Cybernetic Modeling of Bacterial Cultures at Low Growth Rates: Single-Substrate Systems

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Accepted for publication September 23, 1988

The cybernetic framework developed by Ramkrishna and co-workers has been expanded to include the effects of cellular maintenance energy requirements on biomass levels in slow-growing, carbon-substrate-limited cultures. A simple structured model, based on the existence of distinct key enzymes for growth and maintenance functions, is presented. Comparisons of the model with experimental data for the growth of Klebsiella oxytoca in constant fed-batch culture on glucose, fructose, arabinose, and xylose show good agreement. In addition, perturbed fed-batch culture experiments indicate that slow-growing cultures respond less rapidly to a removal of the growth limitation than do faster-growing ones. The possibility of a growth-rate dependent "critical resource" is discussed.

INTRODUCTION

The cybernetic view of microbial growth, advanced by Ramkrishna, holds that microorganisms use their internal regulatory machinery to "steer" themselves toward some goal. Thus far this perspective has been fruitfully applied to predict diauxic growth in batch cultures of the bacterium Klebsiella oxytoca. Moreover, the so-called "matching law" model was shown to be capable of simulating simultaneous utilization of substrates at low growth rates. However, other low growth-rate phenomena, particularly maintenance energy effects, were not considered. In this article we will develop a simple structured model that acknowledges the consumption of substrate for non-growth-associated processes. The chief merit of this model is that it is easily extended to describe growth and maintenance in mixed-substrate systems elsewhere. We also present experimental data for the growth of K. oxytoca on glucose, fructose, arabinose, and xylose in constant fed-batch culture. Finally, the results of some highly transient perturbed fed-batch culture experiments are presented and discussed.

A heterotrophic bacterium growing on a carbon substrate (such as glucose) will use the substrate to provide energy as well as the carbon skeletons used in biosynthesis. The mass of microorganisms (g dry w) formed per unit mass of substrate consumed, called the yield coefficient $Y_G$, is usually found to be constant in substrate-sufficient batch cultures. At lower growth rates prevailing in continuous culture, however, the yield coefficient is reduced. Herbert attributed this phenomenon to an "endogenous" metabolism, whereby microorganisms oxidize their own cell mass in order to satisfy their energetic needs, resulting in lower apparent growth yields. An alternative approach to growth-rate-dependent yield was taken by Mallette and Marr and co-workers. They assumed that the carbon substrate consumed is used directly to meet cellular maintenance energy requirements, which they suggested was needed for maintenance of ion gradients, enzyme turnover, motility, and so forth. The latter approach was subsequently found to be more common if substrate is present in the environment.

Pirt introduced the concept of the maintenance coefficient to quantify the data presented by himself and others. He assumed that some of the substrate is consumed at a small, constant rate by organisms for non-growth-associated functions, resulting in a specific substrate uptake rate of the form

$$q_s = \frac{1}{Y_G} r_G + m$$

where $r_G$ is the specific growth rate (h^{-1}), $m$ is the maintenance coefficient (g/g dry wt h) and $Y_G$ is the growth yield (g dry wt/g) that would be observed if $m$ were zero. The actual yield (as a function of growth rate) is then

$$Y = \frac{r_G}{q_s} = \frac{r_G}{\left(\frac{1}{Y_G} r_G + m\right)}$$

The maintenance coefficient $m$ is obtained from steady-state continuous culture data because a plot of $1/Y$ vs. $1/r_G$ ($= 1/D$) gives a straight line of slope $m$ and intercept $1/Y_G$. 

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Although the use of a constant maintenance coefficient is often adequate to describe variable growth yields in steady state continuous culture, it poses some problems. First, there is no firm basis for assuming that the sum total of maintenance functions is constant. Esener and co-workers\textsuperscript{10} have noted that decreased cell volume at low growth rates (and, hence, increasing biotic-phase interfacial area) could result in increased osmotic work to maintain transmembrane concentration gradients. Second, enzymes are known to degrade more rapidly in nongrowing cultures than in actively growing ones, likely requiring more energy for re-synthesis at low growth rates.\textsuperscript{11} Third, other nongrowth-associated processes such as futile cycles\textsuperscript{12} appear to be strongly dependent upon growth rate.

Aside from biological objections to a constant maintenance coefficient, it also presents difficulties from a mathematical viewpoint. Specifically, eq. (1) predicts the consumption of substrate even when it is not present in the environment. Ramkrishna and co-workers\textsuperscript{13} were able to circumvent this problem by assuming that microorganisms are able to consume substrate even when it is not present in the environment. The above model is a highly simplified picture of the overall growth process and does not reflect the existence of non-growth-associated functions. Let us introduce a key enzyme $E_M$ that is used in non-growth-associated processes. Furthermore, we will assume that the presence of a substrate $S$ in the bacterial environment induces the synthesis of the enzymes that are instrumental in both growth- and non-growth-associated processes. In growing cultures of microorganisms these enzymes are no doubt synthesized independently of each other, and a realistic mathematical description of these events should reflect this fact. However, the additional rate expressions would contain parameters for which values could only be guessed. As a first modeling step, and for the sake of simplicity, we will assume that these enzymes are synthesized at equal rates, i.e.,

$$r_{E_G} = r_{E_M} = r_E$$

Then the specific maintenance rate (3) may be written as

$$r_M = \frac{(\mu_M e_M)S}{K_M + S}$$

Thus, $E_G$ and $E_M$, although distinct, are nonetheless present intracellularly at equal levels. However, the activities of $E_G$ and $E_M$ may be different, so that the actual specific growth and maintenance rates are modified as

$$r_G v_G \quad 0 \leq v_G \leq 1$$
$$r_M v_M \quad 0 \leq v_M \leq 1$$

The cybernetic variables $v_G$ and $v_M$ control the activities of $E_G$ and $E_M$, respectively.

Before we develop a strategy for the forms of the cybernetic variables $v_G$ and $v_M$, we state our assumptions about maintenance metabolism in the form of postulates. The enunciation of these postulates is to identify the basic assumption of our development at present rather than to assign them a permanently non-negotiable status. They are based on data accumulated to date and it is not unlikely that future experience could force their revision.

**Maintenance Postulates**

- **Postulate 1:** The primary goal of microorganisms is to **maximize** growth rate.
- **Postulate 2:** A non-growth-associated maintenance metabolism manifests at growth rates **less** than the maximum growth rate and becomes active at **lower** growth rates.
- **Postulate 3:** The activity of non-growth-associated maintenance metabolism is such that the **total** rate of consumption of substrate for growth **and** maintenance is maximized.
We discuss each of these postulates individually. Let it first be noted that “activity” of any metabolic process refers to the activity of the key enzyme for that process. In other words, growth activity is described by \( v_G \) and that of non-growth-associated maintenance by \( v_M \).

Postulate 1 implies essentially that growth rate is of paramount interest to the organism. It implies that the organism will keep growth metabolism fully active (\( v_M = 1 \)) under all conditions. When mixed (substitutable) substrates are present resource allocation for syntheses of growth enzymes and control of their activity will precede maintenance considerations.

Postulate 2 primarily takes a position against a constant maintenance activity. It contends that maintenance requirements are satisfied by growth processes automatically when they occur at the maximum growth rate thus not necessitating a separate maintenance metabolism. However, when growth occurs at less than the maximum growth rate (for the given substrate) a separate, non-growth-associated maintenance metabolism is activated.

Postulate 3 would seem at first confusing since the obvious way to maximize the rate of consumption of substrate for growth and maintenance is to keep the activities of both growth and maintenance enzymes at their absolute maximum values of unity. For the growth enzyme the activity is already assumed to be unity so that the issue just raised refers to the maintenance enzyme alone. However, it would be wasteful to promote activity to its absolute maximum value of unity, if the maintenance requirements are not that intense. Thus for the application of this postulate we must make an assumption in regard to the requirement of non-growth-associated maintenance metabolism. Here, we assume that the maintenance activity is proportional (i.e., linearly) to the difference between the absolute maximum specific growth rate \( \mu_G^{\text{max}} \) and the prevailing growth rate, \( \mu_G \). Note that this proportionality is consistent with Postulate 2 since it says that no non-growth-associated maintenance activity is required at the absolute maximum growth rate. When the growth rate falls to zero the maintenance requirement is at its maximum and we provide for the activity of the maintenance enzyme to be at its maximum absolute value of unity.

From the foregoing discussion, it is easy to arrive at an expression for the cybernetic variable \( v_M \) for the model under discussion. Postulate 1 requires that \( v_M = 1 \) for all \( r_G \), the second that \( v_M = 0 \) when \( r_G = \mu_G^{\text{max}} \). We note that the specific substrate uptake rate, \( q_s \), is given by

\[
q_s = \frac{1}{v_G} r_G + r_M v_M \tag{12}
\]

Since \( v_M \) is assumed to be proportional to \( \mu_G^{\text{max}} - r_G \), we write

\[
v_M = \lambda (\mu_G^{\text{max}} - r_G) \tag{13}
\]

Because \( v_M \leq 1 \) we have

\[
\lambda \leq 1/(\mu_G^{\text{max}} - r_G) \tag{14}
\]

which must hold regardless of \( r_G \). Since \( r_G \) is non-negative we clearly must have

\[
\lambda \leq 1/\mu_G^{\text{max}} \tag{15}
\]

At zero growth rate we must have the maximum maintenance activity, i.e., \( v_M = 1 \) which follows from Postulate 3. Thus,

\[
v_M = \lambda \mu_G^{\text{max}} = 1 \quad (r_G = 0) \tag{16}
\]

from which \( \lambda = 1/\mu_G^{\text{max}} \), and at all other \( r_G \) values, we have

\[
v_M = 1 - r_G/\mu_G^{\text{max}} \tag{17}
\]

Thus the specific uptake rate of substrate for non-growth-associated process is

\[
q_M = r_M (1 - r_G/\mu_G^{\text{max}}) \tag{18}
\]

which has the same form as the growth-rate dependent term developed by Pirt\textsuperscript{14} to describe maintenance effects in carbon-sufficient continuous cultures. However, it must be remembered that we are concerned with carbon-limited growth, and care must be taken in drawing parallels between the two rate expressions.

It is fruitful to digress momentarily to the case of mixed-substrates environment considered by the authors.\textsuperscript{19} Postulate 1 requires that control of enzyme syntheses and activities be based on growth considerations as by Kompala et al.\textsuperscript{5} We postulate here that control of the maintenance activity is the same for each maintenance enzyme (specific to substrate). Thus eq. (17) should be applicable with appropriate substitutes for \( r_G \) and \( \mu_G^{\text{max}} \). For \( r_G \) we employ the combined growth rates used by Kompala et al.,\textsuperscript{5} i.e.,

\[
r_G = \sum r_G v_G \tag{19}
\]

where \( r_G \) is the growth rate on the \( i \)th substrate. For \( \mu_G^{\text{max}} \) in eq. (16), the proper substitute is determined by the growth rate in the mixed-substrate environment which automatically satisfies the maintenance requirements of the organism. From our experiments with various mixed substrates model predictions were found\textsuperscript{19} to be best when this growth rate was chosen to be \( \sum \mu_G^{\text{max}} v_G \). This choice arises from the fact that it represents a good approximation the maximum growth rate that can be expected in the mixed substrate environment. We now return to the case of growth in single substrate, the topic of main interest to the present article.

The complete mass balance equations for biomass, substrate, and enzyme level in either continuous (or fed-batch) culture are given by

\[
\frac{dc}{dt} = r_G c - D c \tag{20}
\]

\[
\frac{ds}{dt} = D (s_f - s) - c \left( \frac{1}{v_G} r_G + r_M v_M \right) \tag{21}
\]

\[
\frac{de}{dt} = r_E - e (\beta + r_G) \tag{22}
\]

where \( c \) is the biomass concentration; \( D \) is the dilution rate; \( s_f \) is the concentration of substrate in the feed; and \( \beta \)
is a first order decay constant, assumed to be equal for the two key enzymes \(E_G\) and \(E_M\).

The identification of the growth and maintenance parameters \(\mu_G\) and \(\mu_M\) is done by recognizing that \(e = e^{max} (= e^{max}_G = e^{max}_M)\) when \(r_G = \mu_G\), i.e., \(\mu_G\) and \(\mu_M\) are defined by

\[
\mu_G^{max} = \mu_G e^{max}_G \\
\mu_M^{max} = \mu_M e^{max}_M
\]

The value of \(e^{max}\) is attained when \(de/dt = 0\); eq. (22) yields

\[
e^{max} = \alpha/\left(\beta + \mu_G^{max}\right)
\]

so that

\[
\mu_G = \frac{\mu_G^{max}(\mu_G^{max} + \beta)}{\alpha} \\
\mu_M = \frac{\mu_M^{max}(\mu_M^{max} + \beta)}{\alpha}
\]

The parameters \(\mu_G^{max}, Y_G,\) and \(K_G\) are determined directly from batch culture data. The maintenance parameters \(\mu_M^{max}\) and \(K_M\) may be determined by fitting steady-state continuous culture data, or, alternatively, from constant fed-batch culture experiments.

**MATERIALS AND METHODS**

**Organism**

*Klebsiella oxytoca* B199 (ATCC 8724), obtained from the U.S. Department of Agriculture (Peoria, IL), was used in all experiments.

**Medium**

The carbon-free salts medium used in all experiments has been described elsewhere. The medium was prepared and autoclaved in two parts, namely, a concentrated solution containing the phosphate and ammonium salts. Concentrated sugar solutions were made containing only glucose, fructose, arabinose, or xylose. After autoclaving, these components were mixed together and topped off with sterile water to give the desired final concentrations. The total sugar concentration never exceeded 5 g/L, ensuring carbon-substrate-limited growth.

**Inoculum Preparation**

The organism was stored in small vials in a biofreezer at −60°C in a glycerol-rich medium. Inocula were prepared in 250-mL shake flasks containing 50 mL minimal salts medium with glucose at a concentration of 5 g/L. Flasks were incubated for 24 h at 37°C, and the organism was then transferred to another flask containing identical medium. At the stationary phase of this culture, ca. 20 mL were aseptically injected into the fermentor.

**Fermentor Description**

All experiments were carried out in a 2-L New Brunswick fermentor modified for use in either fed-batch or continuous culture mode. The fermentor was placed in a constant temperature water bath (\(T = 37°C\)). The medium (pH 7.1) used in the fermentations provided adequate buffering ability for the low cell concentrations used in the experiments, and the maximum pH decrease during any run was always less than 0.2 unit. Air was supplied to the fermentor at a rate of 1.3 L/min. This ensured that growth was always aerobic, as verified by dissolved oxygen measurements.

Feed was supplied to the fermentor by means of a precision peristaltic pump (Harvard Apparatus, Natick, MA). Before entering the fermentor, the feed stream was passed through two Pyrex “break” tubes,6 mixed with air, and discharged immediately beneath the fermentor impeller (900 rpm). In order to minimize the evaporation of culture broth, the air was bubbled through 250 mL sterile water before being mixed with the liquid feed stream.

The flow rate of liquid medium to the fermentor was accurately controlled throughout a run at approximately 6.5 mL/h. In order to maintain constant culture volume, between 5 and 8 mL culture broth were withdrawn hourly for sampling purposes. In this way the effect of dilution on the culture was minimized.

**GROWTH MEASUREMENT**

The cell dry weight was estimated from absorbance measurements conducted at a wavelength of 540 mm. One absorbance unit was determined to be equivalent to 0.35 g dry wt biomass/L. This correlation (or extinction coefficient) was found to be linear up to an absorbance of 0.2 units. Dense cultures were diluted to ensure that the absorbance readings were within the linear range.

Because the extinction coefficient can depend on growth rate, it was necessary to check for any variation in this correlation. At the end of a fed-batch culture experiment, samples were withdrawn from the fermentor and assayed for biomass level by photometric and direct dry weight determination. In all cases the extinction coefficient was found to have a value of 0.35 ±0.01 g dry wt/L.

**RESULTS AND DISCUSSION**

**Constant Fed-Batch Culture**

A constant fed-batch culture (CFBC) is one for which the flow rate of nutrients to the fermentor does not vary with time. The type of CFBC experiments we have performed is simulated in Figure 1. At time \(t = 0\), a carbon-free medium is inoculated with microorganisms. Simultaneously, the flow of nutrients to the fermentor is begun and growth continues. After an initial transient, the cells normally enter a linear phase of growth during which nearly all of the incoming substrate is consumed and \(dc/dt\) is approximately equal to \(Y_c D S_i\) (but see the letter of Lim17 for an interesting
exception). As the experiment progresses, the specific growth rate diminishes and the effect of maintenance is manifested in a departure of the biomass curve from linearity because less substrate is used for growth.

*Klebsiella oxytoca* was grown in single-substrate environments in CFBC to determine quantitatively the effects of maintenance on yields at low growth rates. The sugars used were glucose, fructose, xylose, and arabinose. Figure 2 shows the results of growth on glucose at four different mass flow rates of the sugar to the fermentor. Notice that the cultures typically enter the linear phase of growth within 1-2 h after start-up. Decreased growth yields are noticeable after 3-4 h.

In order to calculate the experimental cellular growth yields, it was necessary to assay the culture broth for glucose concentration. A modified glucose oxidase procedure (Sigma Chemical Co., St. Louis, MO) enabled the determination of glucose levels down to ca. 2 mg/L. Unfortunately, samples taken after time \( t = 2 \) h showed no traces of residual glucose. It was therefore necessary to assume that \( s = 0 \) (and, consequently, \( ds/dt = 0 \)) after the linear growth phase had been entered. Using these approximations, eq. (21) can be solved for the specific rate of glucose uptake \( (= r_G/Y_G + r_M v_M) \) to give

\[
q_s(t) = D_S f/c(t) \tag{24}
\]

which results in a growth yield \( (= r_G/q_s) \) of the form

\[
Y(t) = r_G(t)c(t)/D_S \tag{25}
\]

The “smoothed” experimental growth rate \( r_G(t) \) can be determined by fitting the biomass data using a cubic spline function. It will be seen that the approximate eqs. (24) and (25) give reasonable results for the experimental values of the substrate uptake rate and the growth yield.

Figure 3 demonstrates explicitly the decrease in the instantaneous cellular growth yield during the course of CFBC. At time \( t = 2 \) h the observed yield coefficient is roughly 0.5 g dry wt/g glucose, or approximately the same value determined from batch culture data. With increasing time, however, the organisms assimilate into biomass substantially less glucose because the growth rate is steadily decreasing. When the run was ended the growth rate had declined to ca. 0.06 h\(^{-1}\), and the instantaneous yield to 0.35 g dry wt/g glucose.

The change in specific rate of glucose uptake is plotted as a function of time in Figure 4. After an initial rise, notice that \( q_s \) decreases quickly and then levels off somewhat. Inasmuch as growth rate and specific substrate uptake rate are linearly related, Figure 4 brings out a fundamental shortcoming of CFBC: only very low growth rates can be maintained for extended periods of time. This problem can only be overcome by increasing the mass flow rate of substrate to the fermentor as the experiment progresses.

The yield as a function of growth rate is presented explicitly in Figure 5. The discrepancy between the experimental and simulated yield values at the higher growth rates is primarily a result of the assumption that \( ds/dt = 0 \). Nonetheless, the effects of maintenance on yield are clearly shown. The specific glucose uptake rate as a function of growth rate is shown in Figure 6, and gives a straight line relationship, as expected.

Single-substrate experiments were also performed to determine maintenance parameters for fructose (Fig. 7), arabinose (Fig. 8), and xylose (Fig. 9). The maintenance parameters \( \mu^\text{max} \) are listed in Table I along with the growth parameters determined from batch culture experiments. The maintenance-rate expression saturation constants were set equal to \( 10^{-6} \) as adequate fits of each set of data were obtained using the one parameter \( \mu^\text{max} \).
**Perturbed Fed-Batch Culture**

Suppose that we start up a constant fed-batch culture and wait for it to enter the linear growth phase. Normally, the residual substrate concentration is very low and the microorganisms are growing under a true carbon-substrate limitation. If we add a small volume of concentrated substrate solution to the culture, the growth limitation will be suddenly removed. The organisms will respond to the nutrient-rich environment by regulating their metabolism so as to exploit the newly favorable conditions (Fig. 10). Because the cells are going from a state of decreasing growth rate to one of increasing growth rate, simulating perturbed fed-batch culture (PFBC) behavior is a much more rigorous test of the cybernetic model than is simulating CFBC behavior.

If bacteria are growing in a glucose-limited fed-batch culture and a cell-saturating pulse of glucose is added to the fermentor, presumably the organisms do not need to synthesize different enzymes to consume the excess sugar (as would be true if we added a sugar other than glucose). However, a slow response to a glucose pulse may indicate that, prior to the perturbation, the capacity of the enzyme-synthesis machinery is not maximal. At very low growth rates, for example, it is unlikely that the cells will synthesize as many enzymes as they need at high growth rates, as this would be grossly inefficient. Since the capacity of the enzyme-synthesis machinery is limited by the availability of resource(s), we need to consider the possibility that the total resource-availability may be growth-rate dependent.

Single-substrate PFBC experiments were performed in order to determine the speed of the response of the organisms.
to a removal of the growth limitation. Glucose-limited fed-batch cultures of *K. oxytoca* were pulsed with cell-saturating quantities of glucose at $t = 2$ h (0.2 g) and $t = 8$ h (0.4 g). The most relevant feature of Figures 11, 12, and 13 is that the specific growth rate at the time of the first pulse is different in each case. Figure 11 demonstrates that at moderate growth rates (0.3 h$^{-1}$), the culture responds to the presence of excess glucose nearly as fast as the model predicts and the agreement is very good. At lower growth rates (Figs. 12 and 13), the model predicts a somewhat faster response than is observed experimentally. Clearly, the cells cannot fully exploit the favorable growth conditions, and this may be a result of constrained resource (and hence enzyme) level.

The response of the cultures to a second pulse of glucose is quite different. Although the specific growth rate at the time of the second pulse is lower than at the time of the first one, the organisms are able to consume the added glucose with no perceptible lag agreeing closely with model predictions. Resource that was synthesized in response to the first glucose pulse may still be present within the cells when the second pulse is introduced. If so, the organisms would be able to respond more quickly when the growth limitation is removed a second time.

Finally, a few words must be said regarding the results shown in Figure 13. For $t > 9$ h there appears to be continued growth of the culture, whereas the simulation predicts a decrease in biomass concentration. Because the rate
expression for nongrowth-associated processes is a simple function of substrate concentration, the fed-batch culture eq. (21) places an upper bound on the total culture biomass level that can be supported by a particular rate of substrate addition. In adding glucose to the culture by way of concentrated pulses, we have allowed the actual biomass level to exceed the theoretical limit. In fact, the organisms most likely effect a drastic change in metabolism at extremely low growth rates, which is not reflected in our model equations. Esener and co-workers\(^\text{18}\) have reported similar behavior for the growth of *Klebsiella pneumonia* on glycerol.

**CONCLUSIONS**

In this article we have addressed the low growth-rate behavior from a cybernetic perspective. A simple structured model based on the existence of distinct key enzymes for growth- and nongrowth-associated processes was developed. Single-substrate fed-batch culture experiments were conducted to determine maintenance parameters for the growth of *Klebsiella oxytoca* on glucose, fructose, arabinose, and xylose. Perturbed fed-batch culture experiments were also performed in order to determine the response of glucose-limited fed batch cultures to a removal of the growth-rate dependent critical resource was discussed in the light of experimental data and model predictions.
Table I. Parameter values obtained from single-substrate data.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( \mu_{\text{max}}^S )</th>
<th>( K )</th>
<th>( Y_0 )</th>
<th>( \mu_{\text{max}}^M )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.10</td>
<td>0.01</td>
<td>0.51</td>
<td>0.086</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.94</td>
<td>0.01</td>
<td>0.51</td>
<td>0.079</td>
</tr>
<tr>
<td>Arabinose</td>
<td>1.00</td>
<td>0.05</td>
<td>0.49</td>
<td>0.080</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.85</td>
<td>0.2</td>
<td>0.40</td>
<td>0.081</td>
</tr>
</tbody>
</table>

For all substrates, \( \alpha = 0.001 \) and \( \beta = 0.05 \).

Units are as follows: \( \mu_{\text{max}}^S, \alpha, \beta \) in h\(^{-1}\); \( K \) in g/L; \( Y_0 \) in g dry wt/g; \( \mu_{\text{max}}^M \) in g/g dry wt h.

The primary goals of our single-substrate studies were to develop a simple structured model describing maintenance effects in single-substrate systems and to determine experimentally the relevant parameters for glucose, fructose, arabinose, and xylose. The model and the data have been used to predict behavior in mixed-substrate systems.\(^\text{19}\)

Figure 11. Response of a glucose-limited fed-batch culture to additions of glucose (run FB81). The growth rate at the time of the first pulse was 0.3 h\(^{-1}\).

Figure 12. Response of a glucose-limited fed-batch culture to additions of glucose (run FB75). The growth rate at the time of the first pulse was 0.2 h\(^{-1}\).
Support from the National Science Foundation through Grant No. CPE-8405138 is gratefully acknowledged. The authors also thank Purdue University for a David Ross Grant which supported this work.

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