his continuous systems and the magnitude of such increase.

In the last twenty years a concerted effort has been exerted on many fronts to model microbial systems so that the behavior of batch and steady and transient state continuous systems could be predicted. Most effort has been concentrated on the development of intrinsic kinetic expressions relating growth rate to concentrations of substrates, products, and organisms. This approach has been fairly successful for some steady state continuous systems, the form of b and s vs. D at least conforming qualitatively to the forms of \( \mu(s,p) \) usually found in batch systems. However, quantitative predictions of continuous system performance from batch system performance with the same organisms and substrate are rare (but see references 38,75,41,2,50,110,90) and sometimes questionable 41,110. Predictions of transient behavior, when attempted, have been largely unsuccessful 86,32 except by unconventional concepts 91,90. The importance of physiological state has been pointed out often. Herbert's review 52 provides considerable evidence for the effect of growth rate on chemical composition
and metabolic activity of organisms. Other results also relate metabolic activity to growth rate \(^{51,18,95,47}\). Much of the failure in predictive ability can probably be attributed to factors complicating the correct formulation of \( \mu(s,p) \) and the lack of incorporation of physiological state into models.

C. Segregation Theory

As mentioned above proper kinetic expressions must be combined with proper fluid flow models. Only for an ideal plug flow reactor can kinetic expressions be obviously used to determine continuous process performance. In all other cases mixing and segregation effects must be distinguished. The usual concept describes two components of mixing, macro- and micromixing, the separation depending on the scale of mixing. Residence time distributions determine only macromixing because sample scale is relatively large, however effects causing such distribution can also affect micromixing. The causes can be large scale effects of convective bypassing due to non uniform velocity distribution, or smaller scale effects of dispersive and diffusive mixing due to concentration gradients. Whereas macromixing is defined by residence time distributions of molecules, micromixing is related to concentration histories for molecules. The study of the separation of macro-
and micromixing developed into a segregation theory, the origins and developments of which are discussed below.

The origins of segregation theory are associated with the original treatises on continuous stirred tank reactors (CSTR's). Apparently the first treatment of well stirred tanks was by Ham and Coe\(^48\) for conservative substances. MacMullin and Weber\(^76\) extended the problem to homogeneous reactions and derived steady state design equations. Kirillov\(^65\) considered transient equations and more complicated reactions. Kirillov\(^66\) was apparently the first to present experimental data for conservative substances and for a first order reaction. Denbigh\(^29,27,28\) extended the treatment in many ways. Other work on various continuous reaction schemes was done by MacMullin and Weber\(^77\), Olsen and Lyons\(^106\), and Brothman\(^5\).

In 1950 Eldridge and Piret\(^33\) reviewed the previous work, gave their version of the derivation for a cascade of CSTR's, and gave the first extensive data for verification of the model for one to five stages and a first order reaction. They also discussed stepwise solutions, algebraic and graphical, for reactions of known order.
In 1951, Jones presented a graphical method to do away with the lengthy computations found in the various analytical derivations. His work was a generalization of the work of Eldridge and Piret because he did not confine himself to reactions of known order. In Jones' paper was the first hint of a discrepancy involving the basic assumptions made in the derivations. The two basic ones in almost all previous work were

1. concentration of effluent equals concentration everywhere in reactor, and

2. rate of reaction is the same as would be given from a batch experiment at that concentration.

In the CSTR work by MacMullin and Weber a special case was considered in which rate was not a single valued function of concentration, so that the analytical approach could not be used. They suggested a graphical integration approach different from the ones that followed (up to Jones' time):

They defined $Q = f(t)$, a property like conversion, a function of time and $y = 1 - e^{-t/\theta} \left[ 1 + \frac{t}{(\theta)} + \cdots + \frac{1}{(n-1)!} \left( \frac{t}{\theta} \right)^{n-1} \right]$

a residence time distribution for n CSTR's in series and let $Q$ in $n^{th}$ vessel $= \int_{0}^{y} Q \, dy$ (11)
This approach required different assumptions. In place of (2) MacMullin and Weber used

(3) no interaction between fluid elements.

Jones pointed out that either model gave the same results for first order reactions. He thought they differed in a regular fashion for orders other than one, but he couldn't prove it. He concluded that the proper set of assumptions would have to be determined by experiment.

In 1953 Weber\textsuperscript{136} presented a paper in which a plug flow reactor with recycle was considered, and he presented both analytical and graphical methods of solution. In a discussion of the paper Jones\textsuperscript{63} pointed out that here again two different viewpoints were evident:

(1) Conversion of a particular fluid element depends only on its residence time in the reactor (special case of MacMullin and Weber\textsuperscript{76}, Weber's analytical method\textsuperscript{136}).

(2) Conversion depends only on the concentration of the fluid element (graphical methods of Weber\textsuperscript{136}, Eldridge and Piret\textsuperscript{33}, Jones\textsuperscript{62}).

Thus Jones seems to be the first to clearly understand the existence of two distinct models of CSTR's.
About this same time work on non ideal residence time distributions (RTD's) was being done. Schoenemann\textsuperscript{122} presented a graphical solution for arbitrary batch data and arbitrary RTD's in continuous systems using segregation theory (i.e. the approach leading to equation (11)). He realized that this approach could strictly be applied only for first order reactions. Gilliland and Mason\textsuperscript{43} distinguished between bypassing (affecting only residence time distribution) and mixing (affecting homogeneity). Danokwerts\textsuperscript{21} in 1953 summarized RTD concepts. He stated that in liquids hydrodynamic effects are more important than diffusion effects in determining age distribution phenomena. He also presented the usual segregation formula (equation (11)) but pointed out that for reaction orders other than one, the chance of conversion of a molecule depends on the other molecules it encounters.

Gilliland et al.\textsuperscript{44} discussed non ideal RTD's and the use of segregation theory to predict conversions in fluidized bed reactors. They realized this approach was not sufficient for reaction orders other than one but stated "however an estimate of the magnitude of the effect of gas flow pattern can be obtained by considering bypassing as being distinct from mixing".
They presented interesting experimental data for a quasi second order reaction which seemed to confirm the conventional mixing model when mechanical mixing was used, but seemed to confirm the segregation model when mixing was due solely to gas flow and particle movement in the fluidized bed. They were perhaps the first to demonstrate theoretically that the segregated model gave higher conversions for second and third order homogeneous reactions than the well mixed model in a vessel with the RTD of a CSTR.

The problems involved in predicting chemical conversions in continuous flow reactors were well summarized by Danckwerts in 1958. He also conceptually organized the two limiting cases of segregation and mixing with clarity, defined quantitative measures of the intensity and degree of segregation, and qualitatively defined the scale of segregation. Zwiering extended Danckwerts' treatment and concluded that the distinction between the two limiting cases was whether "mixing" took place early or late in the residence time or a particular molecule. He gave a better representation of the degree of segregation, defined the state of "maximum mixedness", and presented a mathematical scheme for calculating conversions for maximum mixedness.
The application of segregation theory to heterogeneous reaction systems was initiated by Rietema\textsuperscript{115} in an attempt to find a "real world" application of the theory. He considered liquid-liquid and liquid-solid dispersions with reactions occurring in the dispersed phase or in a boundary layer around that phase. Heterogeneous segregation theory was extended by Curl\textsuperscript{19} and Spielman and Levenspiel\textsuperscript{126} to systems in which coalescence and redispersion of the dispersed phase could occur. Rietema\textsuperscript{116} reviewed segregation effects in liquid-liquid dispersions in 1964.

After Zwietering's\textsuperscript{143} formulation of the two extreme cases of complete segregation and maximum mixedness, the next logical step was the formulation of partial segregation models to study chemical conversions for intermediate micromixing cases. Zwietering\textsuperscript{143} assumed that a molecule spent all of its residence time in either an entering environment (complete segregation) or a leaving environment (maximum mixedness). Ng and Rippin\textsuperscript{101} proposed an intermediate model in which rate of transfer of material from the entering to leaving environment was proportional to the amount of material remaining in the entering environment. They presented theoretical results as a function of a transfer parameter, R.
and showed that the two extreme cases of complete segregation and maximum mixedness corresponded to their model with \( R = 0 \) and \( R = \infty \), respectively. They also presented preliminary data indicating a small, finite value of \( R \) in an experimental system. A later paper by Rippin\(^{117}\) demonstrated the calculation of degree of segregation, \( J \), as a function of \( R \) for a given RTD, using the model of Ng and Rippin\(^{101}\). Weinstein and Adler\(^{137}\) presented two different one-parameter partial segregation models. Rippin\(^{117,118}\) has discussed macromixing-micromixing interactions and the necessity of having a micromixing model to separate the two effects.

The application of segregation theory to microbial reactors was probably initiated by Danckwerts\(^{23}\) in discussion of a paper by Kramers. He pointed out that if the influent to a microbial reactor had no organisms, and complete segregation occurred, then no growth could occur because the influent would not mix with the contents of the reactor which contained the organisms. Evidence of possible partial segregation phenomena has been found, however. Hansford and Humphrey\(^{49}\) observed an effect of influent location on mixing parameters and yield in continuous
cultures of yeast. Gaudy et al.\textsuperscript{41}, Ramanathan and Gaudy\textsuperscript{110}, and Herbert et al.\textsuperscript{53} observed a "tailing off" phenomenon in continuous bacterial cultures in which cells were not completely washed out as dilution rate approached $\mu_m$, but were washed out gradually with steady state levels existing for $D > \mu_m$. Herbert et al.\textsuperscript{53} suggested imperfect mixing as a possible explanation. Tsai et al.\textsuperscript{135} presented theoretical results for complete segregation and maximum mixedness in CSTR's for microorganisms growing according to a Monod model modified with an endogenous respiration term. Fan et al.\textsuperscript{34} extended the consideration to a series of CSTR's and to a CSTR-PFR sequence. They considered complete segregation, maximum mixedness, sequential segregation, and sequential mixedness. The general conclusion of the papers of Tsai et al.\textsuperscript{135} and Fan et al.\textsuperscript{34} was that for any RTD segregation effects are unfavorable to substrate conversion and to growth. They gave a specific example showing that RTD and degree of segregation, $J$, are not sufficient to determine conversion, as explained by Rippin\textsuperscript{117,118}.

The effects of partial segregation on the results of continuous cultivation of microorganisms are now considered for the one-parameter model of Ng and Rippin\textsuperscript{101}. 
The kinetic model is
\[ \frac{db}{dt} = \mu m b \quad s > 0 \quad (12) \]
\[ \frac{db}{dt} = 0 \quad s = 0 \quad (13) \]

Influent microorganism concentration is taken to be zero. The theoretical implications of the approach are illustrated in Figure 4, and the theoretical developments are shown in the APPENDIX, section E.

A second model of imperfect mixing is considered in Figure 4 and the APPENDIX, section E. This model considers a CSTR with partial segregation as above and with recycle of microorganisms to the influent. The volume of the recycled material is assumed to be negligible. This model corresponds physically to cases with some internal settling of microorganisms or to cases with some wall growth. It can also be used when there is an actual positive value of \( b_{in} \). It is not intended for homogeneous systems.

It can be seen that segregation effects provide yet another possible source of trouble in evaluating \( \mu(s) \) in continuous systems. "Tailing off" phenomena are not the result of an intermediate micromixing state but can be explained by other imperfect effects.

Physically, segregation effects can occur if significant concentration gradients exist on a scale
small in comparison to the scale of RTD measurement. RTD's usually are the consequence of turbulence within a reactor. The scale of turbulence might be expected to be large with respect to the scale of reaction centers (i.e. cells for microbial systems, molecules for homogeneous systems) and somehow analogous to the scale of segregation. In fact, the scale of turbulence is often the same order of magnitude as the objects causing the turbulence. However, in a crude sense, turbulent eddies are constantly formed, broken up, and reformed, so that mixing between eddies occurs. Even without such mixing, molecular diffusion should suffice to prevent segregation in fairly dilute, dispersed microbial cultures as discussed in section A (4). For dense, dispersed cultures and for cultures with large diffusion-resistant structures such as floc particles or capsular material, segregation effects could occur due to diffusion limitations. Partial segregation models could have utility in explaining and predicting results in such cases.
III. MATERIALS AND METHODS

A. Bacteria

Pure culture studies used *Escherichia coli* ML 35. The organism was maintained on tryptcase soy agar slants in a refrigerator.

Bacteria for continuous mixed culture studies were originally derived from raw sewage as it entered the primary clarifier of the activated sludge plant in Bellaire, Texas. The subsequent selection of organisms is described under section III.D. and in the RESULTS.

B. Nutrient Media

The following stock solutions were used in the amount of 1 ml per liter final solution volume (more was used in special cases).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO₄·7H₂O</td>
<td>22.5 g/l</td>
</tr>
<tr>
<td>FeCl₃·6H₂O</td>
<td>0.25 g/l</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>27.5 g/l</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>150 g/l</td>
</tr>
</tbody>
</table>

The following phosphate buffer was used in the amount of 10 ml per liter final solution volume (more in special cases). The initial pH of solutions with this buffer was about 7.4.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>34.0 g/l</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>87.0 g/l</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>70.8 g/l</td>
</tr>
</tbody>
</table>
A 15 g/l stock glucose solution was used in appropriate amounts.

For cultures using NO$_3^-$ as an electron acceptor, KNO$_3$ was used at the same concentration 720 mg/l.

All solutions were made up with deionized water.

C. Apparatus

Batch cultures were grown in various sizes and shapes of containers from 25 ml test tubes to 5 liter vessels. Systems were maintained at approximately constant temperature using a water bath or constant temperature incubator room. Aeration and mixing were provided by either sparged air or shaking on a shaker platform.

The continuous culture system consisted of a chemostat type system with control of influent flow rate, reactor volume, temperature, and air flow rate. A schematic diagram of the apparatus is shown in Figure 5. Media was pumped from two calibrated 20 l bottles by two Beckman Model 746 Solution Metering Pumps. Flow rates were determined from observation of the volumes of the feed bottles or by pumping rates into graduated cylinders. The Beckman pumps were found to be extremely consistent. Diluted phosphate buffer was in one feed bottle and glucose and salts were in the other. An effluent aspirator maintained a constant reactor volume (2-3 liter). Temperature
control was provided by an immersible Jumo thermoregulator and a Precision Scientific Co. no. 62690 electronic relay which controlled a five foot length of Electrothermal No. HT341 heating tape which was wound around the reactor. Temperature was controlled better than 25.0 ± 0.1°C. Air flowed through a diffuser stone at 10-20 scfh. The reactor was shown to have the RTD of a CSTR using step changes in influent tracer concentration. Cell suspensions were used as tracers. The oxygen transfer coefficient, \( K_{L/A} \), was between 40 and 90 hr\(^{-1}\), depending on air flow rate and volume.

D. Procedures

1. Preliminary Batch Experiments

Preliminary batch experiments with \textit{E. coli} ML 35 tested effects of initial glucose and bacterial concentrations on batch growth curves and compared agitated systems to quiescent systems. Glucose, soluble carbon, cell mass, optical density, dissolved oxygen, and pH were measured as necessary. Three sets of experiments were performed for three different basic environments: agitated aerobic, quiescent aerobic, and quiescent anaerobic with NO\(_3^{-}\). The NO\(_3^{-}\) systems used NO\(_3^{-}\) as an electron acceptor. For these systems O\(_2\) was purged from the medium by bubbling with N\(_2\), the cells were stirred or mixed
with the O₂-free medium, and culture was dispensed into tubes which were filled and sealed. For all batch experiments nutrient media was inoculated with E. coli ML 35 from an agar slant, and the culture was allowed to grow in a shake flask overnight at about 28°C. This stock culture was used to inoculate test systems. Aseptic procedures and autoclaved materials were used.

2. Continuous Culture Experiments

The bacteria for continuous mixed culture studies were originally obtained from raw sewage. The raw sewage was aerated overnight, homogenized, and filtered through Whatman No. 2 filter paper, and then used to seed a 4 liter culture vessel. Prior to continuous operation the resulting culture was maintained on a fill and draw basis at room temperature with sparged aeration. The organisms retained each day were filtered through Whatman No. 2 filter paper if there was any evidence of clumping. After four weeks continuous operation was initiated, no problems with maintaining a completely dispersed culture having been encountered. General maintenance of the continuous system included periodic chromic acid cleaning of pumps, feed bottles, and feed lines and prevention of wall growth by manually wiping the sides of the reactor. Contamination of the influent was virtually
eliminated by separation of the media components. Parameters measured during operation included glucose, soluble carbon, cell mass, cell numbers, optical density, pH, and temperature.

The continuous system was operated without temperature control except by laboratory air conditioning for about three months. Careful temperature control as described above was used for the final four months of experimentation. During both periods steady states were investigated over a range of dilution rates with influent glucose concentrations of 50-1,000 mg/l. Batch experiments were performed periodically to determine $\mu(T)$ and $\mu(s)$ for comparison with continuous steady state results.

After temperature control was established systematic sets of batch experiments using steady state continuous culture organisms for inocula were performed, with reaction vessels placed in a 25.0 ± 0.5°C water bath. Reaction volumes of 50-200 ml were used, and aeration and mixing were accomplished by sparged air. Initial glucose concentrations of 20-500 mg/l and initial bacterial concentrations of 1.5-100 mg/l were used. These experiments were used to define growth rates and yields as functions of time and dilution rate for prediction of transient responses.
Transients involved step changes in dilution rate and step or impulse changes in influent glucose concentration. Transients were initiated only after steady states had existed for at least three residence times. Changes in dilution rate were accomplished by changing pumping rates or culture volume. Changes in influent glucose concentration were accomplished by stopping the pumps, draining the glucose-salts feed line and pump, changing to a new glucose-salts feed bottle, and initiating flow again. This operation took only about two minutes.

E. Analyses

1. Sampling

Samples were taken by passing aliquots of culture through 0.45μ filters (Gelman no. 01227, 47 mm). For b > 100 mg/l one or two fiber glass filters (Gellman, Type A, 47mm) were placed on top of the membrane filter. Filters were held in place by a Millipore pyrex filter holder which was placed in the mouth of a 1 liter vacuum filtering flask. Filtrate was collected in 75 ml test tubes inside the flask. Filtrate was then transferred to small test tubes which were sealed with parafilm and frozen until analyses were convenient.

2. Glucose (G)
Glucose was determined by the glucostat test (Worthington Biochemical Corp.) on thawed filtrate. One vial each of Chromagen and glucostat reagent were dissolved in 50 ml deionized water; four ml of reagent were added to 2 ml of each sample and the mixtures allowed to react for exactly 1 hour at room temperature. The reactions were stopped by the addition of 1 drop of 4M HCl. Optical densities were then read either by scanning around or direct reading at 400 με using a Beckman DK-2A recording spectrophotometer, and the results were compared to those for standard glucose solutions. This procedure gave very accurate results in the range 1 to 50 mg/l of glucose. Results indicating less than 1 mg/l were assumed to be zero.

3. Soluble carbon

Soluble organic carbon was measured with a Beckman Carbonaceous Analyzer on thawed filtrate. Samples were acidified with one drop of reagent grade HCl and stripped of CO₂ by bubbling with N₂ for ten minutes. Filters prewashed with 150 ml deionized water were used when carbon determinations were to be made. Soluble organic carbon was found to correspond directly with glucose concentration so that carbon data is not reported.
4. Optical density (OD)

Cell mass was determined by optical density of culture samples at 550 μm with a Bausch and Lomb Spectronic 20 or at 450 μm with a Beckman DK-2A spectrophotometer. When using the Spectronic 20 culture samples were diluted so that the OD was less than 0.12, or predetermined correction graphs were used to correct for nonlinearity. The DK-2A gave linear results up to 1.00, and no dilution was necessary. OD was correlated to cell mass by periodic dry weight determinations. Almost all quantitative experimental measurements were for OD, but values have been converted to cell mass (dry weight basis) in the tables and figures which follow by using the conversion factor dry weight (mg/l) = 300 x OD (550, Spec. 20) and by converting OD (450, Beckman) to OD (550, Spec. 20) by daily comparisons of the two measurements. However, logarithmic plots retain the OD measurement values. OD measurements were very consistent and repeatable and were judged superior to other cell mass measurements. OD measurements were used for this reason in the pioneering works of Monod93,92.

5. Dry weight
Dry weight measurements of cell mass were made by the membrane filtering technique. Filters (see (1)) were prewashed with 150 ml deionized water, placed in aluminum weighing pans, heated to dryness overnight at 100°C, dessicated at room temperature for at least 30 minutes, and tared. Known volumes of culture were passed through the prepared filters, and the filters were again placed in their respective pans, dried at 100°C, dessicated, and weighed. The change in filter weight and the volume of culture passed through the filter gave the desired dry weight concentration of bacteria.

6. Dissolved oxygen (DO)

Dissolved oxygen measurements were made by the azide modification of the Winkler method\textsuperscript{127}.

7. pH

pH measurements were made with a Beckman Zeromatic pH Meter.

8. Cell numbers

Occasionally, culture samples were diluted and plated on trypticase soy agar plates, incubated in the dark overnight at room temperature, and observed. Counts were not used quantitatively but rather to provide a source of organisms as well as a qualitative description of the culture in terms of plate culture morphology.
IV. RESULTS

A. Preliminary Batch Experiments

Some 39 batch experiments with *E. coli* ML 35 were performed---56 for agitated aerobic systems, 10 for quiescent aerobic systems, and 23 for quiescent anaerobic with \( \text{NO}_3^- \) systems. Table 1 summarizes key results for representative experiments.

Figure 6 demonstrates the effect of the initial glucose concentration, \( G(0) \), in agitated aerobic systems. \( G(0) \) ranges from 23.2 to 567 in these experiments. The effects of the dilution of the cultures and/or the addition of glucose to cultures are illustrated in Figure 7. Figure 8 illustrates the effect of varying the initial bacterial mass concentration, \( b(0) \).

Figure 9 demonstrates the effect of altering \( G(0) \) and \( b(0) \) in quiescent aerobic cultures, and Figure 10 illustrates the consistency of the stoichiometry involved by showing logarithmic plots of OD, \( E_G^s \), and \( E_{DO}^s \) vs. time. \( E_G^s \) and \( E_{DO}^s \) are related to glucose and dissolved oxygen uptake, respectively, and their logarithmic plots should have the same slope as that of \( \ln \text{OD} \) vs. time. Their significance is explained in the APPENDIX, section F. Some quiescent aerobic cultures were initially saturated with \( O_2 \) instead of air to allow longer growth phases. However, either \( O_2 \) was toxic at the high concentrations achieved (about 24 mg/l) or sufficient \( CO_2 \) was stripped
### TABLE 1
CHARACTERISTICS OF PRELIMINARY BATCH EXPERIMENTS WITH *E. COLI* ML 35

<table>
<thead>
<tr>
<th>Date</th>
<th>No.</th>
<th>b(0) mg/l</th>
<th>G(0) mg/l</th>
<th>Y</th>
<th>$\mu_m$ hr$^{-1}$</th>
<th>$T_C$ °C</th>
<th>Culture System</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-14</td>
<td>(1)</td>
<td>1.2</td>
<td>23.2</td>
<td>0.53</td>
<td>0.61</td>
<td>28.3</td>
<td>Agitated Aerobic</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>1.2</td>
<td>47.9</td>
<td>0.55</td>
<td>0.62</td>
<td>28.3</td>
<td></td>
</tr>
<tr>
<td>7-6</td>
<td>(1)</td>
<td>2.4 excess</td>
<td></td>
<td>-</td>
<td>0.60</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td></td>
<td>185</td>
<td>0.56</td>
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<td>483</td>
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<td>0.33</td>
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<td>4.8</td>
<td>30.4</td>
<td>0.38</td>
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<td>29.7</td>
<td></td>
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<tr>
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<td>(7)</td>
<td>4.8</td>
<td>60.8</td>
<td>0.36</td>
<td>-</td>
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<td>(8)</td>
<td>4.8</td>
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<td>0.48</td>
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<td>(9)</td>
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<td>0.34</td>
<td>0.47</td>
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<td>(10)</td>
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<td>483</td>
<td>-</td>
<td>0.48</td>
<td>29.7</td>
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</tr>
</tbody>
</table>
from solution to affect initial growth rates severely
(see Dagley and Hinshelwood\textsuperscript{20}) because long lag phases
in quiescent cultures and extremely slow growth in agitated
cultures (pure \textit{O}_2 in gas phase) resulted.

The use of \textit{NO}_3^- as an electron acceptor in quiescent
anaerobic with \textit{NO}_3^- systems also allowed longer growth
phases. Figure 11 illustrates results for such cultures
for ranges of temperature and \textit{G}(0).

In the above mentioned figures (6-11) the time axis
was shifted for a few experiments in order to account for
slightly different lag times or \textit{b}(0) values, and to
facilitate comparison with other experiments.

\textbf{B. Continuous System – Steady States}

Steady states in the continuous flow system were
studied by varying dilution rate (\textit{D}) and influent glucose
concentration (\textit{G}_{in}). Figure 12 summarizes the results of
many steady states. Figure 13 demonstrates a long term
steady state and Figure 14 demonstrates a short term
steady state. The attainment of steady states was con-
cluded and measured for time periods of 1 to 25 days,
depending on residence times. Steady state values of
glucose (\textit{G}) and bacterial mass (\textit{b}) for \textit{D}/\mu_m > 1 were
determined by extrapolating transient results after \textit{D}/\mu_m
was increased from slightly below 1 to greater than 1 in
a stepwise manner. It was assumed that \textit{b} would decrease
according to
\[ b = b(0)e^{(\mu - D)t} + \frac{D b_{st, st.}}{D - \mu} (1 - e^{-\frac{(D - \mu)t}{D - \mu}}) \quad (14) \]

where \( b(0) \) is the initial steady state value and \( b_{st, st.} \) is the steady state value for the particular value of \( D/\mu_m > 1 \). This equation is derived in the APPENDIX, section G. A plot of \( \ln b \) vs. time would yield a straight line of slope \( \mu_m - D \) (known) only for \( b_{st, st.} \neq 0 \). The results of such an experiment are shown in Figure 15.

For the case shown it is evident that \( b_{st, st.} < .01b(0) \), leading to the conclusion that \( b_{st, st.} \neq 0 \) and \( G_{st, st.} = G_{in} \) where \( G_{st, st.} \) is the final steady state glucose concentration in the reactor.

Batch experiments with inocula from the continuous system were performed for the same purposes as the preliminary pure culture batch experiments. Although experiments were not as extensive as their pure culture counterparts, the same type results were clearly evident. That is there were abrupt transitions from log phases to stationary phases for both agitated and quiescent cultures, quiescent culture had the same maximum growth rates as agitated cultures, and at high dissolved oxygen concentration \( O_2 \) was apparently toxic. A representative result is shown in Figure 16, and pertinent characteristics of agitated batch experiments are listed in Table 2.

C. Continuous System - Transients
TABLE 2
CHARACTERISTICS OF BATCH MIXED CULTURES USING CONTINUOUS CULTURE INOCULA

<table>
<thead>
<tr>
<th>Date</th>
<th>$\frac{D}{\mu_m}$</th>
<th>b(0) mg/l</th>
<th>c(0) mg/l</th>
<th>Batch T °C</th>
<th>Y</th>
<th>$\mu_m$ hr⁻¹</th>
<th>Culture Type</th>
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<td>0.32</td>
<td>lin</td>
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<td>4.5</td>
<td>89</td>
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<td>0.49</td>
<td>0.35</td>
<td>lin</td>
</tr>
<tr>
<td>1-30</td>
<td>1.24</td>
<td>4.5</td>
<td>89</td>
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<td>0.59</td>
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<td>$G(0)$</td>
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<td>$Y$</td>
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Transients induced by a step change in $D$ are termed set A transient experiments, transients induced by a step change in $G_{1n}$ are termed set B transient experiments, and transients induced by an impulse change in $G_{1n}$ are termed set C experiments. Table 3 catalogs the pertinent variable changes for all transient experiments.

Table 3 also lists culture type as being logarithmic or linear. Batch experiments using continuous culture inocula indicated the existence of these two distinct culture types which are discussed below in the DISCUSSION and the APPENDIX, section I.

As explained in the DISCUSSION and in the APPENDIX the results of such batch experiments are vital for predicting transient responses. The pertinent parameters for batch growth as a function of $D$ and culture type are summarized in Table 4. Figure 17 shows an extensive set of batch experiments for a linear culture type, $D/\mu_m \leq .30$, and with varying $b(0)$ and $G(0)$. Figure 18 shows the corresponding apparent log growth rates as functions of time for each $b(0)$.

The results of transient experiments are shown in Figures 19 through 33. Also shown are the predictions of the results made from the batch experiments summarized in Table 4. The methods of prediction are outlined in the APPENDIX, sections I and J.
TABLE 3

CATALOG OF TRANSIENT EXPERIMENTS AND PARAMETERS FOR PREDICTIONS

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<th>Final</th>
<th>Parameters Used In Predictions</th>
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<td></td>
<td></td>
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<td>Final</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>$G_{in}$</td>
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V. DISCUSSION

As mentioned in the BACKGROUND there is quite a controversy over the dependence of growth rate on substrate concentration. Even for such an easily metabolizable substrate such as glucose, reported $K_m$ values vary from less than 1 mg/l\textsuperscript{131,132} to more than 100 mg/l\textsuperscript{141}. Problems complicating $\mu(s)$ interpretations are also discussed in the BACKGROUND.

At least two approaches can be used for investigating $\mu(s)$ in batch cultures. For a single batch culture the point of deviation from log growth can be determined, and the subsequent transition to the stationary phase investigated. Practical difficulties in this approach include problems with accuracy and the provision of sufficient time in the log phase for very dilute cultures and problems with rapidly changing variables and less constancy of the chemical environment for more concentrated cultures.

Alternatively, sets of batch cultures in which individual cultures differ only in $s(0)$ can be investigated with attention centering on the deviation of growth curves with lower $s(0)$ from curves with higher $s(0)$.

Both approaches were used in this investigation. Individual cultures typically demonstrated very sharp transitions from log growth to the stationary phase, as
evidenced in Figures 6 through 11. The three sets of experiments illustrated in Figure 11 provide particularly convincing evidence of the lack of measurable dependence of \( \mu \) on \( s \). Figure 7 demonstrates that wide changes in glucose concentration effected by addition of glucose or dilution of the culture have no effect on the growth rate of log phase cells.

The conclusions above hold for both quiescent and agitated cultures, and for both pure and heterogeneous cultures, indicating that mixing and thus turbulent diffusion are not important for the dilute (less than 300 mg/l dry weight) cultures of dispersed bacteria used. Thus the simple molecular diffusion considerations in the BACKGROUND seem to provide an explanation for the lack of any observable dependence of \( \mu \) on \( s \). Kehrberger et al.\(^{64}\) presented results from stirred and quiescent BOD bottle systems which indicated that mixing accelerated the growth rate. They concluded that substrate diffusion limitation provided a reasonable explanation of this phenomenon. Results of the present investigation indicate that the maximum log growth rates are the same for both agitated and quiescent cultures (see Table 1). However lag phases are longer in quiescent systems (Figures 9 and 10). When parallel agitated cultures were compared to quiescent cultures, it was found that agitated cultures grew at a maximum rate almost immediately but quiescent cultures
required several hours to reach the log phase. It appears that the results of Kehrberger et al.\textsuperscript{64} showed this same effect since the growth phases of their cultures were very short due to the dissolved oxygen restriction in BOD bottle systems. That is, their results showed acceleration of the lag phase, not the log growth rate. The quiescent, anaerobic with \( \text{NO}_3^- \) cultures illustrated in Figure 11 also demonstrate long lag phases before the log phases begin.

The use of CSTR's for kinetic studies is very attractive because the physical and chemical environment can be held constant, and the growth rate can be altered by changing the dilution rate of the system. However the scale of the environmental constancy is very important. It cannot be concluded that rates are directly related to measured concentrations unless verification of mixing on a molecular scale (maximum mixedness) is given. Arguments presented in the BACKGROUND indicate that molecular diffusion alone should prevent significant concentration gradients and thus segregation. These arguments are experimentally verified by the batch culture results reported herein and discussed above. As shown in Figure 12 the steady state CSTR results completely support the maximum mixedness hypothesis for glucose utilization rates up to about 300 mg/l/hr, up to about 500 mg/l, and bacterial growth rates up to about 0.5 hr\(^{-1}\). Thus the
CSTR can be used for basic kinetic interpretations at least within these rate limitations. Glucose concentration is approximately zero (≤ 1 mg/l) up to $D/\mu_m = 0.97$, providing further evidence that $\mu$ is practically independent of glucose concentration for the cultures used in this research.

The prediction of steady state CSTR cultures for $\mu$ independent of s is outlined in the APPENDIX, section C, for the maximum mixedness condition. Section H outlines the effect of endogenous metabolism, as usually modeled, on this prediction. As indicated there, endogenous metabolism and maintenance energy requirements, as usually modeled, are mathematically equivalent. Figure 12 shows that the use of an endogenous metabolism refinement provides excellent representation of experimental results. Systematic investigation of culture behavior after glucose was exhausted and cell mass began to decrease was not made, however several experiments yielded endogenous metabolism coefficients of the same order as the .05 hr⁻¹ value used in Figure 12.

Except for one case steady states from $D/\mu_m = .11$ to 0.97 were evidently stable as the examples illustrated in Figures 13 and 14 demonstrate. The exception is discussed later.

For the prediction of transient responses it might seem reasonable from preliminary batch culture results.
and steady state CSTR results to assume \( \mu \) becomes maximum as soon as glucose increases to a measurable value from its steady state value (\( \leq 1 \text{ mg/l} \)). However, unless \( D = \mu_m \) for the steady state culture, a lag phase would be expected as in batch cultures using non log phase cells for inocula.

The logical step seemed to be to perform small scale batch experiments using cells from the steady state reactor for inocula. The resulting batch growth curve would allow the modeling of \( \mu = \mu(t) \) during the lag and log phases. The results of such batch experiments are shown in Tables 2 and 4 and Figures 17 and 18.

A surprising development was the appearance of linear growth for some cultures (that is \( \frac{db}{dt} \) proportional to \( b(0) \)). There were three separate periods of time in which batch cultures showed this type of growth for a short time before going into a normal lag phase and then log phase where \( \frac{db}{dt} \) is proportional to \( b \). The results for an extensive set of experiments on a culture at \( D/\mu_m = 0.30 \) showing linear growth are shown in Figures 17 and 18. As in other cases, linear growth rate was apparently independent of glucose concentration.

For cultures showing normal log type growth in batch cultures (log type cultures) and those showing a linear phase in batch cultures (linear type cultures), once
log growth started, results were very similar. The initial log growth rate, \( \mu(0) \), was greater than \( D \) but generally less than \( \mu_m \). Transient growth in continuous cultures also demonstrated this instantaneous increase in \( \mu \) from \( D \) to \( \mu(0) \). This immediate increase in \( \mu \) has been observed by others\(^{90,130,86,120,142} \) for transients involving step changes in \( s_{\text{in}} \) or \( D \). Such an immediate increase in \( \mu \) is possible only if \( \mu \) is practically independent of \( s \), because \( s \) increases at most exponentially after step increases in \( D \) of \( s_{\text{in}} \). However, if growth rate is dependent on \( s \) only for very low values, \( s \) can almost immediately increase to the point where \( \mu \) is independent of \( s \).

McLellan\(^{91,90} \) introduced the concept of available reaction potential to explain the immediate increase in \( \mu \), and Storer and Gaudy\(^{130} \) proposed growth rate hysteresis to explain the increase in \( \mu \) with time. A hopefully more meaningful explanation is offered below.

As discussed above, \( \mu \) is independent of \( s \) for \( s \) above a very low concentration. The steady state growth rate, \( D \), determines the chemical composition of cells and thus their maximum possible growth rate unless that composition is changed. This maximum possible growth rate is less than or equal to \( \mu_m \) since, in general, RNA and ribosomes, which ultimately control growth rate\(^{52,67,120} \), are present at lower than maximum concentration\(^{52} \). The effect of \( D \)
on $\mu(0)$ as shown in Table 4 is very similar to the effect of $D$ on cellular RNA$^{52}$ and various metabolic activities$^{79, 51, 52, 18, 56, 104, 95}$. Thus an increase in $D$ or $s_{in}$ increases the possible growth rate above the original $D$ by increasing $s$ very slightly. While $\mu$ is greater than the original $D$, the chemical composition changes so that the maximum growth rate $\mu_m$ is approached. Thus flux of substrate, $\frac{Ds_{in}}{b}$, seems to be the controlling factor rather than concentration of substrate. A step increase in $D$ or $s_{in}$ gives a step increase in this flux. Whether or not $s$ increases measurably depends on the size of the step increase in flux and the maximum possible initial substrate removal rate. For log growth the substrate mass balance is

$$\frac{ds}{dt} = D(s_{in} - s) - \frac{\mu b}{Y} \tag{15}$$

At the instant the transient is applied $s = 0$ and $b = Y(D^0)s_{in}^0$. Then $s$ will increase if

$$D^f s_{in}^f > \frac{\mu(0)Y(D^0)s_{in}^0}{Y(\mu_m)} \tag{16}$$

The superscripts $o$ and $f$ refer to initial and final values, respectively, and if $s$ is to increase it is assumed $Y$ will become $Y(\mu_m)$. Thus comparison of the new flux, $\frac{D^f s_{in}^f}{Y(D^0)s_{in}^o}$, to $\frac{\mu(0)}{Y(\mu_m)}$ determines whether $s$ will increase.

For linear type cultures this same comparison is used to
see if the culture can handle the transient with log
growth without s increasing. If the culture cannot do
this, a comparison of
\[ \frac{D^f \, s^f_{in}}{\bar{Y}(D^f) s^c_{in}} \]  to
\[ \frac{k_L}{\bar{Y}_{linear}} \]
is made, based on the mass balance
\[ \frac{ds}{dt} = D(s_{in} - s) - \frac{k_L b(0)}{\bar{Y}_{lin}} \] (17)
where \( \bar{Y}_{lin} \) is higher than \( \bar{Y}_{log} \) due to the nature of linear
growth (see Table 4).

The procedures for the exact prediction of transient
responses are outlined in the APPENDIX, sections I and J,
and the agreement with actual results is shown in Figures
19 through 33. Set A experiments (Figures 19 through 23)
show that large increases in D lead to an increase of
glucose in the reactor while smaller increases in D can
be handled without such an increase. Transient experiments
A.5 and A.7 show linear type cultures responding linearly
to prevent glucose from increasing, while experiment A.6
shows a linear type culture able to respond logarithmically
and still prevent an increase in glucose concentration.
Figure 22 demonstrates the interesting phenomenon of
both b and s increasing even though \( s_{in} \) is constant. Set
B experiments (Figures 24 through 31) show that slower
growing cultures can respond to increases in \( s_{in} \) better
than faster growing ones. This is due to relatively large
\( \mu(0)/D \) values at low dilution rates. In predicting responses \( Y \) should probably be taken as \( Y(\mu) \), similar to \( Y(D) \) observed in steady state results. Figures 25, 27, and 30 show predictions using both \( Y(\mu_m) \) and \( Y(D) \). Figure 25 shows cultures first following curves for \( Y(\mu_m) \) when \( \mu \) is relatively high and then following curves for \( Y(D) \) as \( \mu \) approaches \( D \) again.

A detailed theoretical comparison of transient responses of linear and log type cultures is shown in Figures 34 through 37. The comparison is made for the case \( D^0 = 0.15 \text{ hr}^{-1} \), \( s^0_{in} = 100 \text{ mg/l} \), \( \mu_m = 0.50 \text{ hr}^{-1} \), \( D/\mu_m = 0.33 \), \( Y(D^0) = 0.47 \), \( Y(\mu_m) = 0.55 \), \( Y_{lin} = 0.80 \), \( k_L = 0.85 \text{ hr}^{-1} \), \( t_L = 3.0 \text{ hr} \), \( \mu(0) = 0.35 \text{ hr}^{-1} \), and \( a = .05 \text{ hr}^{-2} \). It is apparent that the ability to respond in a linear type growth enables cultures to cope better with step increases in \( D \) and \( s_{in} \). This is because \( k_L \) is significantly larger than \( \mu(0) \). The responses shown in Figure 34 are particularly interesting because of the delay in the glucose increase. McLellan\(^9\) observed such behavior in one of his experiments. He also observed a large increase in \( Y \), similar to the results for linear type cultures reported herein.

The one steady state experiment which exhibited instability was for a linear type culture at \( D/\mu_m = 0.11 \). Not enough data was taken for analysis of the instability,
however daily batch cultures using reactor contents for inocula demonstrated changes in the linear growth parameters. This instability could be due to periodic triggering of the linear type growth.

This research investigated several aspects of the prediction of bacterial growth in batch and CSTR systems. All of the batch, steady state, and transient experiments supported the idea of $\mu$ independent of $s$ above very low levels of $s$. However, there were observations of growth phases in which $\mu$ varied with time and even phases in which linear growth with an abnormally high yield were observed. Predictions of process performance would have to deal with these time dependencies. Of course, it would be desirable and gratifying to be able to predict completely growth behavior in batch and continuous systems for a particular culture from traditional batch studies using previously batch grown cells. No such attempt was made in this study. Instead of predicting the effect of growth rate on physiological state and thus the maximum possible growth rate, the physiological state of steady state systems was measured by the batch culture studies using steady state reactor cells for inocula. The parameters of the physiological state were the growth type, yield, and growth rate as functions of time. These are the parameters listed in Tables 3 and 4. The approach used for transient predictions is direct, practical, and accurate. It
should be applicable to the prediction of responses of continuous microbial systems to changes in $s_{in}$, $D$, and even to changes in substrate nature.
VI. CONCLUSIONS

1. Bacterial growth rate was independent of glucose concentration for glucose concentration above an immeasurably small amount (≈ 1 mg/l) for the dispersed cultures used. This was true for linear growth as well as logarithmic growth.

2. Growth rate was independent of glucose concentration for quiescent as well as agitated systems. Vigorous agitation did shorten lag phases, however.

3. The use of NO$_3^-$ as an electron acceptor provided a convenient system in which dissolved oxygen restrictions were avoided. $\mu_m$ and $Y$ were only slightly lower than for aerobic systems. $Y$ was very close to that found by Schroeder$^{123}$ using agitated NO$_3^-$ systems. Only one growth phase was observed rather than the two that Schroeder$^{123}$ observed.

4. There was no evidence of segregation effects in the CSTR cultures. Nor was there evidence of the imperfect mixing in which an effective influent bacterial concentration was considered. These conclusions are for $b < 500$ mg/l, $\mu < 0.5$ hr$^{-1}$, and glucose utilization rates less than 300 mg/l/hr.

5. Transient responses in microbial CSTR's can be predicted from batch cultures using steady state reactor microorganisms for inocula. Predictions must be based
on culture type (log or linear), yield, and growth rates as functions of time, these factors being determined from the batch cultures. Predictions cannot be made from interpretations of $\mu(s)$ for the transition from the log phase to the stationary phase in batch cultures.

6. Several complicating conditions which may lead to incorrect $\mu(s)$ formulations are discussed. These include the use of complex or multisubstrate media, the appearance of linear growth phases, pH and toxic product rate limitations, diffusion and mass transfer limitations, and partial segregation.
VII. FUTURE RESEARCH

The results of this research suggest the following avenues of further research:

1. The use of $\text{NO}_3^-$ as an electron acceptor in continuous flow experiments with dense cultures of dispersed microorganisms. The use of $\text{NO}_3^-$ would circumvent inter-phase $\text{O}_2$ transfer restrictions and allow the use of dense cultures. Such cultures could be used to test substrate mass transfer limitations using various modeling approaches when consideration of diffusion to more than one bacterium might be necessary. Such cultures could also be used to test segregation theories for much higher substrate utilization rates than used in the present research.

2. The use of systematic batch cultures investigating the effect of varying periods of endogenous metabolism after log growth on lag phase phenomena in subsequent subcultures. Attempts could be made to predict continuous system performance from such batch culture results.

3. CSTR studies with an inadequately buffered medium compared to CSTR studies with the same culture but with pH control. Results such as those predicted in Figure 3 could be tested.

4. CSTR studies with substrates and cultures exhibiting diauxic growth to test the ability of the method used in this research to predict responses to changes
in substrate nature and to test the predictions given in the APPENDIX, section C, for steady state results for two substrates supporting different growth rates.

5. The investigation of physiological factors involved in linear growth phases of certain bacterial cultures, and possible oscillations occurring at low growth rates in CSTR's due to this phenomenon.
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APPENDIX

A. Linear Growth in Batch Cultures

Let \[ \frac{db}{dt} = k_L b(0) \]  \hspace{1cm} (A1)

\[ \Rightarrow b = b(0) + k_L b(0) t \]  \hspace{1cm} (A2)

also \[ s = s(0) - \frac{k_L b(0) t}{Y} \]  \hspace{1cm} (A3)

(A1), (A2) \[ \Rightarrow \frac{1}{b} \frac{db}{dt} = \frac{k_L}{1 + k_L t} \]  \hspace{1cm} (A4)

(A3) \[ \Rightarrow t = Y \frac{(s(0) - s)}{k_L b(0)} \]  \hspace{1cm} (A5)

(A4), (A5) \[ \Rightarrow \text{apparent } \mu = \mu^a = \frac{1}{b} \frac{db}{dt} = \frac{k_L}{1 + Y \frac{(s(0) - s)}{b(0)}} \]  \hspace{1cm} (A6)

This is of the form

\[ \mu^a = \frac{k_1}{k_2 - s} \]  \hspace{1cm} (A7)

where \( k_1 = k_L b(0) \)  \hspace{1cm} (A8)

and \( k_2 = b(0) + Y s(0) \)  \hspace{1cm} (A9)

Figure 1 illustrates \( \mu^a(s) \) for various values of \( b(0) \)
and \( s(0) \) assuming \( Y = 1.0 \), and \( k_L = 2 \mu_m \).

B. pH and Toxic Product Effects

Let \[ \mu_m = \mu_m^i - b(pH - pH')^\alpha \]  \hspace{1cm} (A10)

and assume \[ pH - pH'' = a(\Delta s)^\beta \]  \hspace{1cm} (A11)

where \( pH' \) is the pH for maximum \( \mu, \mu_m^i; pH'' \) is the initial pH of the culture medium; \( \alpha, \beta, a, b \) are constants; and \( \Delta s = s(0) - s \). Assume \( pH' = pH'' \) for
simplification. Then (A10) and (A11) imply

$$\mu'_m = \mu'_m - ba \Delta s^{\alpha+\beta}$$  \hspace{1cm} (A12)

Now assume substrate concentration does not affect

$$\mu$$ so that $$\mu = \mu_m$$ for $$s > 0$$  \hspace{1cm} (A13)

Case 1: $$\mu'_m = 1.0$$, $$b = 0.10$$, $$a = 10^{-5}$$, $$\alpha = \beta = 2$$

Thus $$\mu = 1.0 - 10^{-11} \Delta s^4$$  \hspace{1cm} (A14)

Figure 2 illustrates the resulting $$\mu(s)$$ in batch systems for various values of $$s(0)$$. These results can be used for a CSTR with $$b_{in} = 0$$ as follows:

For a particular $$s_{in}$$ ($$s(0)$$ in batch systems), find $$D_c$$ such that $$\mu(s = 0) = D_c$$. Then for $$D < D_c$$ s will be zero; for $$D_c < D < \mu'_m$$ s will be a positive amount such that $$D = 1.0 - 10^{-11}(s_{in} - s)^4$$; for $$D > \mu'_m$$ s will equal $$s_{in}$$. Figure 3 illustrates these results.

Case 2: $$\mu'_m = 1.0$$, $$b = 0.10$$, $$a = 2.5\times10^{-3}$$, $$\alpha = \beta = 1$$

Thus $$\mu = 1.0 - 2.5\times10^{-4} \Delta s$$  \hspace{1cm} (A15)

Figures 2 and 3 also illustrate batch and CSTR results for this case.

C. Growth in a CSTR with $$\mu$$ Independent of $$s$$

When there is no rate dependence on substrate concentration, the following mass balances and steady state solutions apply only for $$s > 0$$:
\[
\frac{db}{dt} = D(b_{in} - b) + \mu b 
\]  \hspace{1cm} (A16)

\[
\frac{ds}{dt} = D(s_{in} - s) - \mu \frac{b}{Y} 
\]  \hspace{1cm} (A17)

steady state: \( b = \frac{Db_{in}}{D - \mu} \) \hspace{1cm} (A18)

\( s = s_{in} - \frac{b_{in}}{Y(D - \mu)} \) \hspace{1cm} (A19)

These solutions hold only for

\[
D \geq \frac{(b_{in} + Ys_{in})}{Ys_{in}} 
\]  \hspace{1cm} (A20)

obtained by setting (A19) greater than or equal to zero.

For \( D < \frac{(b_{in} + Ys_{in})}{Ys_{in}} \), \( s = 0 \), and \( b = b_{in} + Ys_{in} \).

This is intuitively obvious and can be shown to be the limiting case as rate dependence on substrate concentration becomes negligible for various \( \mu(s) \) formulations.

Now consider two substrates with concentrations \( s_1 \) and \( s_2 \), growth rates \( \mu_1 \) and \( \mu_2 \), and influent concentrations \( s_{1,\text{in}} \) and \( s_{2,\text{in}} \). Assume that for \( s_1 > 0 \) substrate 2 is not utilized, as in diauxic behavior. Assume also \( \mu_1 > \mu_2 \), and \( \mu_1, \mu_2 \) are independent of \( s_1, s_2 \) except for the diauxic behavior.

Now consider \( D < \mu_2 \) so that \( s_2 = 0 \). Since \( \mu_1 > \mu_2 \) assume there is an effective \( b_{in} = Ys_{1,\text{in}} \), and the only influent substrate is substrate 2. Then according
to the above development, \( s_2 = 0 \) for
\[
D < \frac{2(Y_{s1, in} + Y_{s2, in})}{Y_{s2, in}}
\] (A21)

By (A19)
\[
s_2 = s_{2, in} - \frac{2(Y_{s1, in})}{Y(D - \mu_2)}
\] (A22)

for \( D > \frac{2(Y_{s1, in} + Y_{s2, in})}{Y_{s2, in}} \)

Thus the maximum allowable \( D \) for complete substrate
utilization is greater than \( \mu_2 \), the maximum \( D \) if only
substrate 2 were present.

D. Diffusion Effects

1. Stagnant boundary layer around a spherical cell

The steady state diffusion equation for diffusion
in the boundary layer with spherical coordinates is
\[
\partial (a^2 \partial c + 2 r \partial c) = 0
\] (A23)

The solution of this equation is
\[
c = A + B/r
\] (A24)

The boundary conditions are
\[
\begin{align*}
\text{at } r = r_0, \quad &- \partial c \bigg|_r = N \\
\text{at } r = r_1, \quad &c = c_b
\end{align*}
\] (A25)

where \( r_0 \) and \( r_1 \) are the radius of the cell and radius of
the total cell-boundary layer sphere, respectively; \( N \) is
the flux of material at \( r = r_0 \); \( \partial \) is the diffusivity;
and \( c_b \) is the concentration at \( r = r_1 \). These boundary
conditions lead to
\[ c = c_b + \frac{N r^2}{D} \left[ \frac{1}{r_1} - \frac{1}{r} \right] \]  \hspace{1cm} (A27)

Then for \( r_o = 10^{-4}\text{cm}, \ r_1 = 2 \times 10^{-4}\text{cm}, \ N = \frac{4.45 \times 10^{-2}\text{gm}}{\text{cm}^2\text{sec}} \)

\[ J = 10^{-5}\text{cm}^2/\text{sec}, \ c_s = c \text{ at } r_o, \]

\[ c_b - c_s = \frac{N r^2}{D} \left[ \frac{1 - r_o}{r_1} \right] \] \hspace{1cm} (A28)
or \[ c_b - c_s = 2.23 \times 10^{-2}\text{mg/l.} \] \hspace{1cm} (A29)

2. Diffusion in an infinite medium

Rashevsky \^[113] considers the case of diffusion with constant reaction rate within a cell, membrane transport, and diffusion without reaction in an infinite medium outside the cell. His solutions are:

\[ c_i = -q(r_o^2 - r^2) - qr_o^2 - qr_o + c_o \] \hspace{1cm} (A30)

and \[ c_e = c_o - qr_o \frac{3}{D_e} \frac{1}{r} \] \hspace{1cm} (A31)

where \( q \) is the reaction rate, \( c \) is the concentration, \( c_o \) is the concentration at \( r = \infty \), \( D \) is diffusivity, \( r_o \) is the cell radius, \( h \) is the membrane permeability, and subscripts \( e \) and \( i \) refer to external and internal quantities, respectively. Then using

\[ q = \frac{N (\text{cell area})}{\text{cell volume}} = \frac{3N}{r_o} \] \hspace{1cm} (A32)
and \( D_1 = D_e = D = 10^{-5} \text{ cm}^2/\text{sec} \), \( r_o = 10^{-4} \text{ cm} \), \( h = \infty \)

\[
c_1 = \frac{-N(r_o^2 - r^2)}{2r_o D} - \frac{Nr_o}{D} + c_o (A33)
\]

\[
c_e = c_o - \frac{N r_o^2 \cdot 1}{D} \frac{1}{r} (A34)
\]

or \( c_o - c_e (r = r_o) = \frac{N r_o}{D} = 4.45 \times 10^{-2} \text{ mg/l}. \) (A35)

Also \( q \) is constant only for \( c_1 (r = 0) \neq 0 \). \( c_1 (r = 0) \) becomes zero for \( c_o = \frac{N r_o (1 + \frac{1}{2})}{D} = 3N r_o = 6.68 \times 10^{-2} \text{ mg/l}. \) (A36)

That is \( c_o \) must be greater than \( 6.68 \times 10^{-2} \text{ mg/l} \) for the above solutions to be meaningful.

**E. Partial Segregation**

The model for Nag and Rippin\(^1\) is considered for a CSTR using the batch kinetics

\[
\frac{db}{dt} = \mu m b \quad \text{for} \ s > 0, \ t \leq t_1 \quad (A37)
\]

\[
\frac{db}{dt} = 0 \quad \text{for} \ s = 0, \ t \geq t_1 \quad (A38)
\]

where \( t_1 \) is the batch time for \( s \) to reach zero. If \( b_{in} = 0 \) no reaction occurs in the entering environment, and the volume of the entering environment is wasted space. The volume of the leaving environment is\(^1\)

\[
V_{LE} = V \left[ \frac{R \tau}{R \tau + 1} \right] \quad (A39)
\]

where \( V \) is the total reactor volume, \( \tau = V/Q \) = residence time, and \( R \) is the transfer parameter. The predictions
are then identical to the usual case for maximum mixedness except that $D_{LE} = Q/V_{LE}$ is used in place of $D = Q/V$. That is for $D_{LE} > \mu_m s = s_{\text{in}}$ and $b = 0$. These results are shown in Figure 4.

For the case of recycled microorganisms it is convenient to assume an effective $b_{\text{in}}$ which is constant. This case is then formally identical to cases with actual influent microorganisms. The development is as follows, using the nomenclature of Ng and Rippin.\(^{101}\)

\[
\begin{align*}
\text{II} &= \frac{1}{\tau} \int_0^\infty (1 - e^{-R\lambda}) e^{-\lambda/\tau} e^{-\lambda/\tau} d\lambda \\
&= \frac{e^{-\lambda/\tau}}{\tau} \left[ \frac{R\tau}{R\tau + 1} \right] \\
\text{III} &= \frac{1}{\tau} \int_0^\infty e^{-R\lambda} e^{-\lambda/\tau} e^{-\lambda/\tau} d\lambda \\
&= \frac{e^{-\lambda/\tau}}{\tau} \left[ \frac{1}{R\tau + 1} \right]
\end{align*}
\]

\[
\begin{align*}
\text{IV}_{\text{b in}} &= \frac{1}{\tau} \int_0^{t_1} b_{\text{in}} e^{-R\alpha} e^{-\lambda/\tau} e^{-\lambda/\tau} d\lambda + \int_{t_1}^{\infty} (b_{\text{in}} + Y_{\text{in}}) e^{-R\alpha} e^{-\lambda/\tau} e^{-\lambda/\tau} d\lambda \\
&\Rightarrow e^{\lambda/\tau} \text{ IV} = e^{-t_1(R+D)} \left[ \frac{e^{t_1D}}{(R+D)(1-R-D)} \right] - \frac{D}{(1-R)(1-R-D)}
\end{align*}
\]

A mass balance for $b_{\text{LE}}$ yields

\[
\frac{db_{\text{LE}}}{d\lambda} = -\mu_m b_{\text{LE}} + \frac{R}{II} (\text{III} b_{\text{LE}} - \text{IV}_{\text{b in}})
\]

but the nature of II, III, and IV $\Rightarrow b_{\text{LE}}$ is independent of $\lambda$ $\Rightarrow b_{\text{LE}} = \text{constant}$, and the boundary condition
\[
\begin{align*}
\frac{db_{LE}}{dt} \bigg|_{\lambda = \infty} &= 0 \quad \Rightarrow \quad b_{LE}(\mu_m - \frac{III}{II}) = -RIV_{bin} \quad \text{(A44)} \\
\Rightarrow b_{LE} &= \frac{b_{in}(R+D)}{(\mu_m - D)} \left[ \frac{D}{1-R-D} - e^{-t_1(R+D)} \left( \frac{e^{t_1D}}{(R+D)(1-R-D)} \right) \right] \quad \text{(A45)}
\end{align*}
\]

Once \( b_{LE} \) is found, the effluent value of \( b, \bar{b} \), is given by
\[
\bar{b} = b_{LE} \left( \frac{R}{R+D} \right) + b_{in} \left( IV_{\lambda = 0} \bar{c} \right) \quad \text{(A46)}
\]

The mass balance for \( b_{LE} \) (A43), is valid only for \( s_{LE} \geq 0 \), or equivalently \( b_{LE} \leq b_{in} + Y_{s_{in}} \). Thus the constraint \( b_{LE} \leq b_{in} + Y_{s_{in}} \) is made, and \( D_c \) may be found for which \( b_{LE} \rightarrow b_{in} + Y_{s_{in}} \) as \( D \) decreases to \( D_c \). For \( D \leq D_c \), \( b_{LE} = b_{in} + Y_{s_{in}} \). \quad \text{(A47)}

Once \( \bar{s}(D) \) is found, \( \bar{s} = s_{in} + b_{in}/Y - \bar{c}/Y \) \quad \text{(A48)}

Results for this case are plotted in Figure 5 for \( s_{in} = 100, \mu_m = 1, b_{in} = 1 \) and 10, and \( R = 0, 1, 5, \) and \( \infty \).

F. The Significance of \( E_G' \) and \( E_{DC}' \)

Assume log growth, \( \frac{db}{dt} = \mu b \) \quad \text{(A49)}

and constant stoichiometry for nutrient \( n \),
\[
\frac{dn}{dt} = adb \quad \text{(A50)}
\]

\( (A50) \Rightarrow b = b(0) + \frac{n - n(0)}{a} \quad \text{(A51)}
\]

\( (A49) \Rightarrow \frac{dn}{dt} = a\mu b = a\mu \left[ b(0) + \frac{n - n(0)}{a} \right] \quad \text{(A52)}
\]

Now let \( E_n = n - n(0) + ab(0) \) \quad \text{(A53)}
\[
\frac{dE_n}{dt} = \frac{dn}{dt} \quad (A54)
\]
\[
\frac{dE_n}{dt} = \mu E_n \quad (A55)
\]

That is, a logarithmic plot of \(E_n\) vs. time should have the same slope as a logarithmic plot of \(b\) vs. time.

\(E_n\) is simply \(E_n\) multiplied by a constant so that \(b\) and \(E_n\) are of comparable magnitudes.

G. Transient Determination of Steady State \(b\) for \(D/\mu_m > 1\)

Assume steady state \(b = b_{st.st.}\) is due to an effective influent concentration \(b_{in}\). Then

\[
\frac{db}{dt} = D(b_{st.st.} - b) + \mu b \quad \text{for } s > 0 \quad (A56)
\]

The solution of this equation is

\[
b = b(0)e^{(\mu - D)t} + Db_{st.st.} \left[1 - e^{-(D-\mu)t}\right] \quad (A57)
\]

H. Endogenous Metabolism or Maintenance Energy Requirement Effect with \(\mu\) Independent of \(s\)

The usual model for endogenous metabolism in a CSTR is

\[
\frac{db}{dt} = \mu b - Db - k_2 b \quad (A58)
\]

\[
\frac{ds}{dt} = D(s_{in} - s) - \mu b/Y \quad (A59)
\]

For substrate maintenance energy requirement the usual model is

\[
\frac{db}{dt} = \mu b - Db \quad (A60)
\]
\[
\frac{ds}{dt} = D(s_{in} - s) - \mu b/Y - k_1 b
\]  
(A61)

Consider steady states with \( D < \mu_m \) where \( s = 0 \).

For the endogenous metabolism model

\[
\mu = D + k_2
\]  
(A62)

\[
Ds_{in} = \mu b/Y
\]  
(A63)

Let apparent yield \( Y^a = b/s_{in} \)

Then

\[
Y^a = \frac{DY}{D + k_2}
\]  
(A65)

A similar development for the maintenance energy model provides

\[
Y^a = \frac{DY}{D + k_1 Y}
\]  
(A66)

I. Linear Model For Transient Predictions

The model determined from batch systems is that cultures growing logarithmically in continuous culture with \( G = 0 \) shift immediately into linear growth when \( G \) becomes measurably more than zero for any reason.

In batch systems the equations are (A1), (A2), and (A3).

For continuous systems (CSTR's) the equations for linear growth are

\[
b = b(0) \left[ \frac{k_L}{D} + (1 - \frac{k_L}{D})e^{-Dt} \right]
\]  
(A67)

and

\[
s = \frac{k_L b(0) - s_{in}(e^{-D_t} - 1) + s(0)e^{-Dt}}{DY_{lin}}
\]  
(A68)

using \( s = G \). Linear growth continues for time \( t_L \) or
until $s$ reaches approximately zero. After time $t_L$ the culture begins to grow logarithmically at a rate

$$\mu = \mu(0) + at', \quad t' = t - t_L \quad (A69)$$

until $\mu = \mu_m$, if $s$ is positive. With respect to transient responses there are five different sets of equations depending on growth type (log or linear), whether $s$ is zero or greater than zero, and whether $\mu = \mu_m$ or $\mu < \mu_m$.

<table>
<thead>
<tr>
<th>Growth Type</th>
<th>$s$</th>
<th>$\mu$</th>
<th>Applicable Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>log $0 \leq \mu_m$</td>
<td>$b = b(0)e^{-Dt} + Y_{\log}sin(1 - e^{-Dt})$</td>
<td>(A70)</td>
<td></td>
</tr>
<tr>
<td>log $0 &lt; \mu_m$</td>
<td>$b = b(0)\exp\left[(-\mu(0)+D)t + at^2/2\right]$</td>
<td>(A71)</td>
<td></td>
</tr>
<tr>
<td>&amp; $s = S(0)e^{-Dt} + s_{\log}(1-e^{-Dt}) - b/Y_{\log}$</td>
<td>(A72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log $0 = \mu_m$</td>
<td>$b = b(0)\exp\left[(D - \mu)t\right]$</td>
<td>(A73)</td>
<td></td>
</tr>
<tr>
<td>&amp; $s = S(0)e^{-Dt} + s_{\log}(1-e^{-Dt}) - b/Y_{\log}$</td>
<td>(A72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lin $0$</td>
<td>$b = b(0)e^{-Dt} + Y_{\log}sin(1 - e^{-Dt})$</td>
<td>(A74)</td>
<td></td>
</tr>
<tr>
<td>lin $0 &lt; \mu_m$</td>
<td>$b = b(0)\left[k_L + \left(1 - k_L\frac{D}{Dy}\right)e^{-Dt}\right]$</td>
<td>(A67)</td>
<td></td>
</tr>
<tr>
<td>&amp; $s = k_Lb(0) + s_{\log}(1-e^{-Dt}) + s(0)e^{-Dt}$</td>
<td>(A68)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

where $S = s + b/Y_{\log}$

The procedures for predicting transient responses of
steady state systems to changes in $s_{in}$ and $D$ are outlined below.

1. Step increase in $D$ from $D^0$ to $D^f = D$
   
   a. If $\mu(0) > D$ glucose will remain zero, and the culture will remain in log type growth.
      Use (A70).
   
   b. If $\mu(0) < D$ the culture will respond in a linear type growth.

(1) If $Ds_{in} < k_Lb(0)/Y_{lin}$, glucose will remain zero. Use (A74) for $t \leq t_L$. For $t > t_L$
   
   (a) If $Ds_{in} < \mu(0)b(t_L)/Y_{log}$, glucose will remain zero. Use (A70), $t = t - t_L$.
   
   (b) If $Ds_{in} > \mu(0)b(t_L)/Y_{log}$, glucose will increase. Use (A71) and (A72) with $t = t - t_L$ until $s = 0$ at time $t_1$, in which case then use (A70) with $t = t - t_1$, or until $\mu = \mu_m$ at time $t_2$, in which case use (A73) and (A72) with $t = t - t_2$. Use these until $s = 0$ at time $t_3$ and then use (A70) with $t = t - t_3$.

(2) If $Ds_{in} > k_Lb(0)/Y_{lin}$, glucose will increase. Use (A67) and (A68) for $t \leq t_L$. For $t > t_L$ use (A71) and (A72) with $t = t - t_L$ until $s = 0$ or $\mu = \mu_m$ and then use (A70) or (A73) and (A72) and then (A70) as outlined
above, section 1. b. (1)(b).

2. Step increase in $s_{in}$ from $s_{in}^0$ to $\approx s_{in}^0 = s_{in}$
   a. If $\mu(0)/D > \approx$ glucose will remain zero, and
      the culture will remain in log type growth. Use
      (A70).
   b. If $\mu(0)/D < \approx$ the culture will respond in a
      linear type growth. Use the approach outlined
      in section 1. b. above.

3. Impulse change in $s_{in}$
   Glucose will instantaneously become $G(0) = s(0)$
   a. Use (A67) and (A68) until
      (1) $s = 0$ at time $t_1$. Then use (A70).
      (2) $t = t_L$. Then use (A71) and (A72) until
      $s = 0$ at time $t_2$, at which time use (A70) with
      $t = t - t_2$, or until $\mu = \mu_m$ at time $t_3$,
      at which time use (A73) and (A72) with $t = t - t_3$.
      Use these until $s = 0$ at time $t_4$ and then use
      (A70) with $t = t - t_4$.

J. Logarithmic Model For Transient Predictions

The basic equations are (A70) through (A73) above.

The procedures for predicting transient responses of
steady state systems to changes in $s_{in}$ and $D$ are out-
lined below.
1. Step increase in D from $D^0$ to $D^f = D$
   a. If $\mu(0) > D$ glucose will remain zero. Use (A70).
   b. If $\mu(0) < D$ glucose will increase. Use (A71) and (A72) until
      
      (1) $s = 0$ at time $t_1$, then use (A70) with $t = t - t_1$.
      
      (2) $\mu = \mu_m$ at time $t_2$, then use (A73) and (A72) with $t = t - t_2$. Use these until $s = 0$
      at time $t_3$ and then use (A70) with $t = t - t_3$.

2. Step increase in $s_{in}$ from $s_{in}^0$ to $\alpha s_{in}^0 = s_{in}$
   a. If $\mu(0)/D > \alpha$ glucose will remain zero. Use (A70).
   b. If $\mu(0)/D < \alpha$ glucose will increase. Follow section 1.b. above.

3. Impulse change in $s_{in}$
   Use (A71) and (A72), (A70), and (A73) and (A72) as indicated in section 1.b. above.

K. Nomenclature

1. Basic variables and parameters
   
   $b =$ bacterial mass concentration
   
   $BOD =$ biochemical oxygen demand
   
   $c =$ substrate concentration
C = soluble organic carbon
COD = chemical oxygen demand
CSTR = continuous stirred tank reactor
DO = dissolved oxygen
$\mathcal{D}$ = diffusivity
D = dilution rate, $Q/V$, or diffusivity
$E_G$, $E_{DO}$ = quantities related to glucose uptake and DO uptake, respectively; see APPENDIX, section F
$f(s,p)$ = fractional factor in growth rate formulation
G = glucose concentration
$G_t$ = generation time
h = membrane permeability
$k_L$ = linear growth rate
$K_La$ = mass transfer coefficient
$K_m$ = constant in Monod$^9$ model
N = substrate flux
OD = optical density
p = product concentration
Q = volumetric flow rate
q = reaction rate
r = radial measurement
R = transfer parameter in model of Ng and Rippin$^{101}$
RNA = ribonucleic acid
RTD = residence time distribution
s = substrate concentration
S = s + b/Y^\log
\text{t = time}
\text{T = temperature}
\text{V = volume}
\text{Y = yield factor}
\mu = \log \text{growth rate} = \frac{1}{\text{db}}
\text{d}t
\mu_m = \text{maximum log growth rate}
\theta, \tau = \text{residence time}
\lambda = \text{life expectation}^{101,143}
\alpha = \text{age}^{101,143}
II, III, IV = \text{quantities defined by Ng and Rippin}^{101}
a, b, k, \alpha, \beta = \text{used to denote constants}
2. Subscripts
\text{in = influent}
\text{LE = leaving environment}^{101}
\text{L = linear}
\text{lin = linear}
\text{log = logarithmic}
\text{c = critical}
0, 1, 2, 3, 4 = \text{used to denote special position, time, or quantity}
3. Superscripts
   a = apparent
   o = initial
   f = final
4. Other
   (0) denotes initial condition
   \(\Delta\) denotes change from initial condition
   - over quantity denotes average
FIGURE 1 - APPARENT $\mu(s)$ FOR BATCH CULTURES WITH LINEAR GROWTH.
FIGURE 2 - APPARENT $\mu(S)$ FOR BATCH GROWTH WITH pH CHANGE DURING GROWTH.

CASE 1, $\mu = 1.0 - 10^{-11}(S(0) - S)^4$
CASE 2, $\mu = 1.0 - 2.5 \times 10^{-4} \,(S(0) - S)$
FIGURE 3 - EFFECT OF pH CHANGE ON $S(D/\mu_m)$ FOR CONTINUOUS CULTURE, CSTR.
FIGURE 4 - EFFECT OF PARTIAL SEGREGATION ON $S(D/\mu_m)$ FOR $b_{in}>0$ AND $b_{in}=0$
FIGURE 5 - SCHEMATIC DIAGRAM OF CONTINUOUS CULTURE APPARATUS.
FIGURE 6—AGITATED AEROBIC CULTURES, EFFECT OF G(O).
Figure 7 - Agitated Aerobic Cultures, Effect of Dilution and Addition of Glucose to Growing Cultures.
**FIGURE 8 - AGITATED AEROBIC CULTURES, EFFECT OF b(0)**

- **1**, \( b(0) = 3.9 \text{ mg/l} \), \( G(0) = 263 \text{ mg/l} \)
- **2**, \( b(0) = 7.5 \text{ mg/l} \), \( G(0) = 263 \text{ mg/l} \)
- **3**, \( b(0) = 11.1 \text{ mg/l} \), \( G(0) = 265 \text{ mg/l} \)
- **4**, \( b(0) = 16.2 \text{ mg/l} \), \( G(0) = 263 \text{ mg/l} \)
- **5**, \( b(0) = 18.9 \text{ mg/l} \), \( G(0) = 265 \text{ mg/l} \)
FIGURE 9 - QUIESCENT AEROBIC CULTURES, EFFECT OF $b(O), G(O),$ AND DO.
FIGURE 10 - QUIESCENT AEROBIC CULTURES, DEMONSTRATION OF CONSISTENT STOICHIOMETRY.
FIGURE II - QUIESCENT ANAEROBIC WITH NO\textsuperscript{3}\textsuperscript{-} CULTURES, EFFECTS OF G\textsubscript{6}(O) AND T.
FIGURE 12 - CONTINUOUS CULTURE STEADY STATE DATA
FIGURE 14 - SHORT TERM STEADY STATE, EXPERIMENT SS 35, \( D/\mu_{\text{max}} = 0.83 \), \( G_{\text{in}} = 425 \text{ mg/l} \)
FIGURE 15 - DETERMINATION OF $b$ IN REACTOR AT STEADY STATE FOR $D/\mu_{\text{in}} = 1.2$

I PREDICTED RESPONSE FOR $b_{\text{st. st.}} = 0$

II PREDICTED RESPONSE FOR $b_{\text{st. st.}} = .01 b(0)$

III PREDICTED RESPONSE FOR $b_{\text{st. st.}} = .05 b(0)$

$b(0) = .214 \text{ OD UNITS} = 64 \text{ mg/l}$

$D = .618 \text{ hr}^{-1}$

$\mu_{\text{max}} = .509 \text{ hr}^{-1}$

TIME, HOURS
FIGURE 16 - QUIESCENT, AEROBIC CULTURES - SEED FROM CONTINUOUS SYSTEM.
Figure 17 - Batch cultures using continuous culture inocula, $D/\mu_m$ approximately 0.30.
FIGURE 18 - APPARENT $\mu$ AS A FUNCTION OF TIME, LINEAR TYPE CULTURE, $D = 0.15$ HRS$^{-1}$
FIGURE 19 - TRANSIENT EXPERIMENT A.I, STEP CHANGE IN $D/\mu_m$ FROM 0.11 TO 0.90.
FIGURE 20 - TRANSIENT EXPERIMENTS A.2 AND A.8.
FIGURE 21- TRANSIENT EXPERIMENT A.3, STEP CHANGE IN D/μm FROM 0.30 TO 0.95.
\textbf{FIGURE 22- TRANSIENT EXPERIMENT A.4, STEP CHANGE IN D/\(\mu_m\) FROM 0.33 TO 1.04.}
A.7 STEP CHANGE IN D/Lm FROM 0.61 TO 0.90

Figure 24 - Transient Experiments A.6 and A.7.
FIGURE 25- TRANSIENT EXPERIMENTS B.1 AND B.2.

B.1  $D/\mu_m = 0.13$
STEP CHANGE IN $G_{in}$
FROM 101 TO 202 MG/L

B.2  $D/\mu_m = 0.29$
STEP CHANGE IN $G_{in}$ FROM 100 TO 202 MG/L
FIGURE 26 - TRANSIENT EXPERIMENT B.3, D/μm = 0.63, STEP CHANGE IN G_{in} FROM 102 TO 204 mg/l.
FIGURE 27 - TRANSIENT EXPERIMENT B.5, D/\mu_m = 0.12, STEP CHANGE IN G_in FROM 99.2 TO 200 mg/l.
FIGURE 28 - TRANSIENT EXPERIMENT B.6, \( \frac{D}{\mu_m} = 0.89 \), STEP CHANGE IN \( G_{in} \) FROM 101 TO 202.
Figure 29 - Transient experiment B.7, D/μ_m = 0.58, step change in
G_in from 103 to 207 mg/l.
FIGURE 30 - TRANSIENT EXPERIMENT B.8, D/μ_m = 0.56, STEP CHANGE IN G_in FROM 214 TO 321 mg/l AT TIME 0 AND TO 428 mg/l AT TIME 4.2 HOURS
FIGURE 31 - TRANSIENT EXPERIMENT B.9, D/\mu_m = 0.88, STEP CHANGE IN G_in FROM 96.4 TO 198 mg/l.
Figure 32 - Transient Experiment CL, D/m = 0.29, impulse in $G_{in}$, $G_{in}(t) = 100 \text{mg}$/l
Figure 33 - Transient experiment C.2, \( D/\mu_m = 0.48 \), impulse change in \( G_{in} \), \( G(0) = 100 \text{ mg/l} \)
FIGURE 34 - RESPONSES OF CULTURE AT $D = 0.15 \text{ HR}^{-1}$, $S_{in} = 100$ TO STEP INCREASES IN $D$ TO $D^f$, LINEAR MODE.
Figure 35 - Response of culture at $D = 0.15$ hr$^{-1}$, $S_{in} = 100$ to step increases in $D$ to $D^f$, log mode.

Curves for $D^f = 0.25$ & 0.30 identical to culture for linear mode.
Figure 37 - Responses of culture at $D=0.15 \, \text{hr}^{-1}$, $S_{in}^o = 100$ to step increases in $S_{in}$ to $\alpha S_{in}^o$. LOG MODE