Batch- and Continuous-Culture Transients for Two Substrate Systems

C. N. STANDING, A. G. FREDRICKSON, AND H. M. TSUCHIYA

Department of Chemical Engineering and Materials Science, and Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455

Received for publication 9 July 1971

Batch growth of Escherichia coli in the presence of equal initial concentrations of glucose and a secondary substrate (xylose) is characterized by sequential utilization of the substrates, whereas continuous-culture systems with equal concentrations of the two substrates in the feed are characterized by complete utilization of both substrates at both high and low dilution rates. Such systems at steady state at a low dilution rate, when suddenly shifted to a higher dilution rate, experience a transient drop in population density accompanied by accumulation of the secondary substrate but virtually no accumulation of glucose. Systems at steady state with 200 mg of glucose per liter were found to undergo a transient population decrease and eventual recovery when switched to feed containing 200 mg of a secondary substrate per liter.

Escherichia coli and certain other organisms are known to utilize glucose preferentially when it is present in a batch culture with one of several other substrates which they can also use as sole energy source. The batch growth phenomenon, termed diauxie by Monod (6), is typically characterized by a two-phase growth curve in which growth in glucose is represented by the first phase of growth and growth on the secondary substrate by the second phase. The extension of the study of these systems to the chemostat provides an opportunity for determining steady-state and transient behavior of continuous cultures in the presence of such substrate pairs. This paper describes batch- and continuous-culture data for E. coli with glucose-galactose and glucose-xylose as the substrate pairs.

MATERIALS AND METHODS

**Organism.** E. coli B/r was used in all experiments.

**Medium.** The basal medium used in these experiments contained (per liter): NaCl, 0.01 g; KH₂PO₄, 1.50 g; K₂HPO₄, 3.68 g; FeSO₄·7H₂O, 0.002 g; MgSO₄·7H₂O, 0.10 g; and (NH₄)₂SO₄, 1.25 g; pH 7.0 ± 0.1. Ethylenediaminetetraacetate was present at 10⁻⁴ M.

D-Glucose, D-galactose, and D-xylose, present singly or in combinations at a total concentration of 200 mg/liter, were the limiting substrates in the medium.

**Method of propagation.** Batch and continuous experiments were carried out in 100-ml glass chemo-

stats similar to those of Novick and Szilard (7). Continuous feed of fresh medium was effected through the use of capillary tube feed (8) in which the feed reservoir and chemostat were connected by a 1.5-meter length of 22-gauge stainless-steel hypodermic tubing (inner diameter, 0.4 cm). The medium flows through this tubing at a rate proportional to the size of the hydrostatic pressure head, maintaining a constant feed rate to within better than 10%. Constant volume was maintained by an overflow siphon leading to a collecting reservoir. Aeration and agitation were effected by air admitted to the bottom of the chemostats at a rate of about 2 liters/min. Water from a constant-temperature bath was pumped through the chemostat jacket, maintaining a temperature control of ±0.2 C. Aseptic precautions were maintained at all times; the cultures were checked by plating out on tryptone-glucose-yeast extract medium (grams/liter: tryptone, 5.0; glucose, 1.0; yeast extract, 2.5; agar, 20).

**Population size.** Measurement of population size was generally taken to be the optical density (absorbance) at 450 nm measured on a Beckman model DU spectrophotometer. Complete utilization of substrate at 0.2 g/liter typically gave an optical density of 0.240 ± 0.030 for all three substrates; this correlated with a dry cell weight of about 85 mg/liter and a total cell count (Coulter Electronics model B particle counter) of about 7 × 10⁶ per ml. Viable counts were made by use of the capillary tube counter of Schoon (Ph.D. Thesis, Univ. of Minnesota, Minneapolis, 1969) and Schoon et al. (9).

**Sugar analyses.** Samples taken for sugar analysis were filtered through a 0.45-μm membrane filter (Millipore Corp.) and stored at −5 C until analyzed.

Glucose was determined by the Glucostat method (Worthington Biochemical Corp., Freehold, N.J.).
Galactose was determined by the Galactosat method (Worthington Biochemical Corp.) as modified by Sempere (10).

Xylose was determined by a modification of the orcinol reaction (1, 2) which involves addition of 3.0 ml of iron solution (0.5 ml of 10% FeCl₃·6H₂O solution in 100 ml of concentrated HCl) and 0.2 ml of orcinol solution (0.6 g of orcinol in 10 ml of methanol) to 1.5 ml of sample, heating in a boiling-water bath for 20 min, and reading absorbance at 665 nm.

RESULTS

Batch growth behavior. Batch growth experiments were carried out in chemostats which were aerated and had a zero feed rate.

Batch growth of E. coli in the presence of glucose and xylose displays the familiar diauxie effect which was described by Monod (6). Figure 1 illustrates results for growth at 25°C with glucose and xylose at initial concentrations of 100 mg/liter each. The preference for glucose is manifested in the typical sequential use of the substrates as well as in the lag period following glucose depletion, during which induction of enzymes for the second substrate presumably takes place.

The data show intermediate lag periods for population density measured by both total-cell counts and viable-cell counts, as well as by optical density. The curves of these three parameters differ in two respects: (i) after glucose depletion, the total and viable cell counts "decreased" slightly and then increased again before xylose utilization began while the optical density remained essentially constant throughout this period; (ii) after all substrate was depleted, the optical density leveled off while the total- and viable-cell counts continued to increase, indicating cell division in the absence of growth at the end of the batch curve.

Diauxie can also occur without an obvious intermediate lag period, as illustrated in Fig. 2 for batch growth of E. coli at 25°C in medium containing equal initial concentrations of glucose and galactose. For this set of substrates, there was no apparent lag period after depletion of glucose, although utilization of the substrates was sequential.

Similar behavior was observed when these two substrate pairs were supplied for batch growth of E. coli at 33°C.

Continuous-culture steady states. Continuous cultures with glucose, galactose, or xylose as the single growth-limiting feed substrate (200 mg/liter feed concentration) reached a steady state after several holding times. The steady state was characterized by a relatively unchanging population density and by residual substrate levels which fell below the limit of sensitivity of the sugar determinations used (glucose, <0.5 mg/liter, galactose, <2 mg/liter; and xylose, <1 mg/liter). The steady-state population density was approximately equal for each of these substrates with optical densities averaging about 0.240, although individual
steady-state densities differed from this figure by as much as ±0.030.

Similar steady states have been observed for cultures with either glucose and galactose or glucose and xylose at equal concentrations in the feed. Optical densities averaged 0.240 ± 0.030, and measurements of residual substrate concentrations indicated that in both systems the two sugars were at concentrations below the sensitivity limits of the sugar determinations for holding times ranging from just above the minimum holding time to several times the minimum holding time.

Continuous-culture transients. The two types of continuous-culture transients examined here are referred to as "feed switching" and "feed rate shift-up" experiments.

In the "feed switching" experiments, the feed rate was held constant while the feed medium itself was changed from one containing glucose at 200 mg/liter to one containing a secondary substrate (galactose or xylose) at 200 mg/liter. In the "feed rate shift-up" experiments, the feed rate or dilution rate was increased abruptly (holding time decreased) while the composition of the feed medium remained unchanged. This is superficially similar to the shift-up concept used by Mateles et al. (5) and is distinguished from shift-up experiments which involve supplementation with richer media (3, 4).

Feed switching. Figure 3 illustrates data for E. coli at 25 C of switches between a feed containing 200 mg of glucose/liter and one containing 200 mg of xylose/liter. The data show that switching from glucose to xylose caused a transient population decrease during which xylose accumulated in the chemostat owing to lack of full utilization of the new substrate; switching from xylose to glucose caused no such transients. Similar results occurred when the culture was subjected to a second series of feed switches, indicating loss of xylose-metabolizing ability during the period of glucose feed which occurred between periods on xylose. In these data, the steady-state population densities on xylose averaged 10% higher than those on glucose, but all steady-state densities fell within the average range of 0.240 ± 0.030, which was typically observed for full utilization of either substrate when present at 200 mg/liter in the feed.

The transients of Fig. 3 occur at a constant holding time of 10 hr, which is about twice the minimum or washout holding time for this system and temperature.

The transients may be described in terms of the parameters: recovery time (time to rebound to 90% of the eventual steady-state population density), population decrease (greatest drop in optical density from previous steady state), and substrate accumulation (peak substrate concentration). For the data of Fig. 3, the recovery times of the two glucose- to-xylose switches averaged 1.08 days, the population decreases averaged 0.097, and the xylose accumulated to a peak value of about 100 mg/liter.

Similar results have been obtained for switching a chemostat culture from a feed containing glucose to one containing galactose.

Feed rate shift-up experiments. In transient states caused by sudden increases in feed flow rate, preferential utilization of glucose was demonstrated in two substrate cultures of E. coli.

Typical results are shown in Fig. 4 for a culture with feed containing glucose and xylose each at 100 mg/liter. The culture, initially at steady state at a holding time of 10 hr, was shifted-up to a holding time of 5 hr. The cul-

---

**Fig. 3.** Transients: feed switches between glucose and xylose.

**Fig. 4.** Transient: glucose and xylose feed, θ = 10 hr to θ = 5 hr.
ture, growing too slowly to utilize the increased inflow of substrate, decreased in population density because of the washing-out effect. Accumulation of growth-limiting substrate(s) must accompany such a population decrease. In this experiment, the glucose did not accumulate but remained at a concentration of less than 0.5 mg/liter, whereas the xylose accumulated to a maximal concentration of 58 mg/liter. The culture eventually attained a fast enough growth rate to utilize all incoming substrate, and the xylose concentration dropped to less than 1 mg/liter.

One explanation of this phenomenon might be that the minimum holding time is slightly greater for xylose utilization than it is for glucose utilization. Indeed, it has been found that the minimum holding time for utilization of both glucose and xylose is about 4.5 ± 0.5 hr at 25 C. However, if for the culture of Fig. 5 the minimum holding time for xylose were 5 hr and the minimum holding time for glucose were 4.5 hr, then the observations in Fig. 4 could be caused by having the actual flow rate at about 4.9 hr early in the experiment and about 5.1 hr later in the experiment. To avoid these difficulties, further feed rate shift-up experiments were performed in which the new holding time was chosen to be well above the minimum holding time of either substrate.

A series of chemostats were shifted to a holding time of 6 hr from initial holding times ranging from 8 to 24 hr, with the results tabulated in Table 1.

In each of these experiments, virtually all of the accumulated substrate was again found to be xylose, as is the case for a shift-up to a holding time (θ) of 5 hr. Shifting from θ = 8 to θ = 6 hr caused virtually no change in the culture, and a shift from θ = 12 to θ = 6 hr caused a small change; shifts from long holding times of 16 and 24 hr caused transients of a considerable extent. The recovery times for these shift-ups to θ = 6 hr are plotted in Fig. 5 as a function of initial holding time, illustrating the dependence of transient washout on the extent to which initial and final holding times differ.

Similar transients have been observed in feed rate shift-up experiments with the glucose-galactose system, as shown in Fig. 6. A chemostat switched from θ = 24 to θ = 6 hr had a population drop of 0.120 and a recovery time of 1.73 days.

Similar transients have also been observed for chemostat cultures whose feeds contain a single substrate, even glucose. Figure 7 shows transients for a switch from θ = 24 to θ = 6 hr for a chemostat receiving 200 mg of glucose/liter in the feed; the population drop was 0.104 and the recovery time was 1.82 days.

The dashed lines marked “theoretical washout” in Fig. 4, 6, and 7 represent curves which the population density and total substrate concentration would follow if the chemostat

---

**Table 1. Transients for feed rate shift-ups for chemostats with feeds containing 100 mg of glucose/liter and 100 mg of xylose/liter**

| Initial holding time (hr) | Avg steady-state OD | Decrease in OD | Recovery time (days) | Low point of OD | Peak concn (mg/liter) |
|--------------------------|---------------------|----------------|---------------------|----------------|----------------------|
|                          |                     |                |                     |                | Xylose | Glucose |
| 8                        | .240                | .009           | 0                   | .234           | 1.0     | <0.5    |
| 12                       | .245                | .024           | 0.48                | .221           | 5.0     | <0.5    |
| 16                       | .243                | .074           | 1.01                | .171           |         |         |
| 24                       | .245                | .095           | 1.43                | .144           |         |         |
| 24                       | .248                | .123           | 2.10                | .125           | 52      | 3.0     |

* Escherichia coli at 25 C.
* The final holding time was 6 hr in all instances.
* Average of steady-state culture optical density (OD) before and after the switch (not significantly different).
* Steady-state sugar concentrations were: xylose, <1 mg/liter; glucose, <0.5 mg/liter.
* No sugar analyses.
cultures ceased growing and straight washout occurred. The experimental data follow curves quite different from these, indicating the existence of considerable growth throughout the transient period.

**DISCUSSION**

Batch growth curves for the glucose-xylose system indicate inhibition of xylose utilization in the presence of glucose, as well as an induction period following glucose depletion evidenced by the lag period prior to xylose utilization.

Batch growth curves for the glucose-galactose system indicate inhibition of galactose utilization in the presence of glucose and virtually no induction period following glucose depletion. This suggests an induction of the galactose enzymes during growth in glucose, with inhibition being caused not by a lack of enzymes but by another phenomenon such as inhibition of galactose transport.

Steady-state continuous cultures fed equal flow rates of glucose and either galactose or xylose have been found to utilize both sugars completely (within limits of detectability) at both high and low dilution rates. This indicates that the small steady-state concentration of glucose (<0.5 mg/liter) is not sufficient to cause inhibition of either galactose or xylose utilization.

Switching feeds from glucose to a secondary substrate such as xylose causes a continuous culture to suffer a transient population decrease during the first hours after switching. The culture is not induced for utilization of the secondary substrate during this period, and the incoming medium simply acts to dilute the culture and cause the population density to decrease through a washout. After the culture begins to utilize the new substrate, the population density first decreases more slowly and then increases when the growth rate exceeds the dilution rate. Eventually, complete utilization of the new substrate is accomplished.

In the feed rate shift-up experiments, two results are of interest: (i) the effect of initial holding time on the extent of the transients, and (ii) the almost exclusive accumulation of the secondary substrate while glucose remains fully utilized in two substrate systems.

The first result indicates that cultures at long holding times not only are growing slowly because they are at steady state with a slow feed flow rate, but also that they are incapable of immediately growing at a faster growth rate. Even when the feed medium contains only glucose, a culture at long holding time ($\theta = 24$) requires an induction period before it is able to utilize fully substrate fed at $\theta = 6$ hr.

With respect to the second point, glucose-xylose chemostats, when shifted up to a faster flow rate, are unable to use all of the substrate at the new flow rate. The fact that the culture tends to utilize all of the glucose and less than all of the xylose during the transient period must be related to one or both of two factors. Either the glucose concentration rises to a higher but still undetectable level which is effective in causing some inhibition of xylose utilization, or the culture is more capable of adapting to higher flow rates of glucose than it is to higher flow rates of xylose.

The former explanation is less likely because the actual glucose concentration in most of these cases remains very low, less than 0.5 mg/liter.

The other explanation may be valid in view of the constitutive nature of glucose metabolism. At the initial holding time, the culture is
adapted to both glucose and xylose to an extent at least sufficient to utilize all (or virtually all) of the incoming sugar. The inducible xylose enzymes might be produced only at about the minimum level to utilize all xylose at the initial flow rate, whereas enzymes associated with glucose utilization may be present at a higher than necessary level for the initial flow rate. This seems reasonable in that the glucose enzymes are constitutive, and may be present in the cells at a level consistent with the total substrate flow rate and total population size. This level of enzyme, however, is greater than needed for the initial pre-shift-up glucose flow rate since only half of the incoming substrate is glucose. This would account for full utilization of glucose during transient xylose accumulation after the shift-up.

Similar arguments can be made for shift-ups in the glucose-galactose system, which shows transient behavior similar to that of the glucose-xylose system.

ACKNOWLEDGEMENT

This work was supported by Public Health Service grant GM 16692 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Brown, A. H. 1946. Determination of pentoses in the presence of large quantities of glucose. Arch. Biochem. 11:269–278.
2. Drury, H. F. 1949. Identification and estimation of pentoses in the presence of glucose. Arch. Biochem. 19: 455–566.
3. Harvey, R. J. 1970. Metabolic regulation in glucose-limited chemostat cultures of E. coli. J. Bacteriol. 104: 698–706.
4. Maaløe, O., and N. O. Kjeldgaard. 1966. Control of macromolecular synthesis. W. A. Benjamin, Inc., New York.
5. Mateles, R. I., D. Y. Ryu, and T. Yasuda. 1965. Measurement of unsteady state growth rates of microorganisms. Nature (London) 208:263–265.
6. Monod, J. 1942. Recherches sur la croissance des cultures bactériennes, Herman et Cie, Paris.
7. Novick, A., and L. Szilard. 1950. Description of the chemostat. Science 112:715–716.
8. Parker, R. B. 1967. Gravity flow systems for continuous culture feed. Appl. Microbiol. 15:197.
9. Schoon, D. J., J. F. Drake, A. G. Fredrickson, and H. M. Tsuchiya. 1970. Automated counting of microbial colonies. Appl. Microbiol. 20:815–820.
10. Sempere, J. M., C. Gancedo, and C. Asensio. 1965. Determination of galactosamine and N-acetylgalactosamine in the presence of other hexosamines with galactose oxidase. Anal. Biochem. 12: 509–515.