The Effect of Methyl-alpha-D-Glucopyranoside on *Escherichia coli* Growth on Lactose and on Diauxic Growth

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ABSTRACT

Diauxic growth in *Escherichia coli* was originally explained by the theory of catabolite repression which proposes that a catabolite of glucose represses the induction of the lactose operon even when the inducer, lactose, is present. A competing theory, known as inducer exclusion, proposes that the phosphoenolpyruvate:carbohydrate phosphotransferase system enzyme III^{glc} in the dephosphorylated state inhibits lactose permease, and that when glucose is being transported into the cell, III^{glc} is in the dephosphorylated state thus preventing lactose from entering the cell. We have tested these theories and have shown that the glucose analog, methyl-alpha-D-glucopyranoside, is not metabolized by *E. coli*, yet it retards growth of the bacteria on lactose, and that the non-catabolizable lactose analog, isopropyl beta-D-thiogalactoside, induces the *lac* operon in the presence of glucose, but lactose is not metabolized while glucose is present. These results support the concept of inducer exclusion, and argue against catabolite repression.

INTRODUCTION

The phenomenon of diauxic growth is characterized by a microbe preferentially utilizing one nutrient before another when both are present (Monod 1945). For example, when Escherichia coli is inoculated into a minimal salts medium containing glucose and lactose, growth on glucose occurs first, followed by a lag period (intermediate lag) characterized by no increase in cell numbers, followed by growth on lactose. Step-like growth such as this is an illustration of diauxic growth. Inhibition of lactose utilization while glucose is present in the cell has been explained by the theory of catabolite repression (Magasanik 1961). This theory proposes that a catabolite of glucose represses the transcription of the lactose operon even when the inducer, lactose, is present.

It was first believed that the gene system that codes for the synthesis of the proteins needed for lactose utilization had only one genetic switch to control transcription (Jacob and Monod 1961 a and b). The switch consisted of a repressor protein which, in the absence of an inducer, was bound to the operator site. The binding of the repressor to the operator inhibited the transcription of the lac structural genes. However, when the inducer (lactose) is present in the cell, lactose binds the repressor protein, and this complex is unable to bind the operator, thus transcription of the lac structural genes can occur, and the enzymes

beta-galactosidase, permease, and transacetylase are produced. Further studies on this gene system have shown that there is a second regulatory mechanism necessary for transcription. For the lac operon to be transcribed, not only must the repressor be off the operator, but also the cyclic AMP receptor protein (CRP)-cyclic AMP(cAMP) complex must be on the promotor (de Crombrugghe et al. 1971). This gene system is reviewed by Beckwith (1987).

Recently, a protein of the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) has been shown to function in a regulatory capacity. Specifically, PTS enzyme III^{glc} (III^{glc}) in the dephosphorylated state has been reported to inhibit lactose permease (Mitchell et al. 1987. Saier et al. 1983). It has been proposed that III^{glc} in the phosphorylated state does not inhibit lactose permease but functions as a positive activator of adenyl cyclase, the enzyme that catalyzes the synthesis of cAMP from ATP (Saier 1989. Postma 1986). When glucose is being transported into the cell, III^{glc} is in the dephosphorylated state and thus adenyl cyclase is inactive, and lactose is prevented from entry into the cell. This phenomenon is known as inducer exclusion. In the absence of glucose, III^{glc} is phosphorylated, thus lactose can enter the cell, adenyl cyclase is active, therefore cAMP can be synthesized, and the lac operon is transcribed.

The purpose of this study was to further investigate the theories of catabolite repression and inducer exclusion by determining whether or not the non-catabolizable analog of glucose, methyl-

alpha-D-glucopyranoside, would repress lac operon transcription. If the analog is not catabolized, it could be assumed that no catabolites would be present in the cells to repress lac operon transcription, hence E.coli should show growth on lactose. However, if the analog was to prevent growth of E. coli on lactose, and no methyl-alpha-glucose catabolites would be present, it would suggest that, in this case, some mechanism other than catabolite repression may exist to explain diauxic growth (i.e. inducer exclusion).

To further test the theories of catabolite repression and inducer exclusion, E.coli would be exposed to isopropyl beta-D-thiogalactoside (IPTG) in diauxic medium. IPTG has the ability to get into the cell and induce the lac operon, but it is not catabolized. If IPTG induces the lac operon in the presence of glucose, and lactose is not metabolized while glucose is present, one can conclude that inducer exclusion is an explanation for diauxic growth in this case. If, however, lactose and glucose were used simultaneously it would suggest that glucose does not inhibit the cellular uptake of lactose. Also, if IPTG induces beta-galactodidase synthesis while glucose is being metabolized, it would argue against adenyl cyclase being inactive in the presence of glucose. On the other hand, if IPTG fails to induce the lac operon in the presence of glucose, it would support the theory of catabolite repression.

METHODS AND MATERIALS

BACTERIUM

The genetically well defined bacterium used in this study was strain MG1655 which was derived from Escherichia coli K-12. It differs from the wild type in that it is lambda minus, F minus and contains no detectable chromosomal copies of the gamma delta insertion sequence. For a history of this strain, see Guyer et al. (1980). Also used in this study were several locally collected field isolates of E.coli. These field isolates are not genetically defined; however, numerous biochemical tests (see page 7) were done on these field isolates to confirm their identity as E.coli (see Table 1). Either type of bacterium, the genetically defined or one of the field isolates, was used to inoculate starter culture media (see page 7) for use in all experiments. The starter cultures were incubated for 24 hours at 37°C. A quantity of 25 microliters of 24 hour starter culture was inoculated into each experimental medium (defined below) at the onset of each experiment.

MEDIA

All experiments were conducted in a minimal salts solution to which carbohydrates were added.

Minimal Salts Solution (MSS). The salt solution to which carbohydrate was added was prepared as previously described by Diehl and Malasavage (1989) except it was composed of 2.0 g NH_4Cl, 12.0 g Na_2HPO_4, 6.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$ in 1.0 L H_2O. The pH was adjusted to 7.0 with KOH.

Stock Carbohydrate Solutions. Carbohydrate solutions were prepared as 10% aqueous solutions and sterilized by filtration through a 0.2 micron Acrodisc filter (Gelman Scientific, Ann Arbor, Mi. 48106) or autoclaved. IPTG (Sigma Diagnostics, St. Louis, Mo.) was prepared as a 5% aqueous solutions. When IPTG was added to culture media its final concentration was 0.0010M.

Starter Culture Medium. One milliliter of MSS was aseptically placed into a sterile test tube along with 33.3 microliters of the 10% glucose solution and 0.3ml of dH_2O in order to make a final concentration of 0.25% carbohydrate in 1.33ml of starter culture medium.

Experimental Media. Three milliliters of the salt solution were placed in a sterile spectrophotometer tube along with any combination of carbohydrate solution. Metabolizable carbohydrate concentration normally did not exceed 0.0035M (except in competition studies), and the final medium volume was adjusted to 4ml with dH_2O. The experimental medium was inoculated with 25 microliters of bacteria from the 24 hour starter culture.

Biochemical Tests. Media used in biochemical tests for the field isolates were prepared according to manufacturer's (Difco, Detroit, Mi.) instructions.

GROWTH CURVES

Bacterial growth curve data were collected spectrophotometrically using an Apple IIE computer that was equipped with an analog/digital conversion board as previously described by Diehl and Angstadt (1989).

BETA-GALACTOSIDASE ASSAYS

Beta-galactosidase (beta-gal) assays of bacterial cultures were performed, and activities calculated, according to the procedure described by Bahl et al. (1980), except absorbance (light

scattering) values of bacterial suspensions were measured at 490 nm. This assay involved placing 50 microliters of culture into 0.5ml of Z buffer (pH 7.0 phosphate buffer containing 16.1g $Na_2HPO_4 \cdot 7H_2O$, 5.5g NaH_2PO_4 H_2O, 0.75g KCl, 0.246g $MgSO_4$ $7H_2O$ per liter, and 2.7ml beta-mercaptoethanol) and adding 50 microliters each of 1.0% sodium dodecyl sulfate (SDS) and $CHCl_3$. Then 0.15ml of o-nitrophenyl-beta-D-galactoside (ONPG) at a concentration of 4.0mg/ml H_2O was added. The time of ONPG addition was recorded. This mixture was incubated at 28°C until a pale yellow color developed. The reaction was then stopped by the addition of 0.25ml Na_2CO_3 (1.0M) and the time was recorded. After mixing, the aqueous phase was pipetted to a 1.5ml polypropylene centrifuge tube (Perfector Scientific, Atascodeno, Calif.) and the cellular debris was pelleted at 9,000 rpm for 10.0 minutes in an Eppendorf 5415 centrifuge. The absorbance of the supernatant was spectrophotometrically measured at 420 nm. To control for non-enzyme catalyzed hydrolysis of ONPG, mixtures were prepared as described above except uninoculated culture medium was substituted in place of bacterial culture. Units of beta-galactosidase specific activity (Z/B) were calculated according to Jobe et al. (1974), namely:

$$Z/B = \frac{\text{net O.D. @ 420 nm}}{\text{net O.D. @ 490 nm X assay time in min.}}$$

CARBOHYDRATE ASSAYS

Concentrations of glucose and lactose were determined with Trinders Glucose 100 reagent, Sigma Diagnostics (St. Louis, Mo.). For the glucose assays, samples of culture were centrifuged at 9,000 rpm for 10.0 minutes and 25.0 microliters of supernatant were combined with 1.5 ml of Trinders. For the lactose assays, 7.5 units beta-galactosidase enzyme purchased from Worthington (Freehold, N.J.) were added to the above mixture. After a 60 minute incubation at 37^{o}C, absorbance was read at 505 nm. A tube of 2.5 ml Trinders with no bacterial culture was used for glucose negative control assays. For glucose positive control assays, 1.5 ml of Trinders was combined with 25.0 microliters of 0.0035M glucose. A tub containing 2.5 ml of Trinders and 5 microliters of beta-gal enzyme wit no bacterial culture was used for lactose negative control assays. Fo lactose positive control assays, 1.5 ml of Trinders and 5 microliters of beta-gal enzyme was combined with 48 microliters of 0.0035M lactose Lactose concentrations were determined by calculating the difference i absorbance of lactose assays and glucose assays.

RESULTS

First, to confirm that methyl-alpha-glucose is a non-metabolizable carbohydrate, MSS containing 0.0035M of methyl-alpha-glucose as the only carbohydrate were inoculated separately with either WT E.coli or a field isolate. These experiments showed no growth after six weeks incubation.

Next, experiments were done to determine what effect methyl-alpha-glucose had on diauxic growth in WT E.coli. Figure 1 is a normal diauxic growth curve with assays for glucose, lactose, and beta-gal for E.coli (genetically defined strain, or WT) cultured in MSS containing 0.0035M glucose and lactose. The initial lag period was one hour long, followed by an 8.0 hour growth on glucose. The intermediate lag period was approximately 1.75 hours long, followed by 4.5 hours growth on lactose. Figure 2 is a growth curve with glucose, lactose, and beta-gal assays for E.coli (WT) cultured in MSS containing 0.0035M glucose, lactose, and methyl-alpha-glucose. The initial lag period for this experiment was 2.0 hours, followed by a 9.0 hour growth on glucose. The intermediate lag period was approximately 2.0 hours, followed by a 4.5 hour growth on lactose. For both types of growth curves, lactose was not utilized until glucose was gone. In the WT experiment involving glucose and lactose only (normal diauxic growth), beta-

galactosidase activity was not detected until glucose was completely utilized. However, in the culture that also contained methyl-alpha-glucose, a small amount of beta-galactosidase activity was found near the end of growth on glucose.

When IPTG was added to WT E.coli cultures containing glucose, lactose, and methyl-alpha-glucose (all at 0.0035M), the intermediate lag period was eliminated (compare Figures 2 and 3). Carbohydrate assays done on these cultures show that lactose was not utilized simultaneously with glucose. However, when IPTG is present, beta-galactosidase assays show that beta-gal activity was present in the cell before lactose was utilized (see Figure 3).

In the experiments involving field isolate E.coli (FI) and the carbohydrates glucose, lactose, and methyl-alpha-glucose (all at 0.0035M), there was no sign of diauxic growth (see Figure 4) as there was when just glucose and lactose were present (see Figure 5). Assays on FI cultures containing glucose, lactose, and methyl-alpha-glucose show that beta-gal was detectable before glucose was gone, but lactose was not utilized until glucose was gone (see Figure 4). In experiments involving field isolate E.coli grown in MSS containing glucose, lactose, methyl-alpha-glucose (all at 0.0035M), and IPTG, there was again no sign of diauxic growth. Assays done on these cultures show that lactose was not utilized simultaneously with glucose, and beta-gal was detectable before lactose was utilized (see Figure 6). The results from both these types of experiments are consistent with the results from the same types of experiments done with WT E.coli, except

methyl-alpha-glucose does not eliminate diauxy with WT E. coli (compare Figures 2 and 4; Figures 3 and 6).

Wild type E.coli experiments involving competition between glucose and methyl-alpha-glucose were done in separate media containing glucose at 0.0035M and methyl-alpha-glucose at concentrations that varied from glucose by factors of 2X and 0.5X. This resulted in initial lags of 2 and 1 hours respectively as compared to a 1 hour lag in MSM containing equal concentrations of glucose and methyl-alpha-glucose (see Figures 7, 8, and 9). The culture incubated with 2X concentration of methyl-alpha-glucose relative to glucose (Figure 7) not only had a retardation in the onset of growth, but once growth started, the rate was not as rapid as that of cultures containing 0.5X methyl-alpha-glucose relative to glucose (Figure 8) or methyl-alpha-glucose and glucose in equal concentrations (Figure 9).

Competition studies were done involving WT E.coli cultured in media containing 0.0035M glucose and lactose, and methyl-alpha-glucose at concentrations of either 0.0070M (2X) or 0.0017M (0.5X). In the experiment involving 2X methyl-alpha-glucose, the initial lag period was 2.5 hours, followed by a 9.5 hour growth on glucose. The intermediate lag period for this experiment was 1.0 hour, followed by a 5.0 hour growth on lactose (see Figure 10). In the experiment involving 0.5X methyl-alpha-glucose, the initial lag period was 2.0 hours, followed by a 10.0 hour growth on glucose. The intermediate lag period for this experiment was 3.75 hours, followed by a 10.5 hour growth on

lactose (see Figure 11). The growth on lactose for this type of experiment was twice as long as in the experiment involving 2X methyl-alpha-glucose concentration, greater than twice as long as in experiments involving just 0.0035M glucose and lactose, and greater than twice as long as in experiments involving 0.0035M glucose, lactose, and methyl-alpha-glucose (compare Figures 2 and 11).

Consequently, it became desirable to study the effects of varying concentrations of methyl-alpha-glucose in MSS containing just lactose. Experiments were done involving WT E.coli cultured in media containing 0.0035M lactose and methyl-alpha-glucose at concentrations of either 0.0035M, 0.0070M (2X), or 0.0017M (0.5X). In the experiment involving equal concentrations of lactose and methyl-alpha-glucose, there was an initial lag of approximately 10.5 hours, with growth on lactose for 25.5 hours (Figure 12). In the experiment involving 2X methyl-alpha-glucose, there was an initial lag of 7.0 hours, and a 23.5 hour growth on lactose (Figure 13). In the experiment involving 0.5X methyl-alpha-glucose, there was an initial lag of 22.0 hours, with a 15.0 hour growth on lactose (Figure 14).

The results of the biochemical tests done on field isolates of E.coli are presented in Table 1. All isolates tested negative for malonate, inositol, and H_2S on Triple Sugar Iron (TSI). All isolates tested positive for acid and gas in mannitol, sorbitol, rhamnose, arabinose, glucose, and lactose. All isolates tested positive for the amino acids lysine and ornithine, and negative for arginine.

DISCUSSION

Initial experiments showed that methyl-alpha-glucose in MSS alone does not support the growth of WT or field isolate E.coli, suggesting that methyl-alpha-glucose is a non-catabolizable carbohydrate.

In the experiments done with WT E.coli in a MSS containing methyl-alpha-glucose, glucose, and lactose (Figure 2), the amount of time required for glucose to be metabolized was approximately 9.0 hours, as compared to the culture without methyl-alpha-glucose, which took 8.0 hours to metabolize glucose (compare Figures 1 and 2). It was concluded that methyl-alpha-glucose appeared to have an inhibitory effect on the growth of E.coli on glucose. Studies involving competition between glucose and methyl-alpha-glucose in which the concentration of methyl-alpha-glucose varied from that of glucose by factors of 2X and 0.5X confirmed that methyl-alpha-glucose caused a delay in the onset of growth and a reduction in growth rate. One interpretation of these results is that methyl-alpha-glucose competes with glucose for a receptor/transporter (namely Enzyme II^{glc}); thus as the concentration of methyl-alpha-glucose increases relative to glucose, competition becomes greater, and growth is progressively retarded. Hagihara, Wilson, and Lin (1963) and Gachelin (1970) report that accumulated (labelled) methyl-alpha-glucoside phosphate is easily

expelled from the cell upon addition of another PTS carbohydrate or (unlabelled) methyl-alpha-glucose. Perhaps in this study, methyl-alpha-glucose is expelling glucose from the cell, thus delaying and decreasing the growth of cells on glucose. Also, as the concentration of methyl-alpha-glucose was increased, the more glucose would be expelled, therefore showing a greater delay and decrease in growth on glucose as the methyl-alpha-glucose concentration increased, as was the case in these experiments.

Carbohydrate and beta-galactosidase assays done on the WT experments involving glucose, lactose and methyl-alpha-glucose confirm that glucose was metabolized before lactose, and beta-galactosidase was present only in small quantities before glucose metabolism was complete (Figure 2). The presence of beta-gal in the cell before the utilization of lactose could be explained by the suggestion that methyl-alpha-glucose caused some type of cellular alteration (such as the phosphorylation of III^{glc}, which comes off the lac permease) which allowed minor amounts of lactose to enter the cell and derepress the lac operon enough to detect small amounts of beta-galactosidase. However, assays done on WT cultures containing glucose, lactose, methyl-alpha-glucose, and IPTG (Figure 3) showed that glucose was metabolized before lactose. Lactose was not utilized until glucose was gone, but larger quantities of beta-galactosidase were present in the cell before utilization of lactose. This supports the concept of inducer exclusion, the reason being that, although beta-galactosidase and presumably permease are produced, lactose remains

outside the cell (inducer is excluded). Also, since beta-gal is being synthesized, this argues against III^{glc} regulating adenyl cyclase. That is, if glucose is present, III^{glc} is dephosphorylated, thus adenyl cyclase would be in the inactive form, cAMP could not be produced, and would not bind with CRP to bind with the promotor. This also argues against the theory of catabolite repression. Beta-gal could not be produced if some catabolite of glucose was repressing the induction of the lac operon.

Methyl-alpha-glucose has been reported to be transported and phosphorylated by enzyme II^{glc} (Postma 1986). Therefore, III^{glc} should be dephosphorylated, thus inhibiting lac permease in the presence of methyl-alpha-glucose. However, this was not true in the diauxic studies with the concentration of methyl-alpha-glucose tested (see Figure 2). Methyl-alpha-glucose did not significantly inhibit the growth of lactose in diauxy, nor did it greatly increase the intermediate lag period in experiments involving glucose, lactose and methyl-alpha-glucose (compare Figures 1 and 2). Postma (1986) also described the preference of E.coli for one PTS sugar to another, and explained this was probably due to the fact that the III^{glc}/II^{glc} system has a higher affinity for one carbohydrate over another. Diehl and Malasavage (1989) reported that the glucose analog, 2-deoxyglucose (2Dglc), has an inhibitory effect on lactose utilization. In this instance, perhaps there was a greater affinity for transport enzymes in the experiments involving 2Dglc than in the experiments involving methyl-alpha-glucose, thus growth on

lactose would not be greatly inhibited in MSS containing methyl-alpha-glucose, as it is in MSS containing 2Dglc.

Experiments involving competition between methyl-alpha-glucose and lactose with WT E. coli in MSS showed inhibition of growth on lactose. In the culture containing 0.0035M methyl-alpha-glucose and lactose, the initial lag period was 11.0 hours, as compared to a 2.0 hour initial lag in cultures containing 0.0035M methyl-alpha-glucose, lactose and glucose (compare Figures 2 and 12). Initial lags for cultures containing 0.0035M lactose and 2X or 0.5X methyl-alpha-glucose are, respectively, 7.0 hours and 20.0 hours (see Figures 13 and 14). The inhibitory effect methyl-alpha-glucose on growth of cells in MSS containing only lactose may be related to cell number. At the onset of these types of experiments, the concentration of methyl-alpha-glucose was relatively high compared to total cells present, thus causing a delay in the onset of growth. However, this does not explain why increased concentrations of methyl-alpha-glucose did not inhibit growth on lactose in the diauxic studies. Perhaps the methyl-alpha-glucose concentrations were not increased enough to significantly inhibit the growth on lactose, considering that cell number had increased several powers of ten while methyl-alpha-glucose concentration was increased only two fold. Also, inhibition of growth on lactose could be related to a weak affinity of methyl-alpha-glucose for II^{glc}, or maybe lactose transport enzymes work more efficiently in diauxic media containing methyl-alpha-glucose. Saier et al

(1983) asserts that an allosteric mechanism, involving the enzyme III^{glc}, is responsible for the inhibition of all of the non-PTS permeases by all PTS sugars. Therefore, it could be that methyl-alpha-glucose was causing an allosteric change that resulted in inhibition of lactose permease, such as the dephosphorylation of III^{glc}, keeping the lac operon repressed due to inducer exclusion, thus retarding the growth of WT E.coli on lactose. Perhaps this allosteric change does not occur in diauxic media due to some type of interaction with methyl-alpha-glucose and glucose (such as competition) in which methyl-alpha-glucose is not available to inhibit lac permease, thus not inhibiting the growth of WT E. coli on lactose in diauxic media. Another possibility is that when methyl-alpha-glucose binds II^{glc}, it is phosphorylated more slowly than is glucose. This slower phosphorylation would allow some of III^{glc} to momentarily become phosphorylated. This would then deinhibit enough permease to allow a little lactose to get into the cell.

Experiments done with the field isolates of E.coli involving glucose, lactose, and methyl-alpha-glucose showed no sign of diauxic growth (Figure 4), as is characteristic of WT E.coli. This could be due to genetic differences in the WT and field isolate E. coli. However, assays done on experimental cultures involving the field isolate E.coli showed the same characteristics as the assays that were done on experimental cultures involving WT E.coli. This suggests that the phenomenon of inducer exclusion is not exclusive to WT E.coli. One thing is certain, methyl-alpha-glucose does not

support the growth of E.coli in MSS, suggesting that it is not a catabolizable carbohydrate. If this is the case, then there would be no catabolites in the cell; and it follows that catabolite repression as originally conceived can not be the explanation for diauxic growth in this instance.

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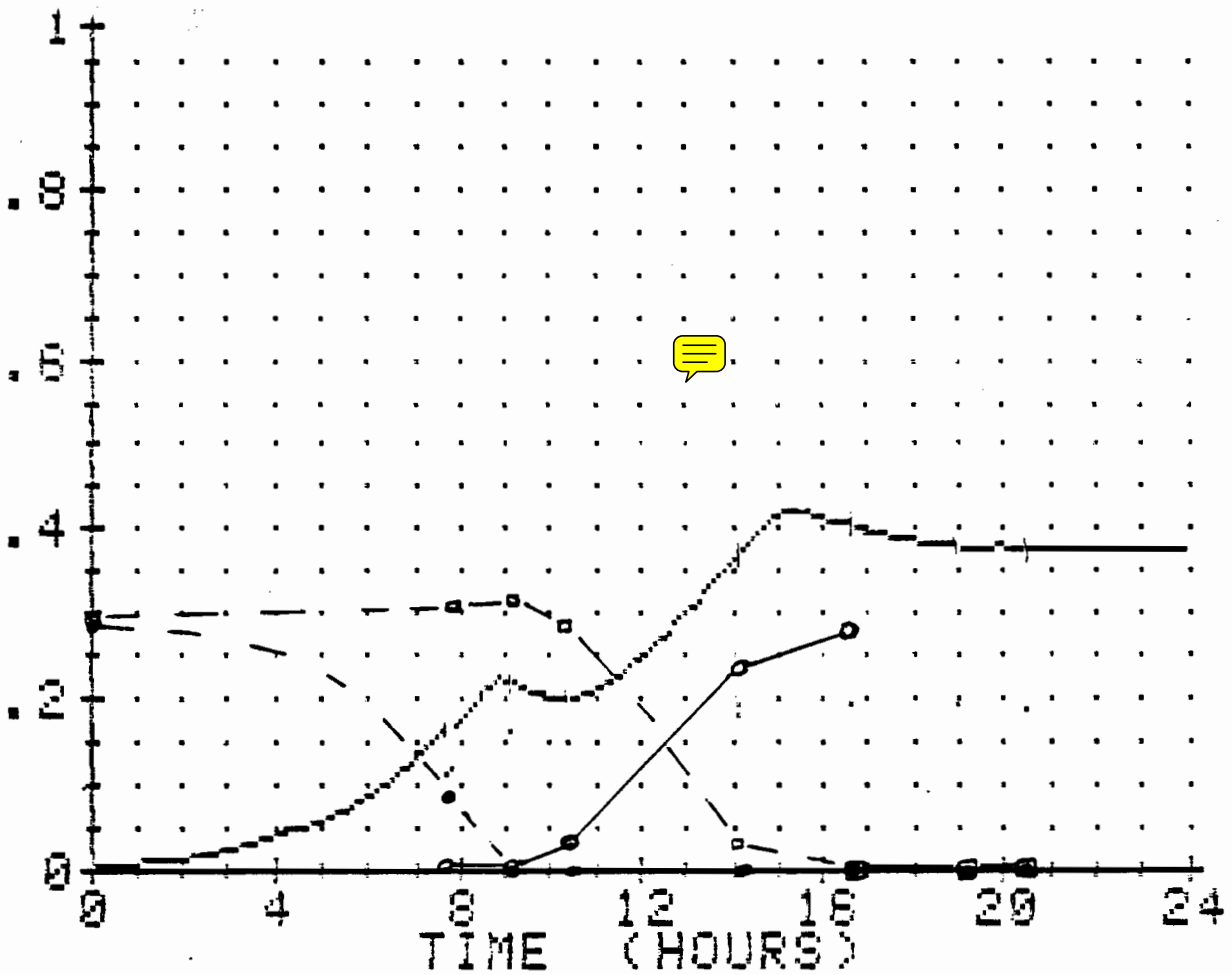

Figure 1

Normal diauxic growth curve for WT E.coli cultured in MSS containing 0.0035M glucose and lactose (intermediate lag = 1.75 hours).

● = glucose
□ = lactose
o = beta-galactosidase

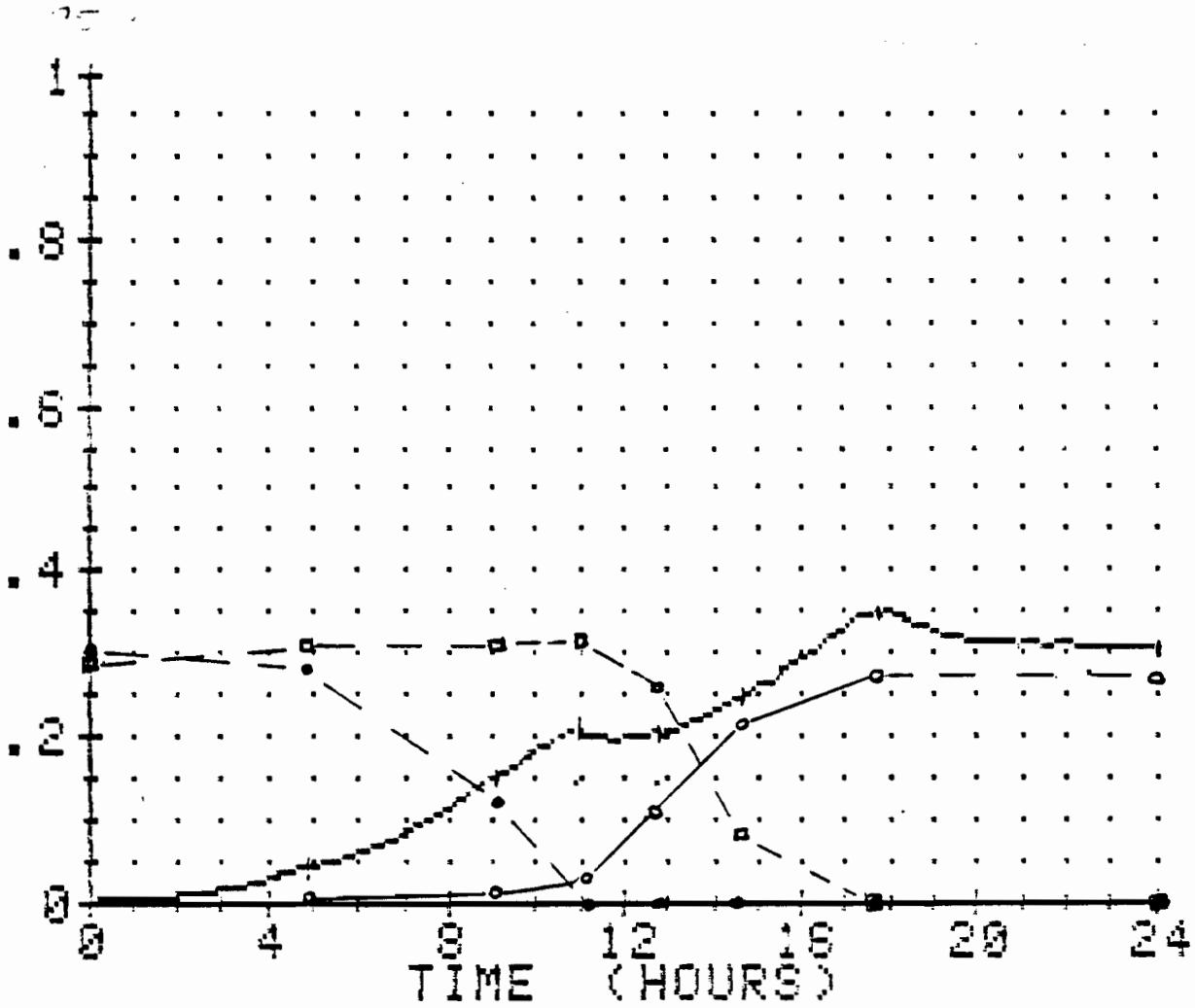

Figure 2

Growth curve for E.coli (WT) cultured in MSS containing 0.0035M glucose, lactose, and methyl-alpha-glucose (intermediate lag = 2.0 hours).

● = glucose
□ = lactose
o = beta-galactosidase

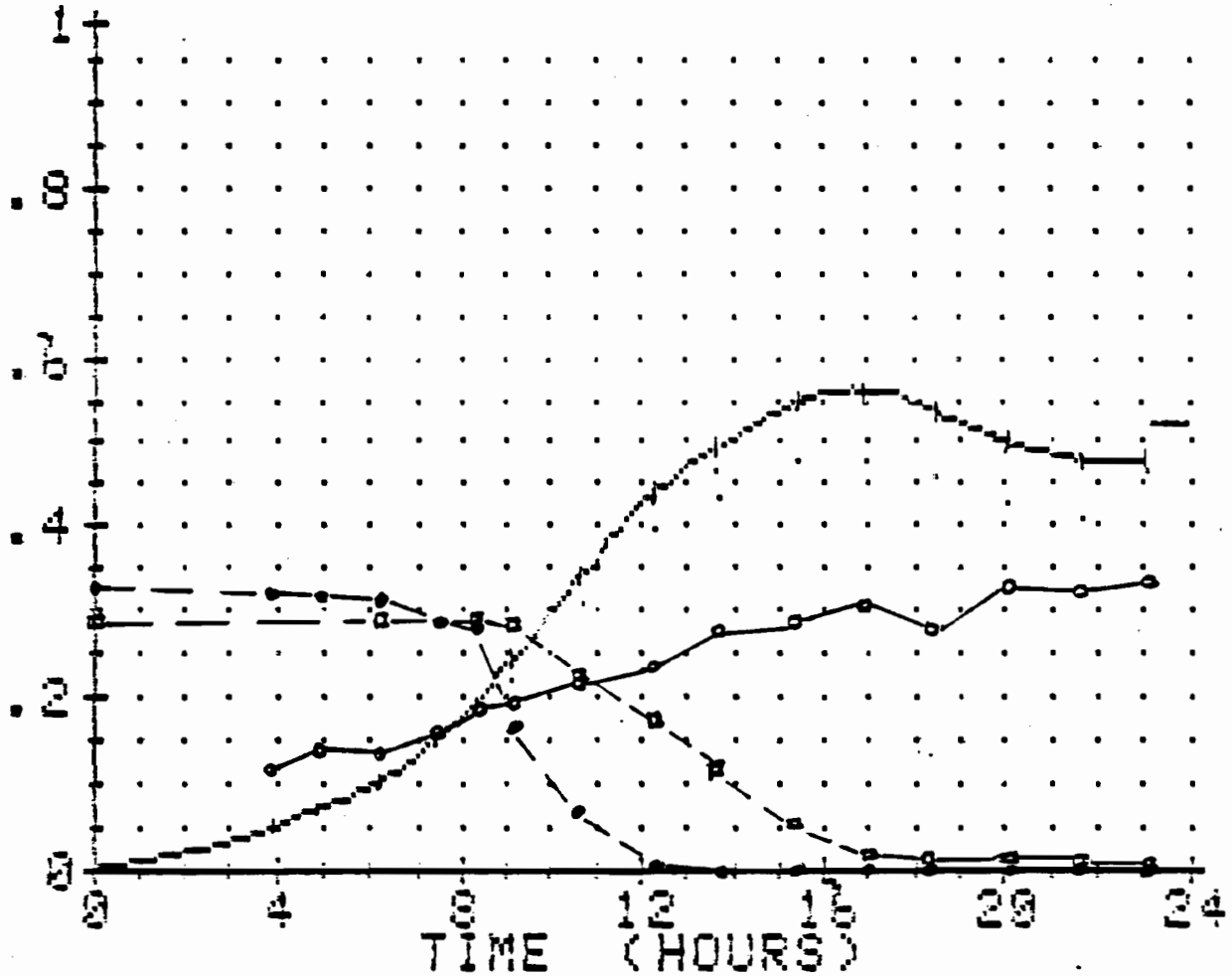

Figure 3

Growth curve for WT E.coli cultured in MSS containing 0.001M IPTG and 0.0035M glucose, lactose, and methyl-alpha-glucose (no intermediate lag).

● = glucose
□ = lactose
o = beta-galactosidase

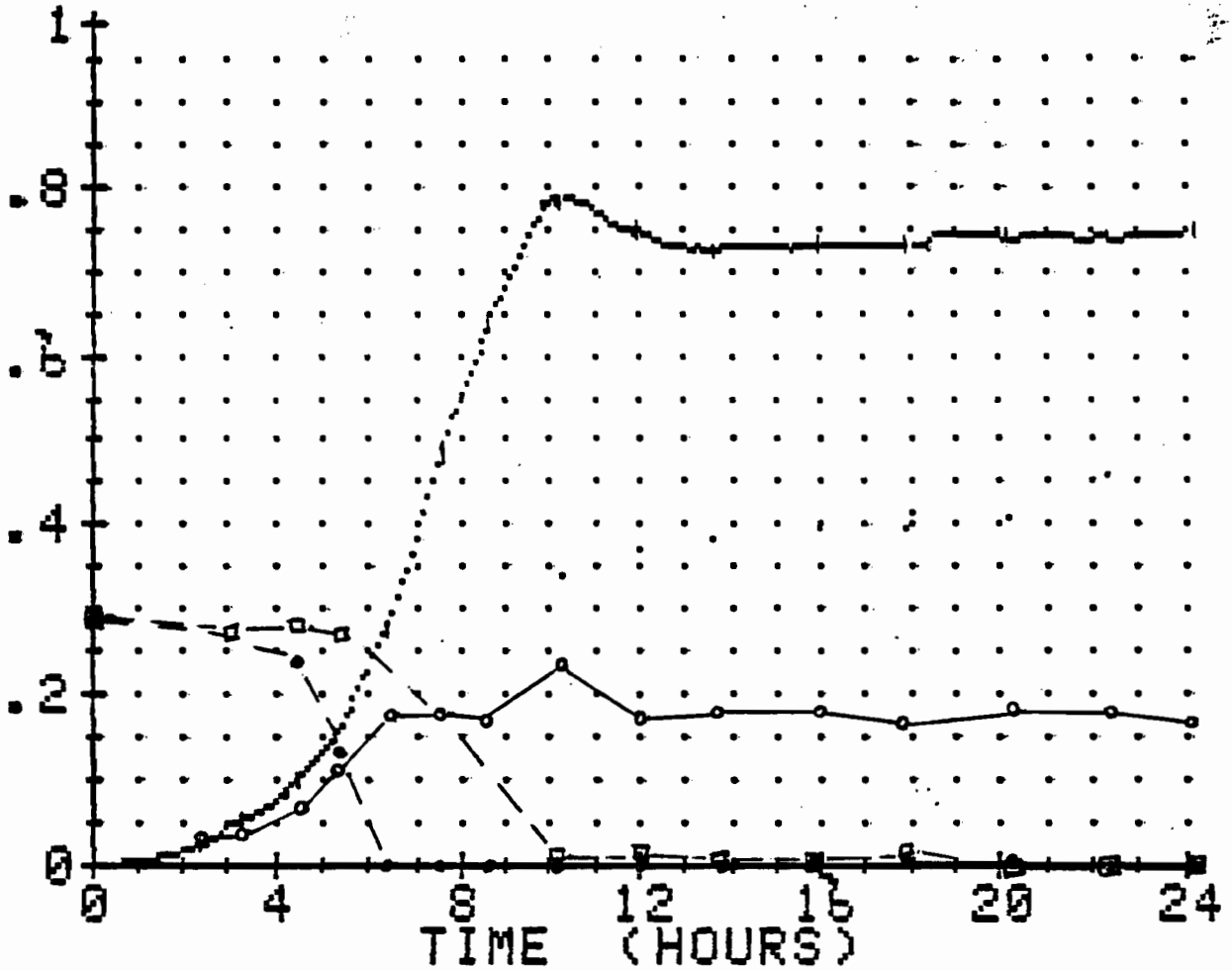

Figure 4

Growth curve for FI E.coli cultured in MSS containing 0.0035M glucose, lactose, and methyl-alpha-glucose.

● = glucose
□ = lactose
o = beta-galactosidase

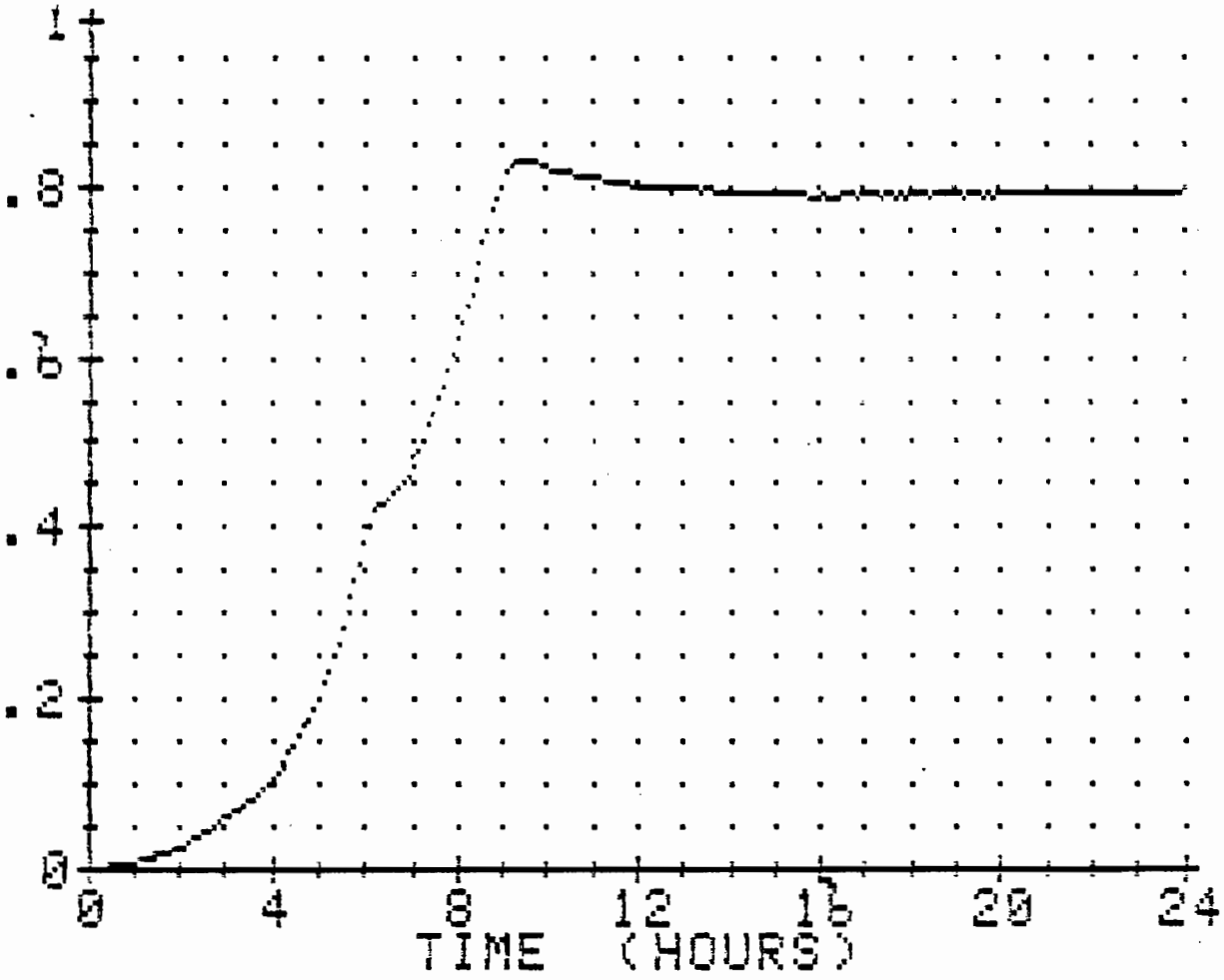

Figure 5

Growth curve for FI E.coli cultured in MSS containing 0.0035M glucose and lactose.

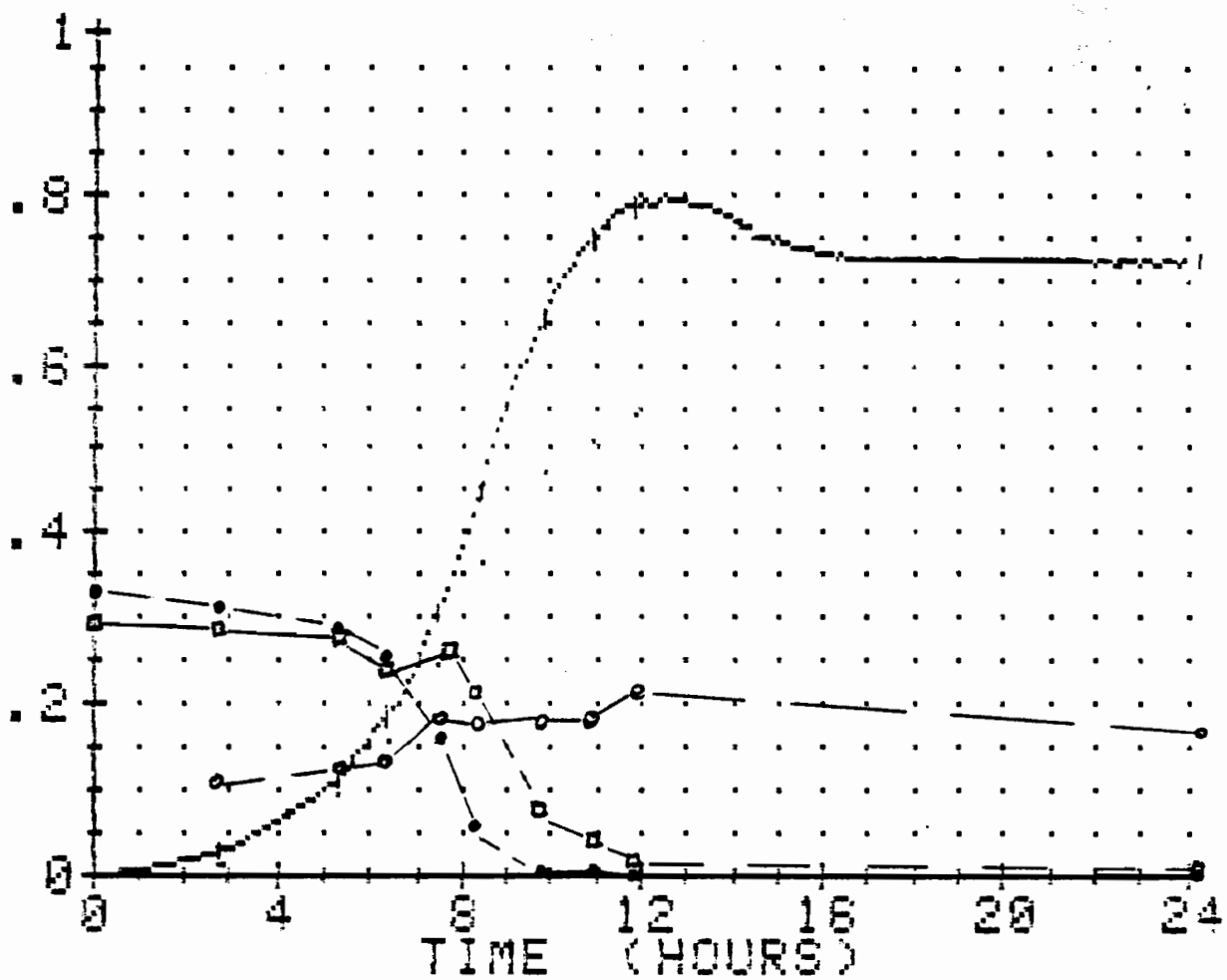

Figure 6

Growth curve for FI E.coli cultured in MSS containing 0.001M IPTG and 0.0035M glucose, lactose, and methyl-alpha-glucose.

● = glucose
□ = lactose
o = beta-galactosidase

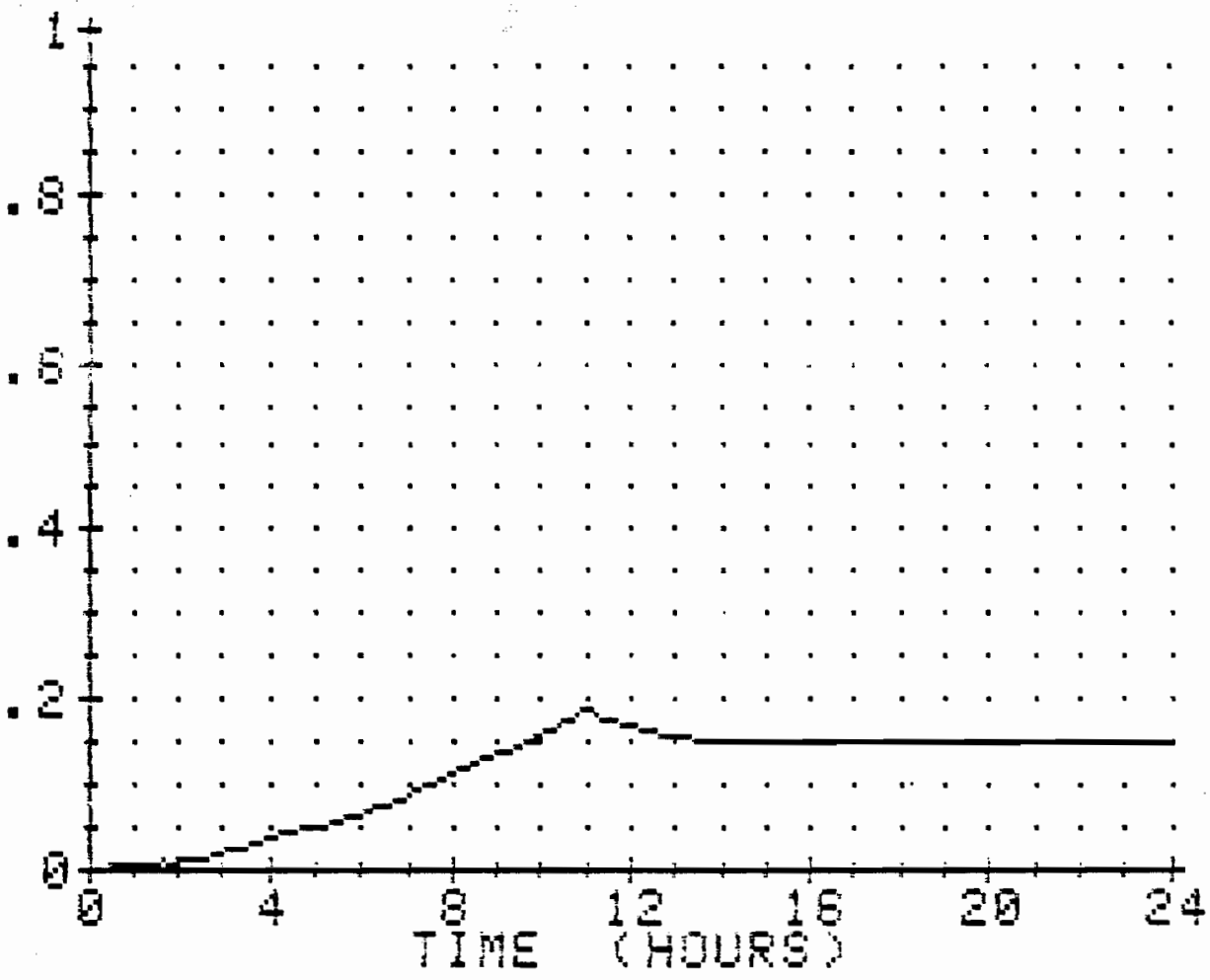

Figure 7

Growth curve for WT E.coli cultured in MSS containing 0.0035M glucose and 0.007M (2X) methyl-alpha-glucose.

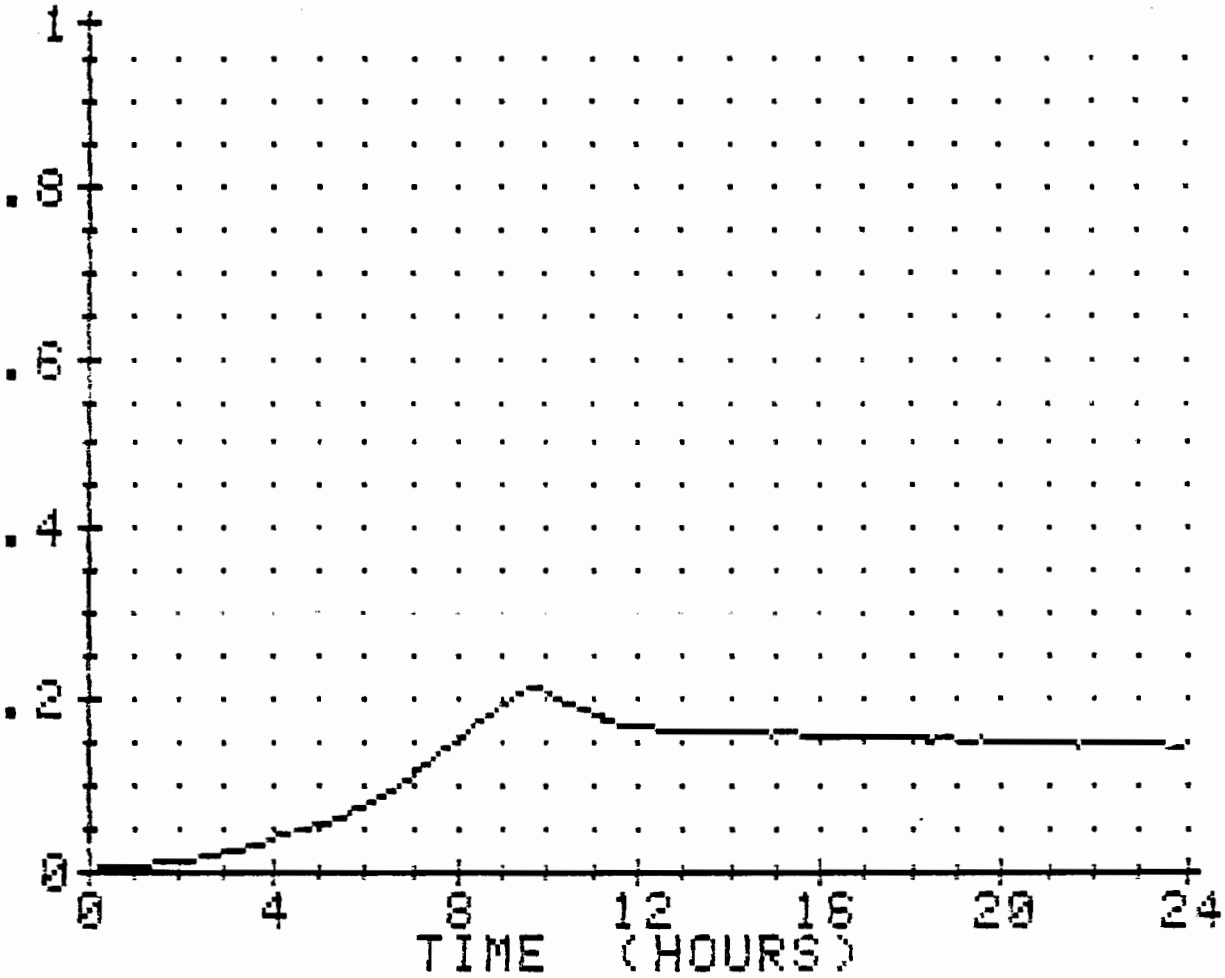

Figure 8

Growth curve for WT E.coli cultured in MSS containing 0.0035M glucose and 0.00175M (0.5X) methyl-alpha-glucose.

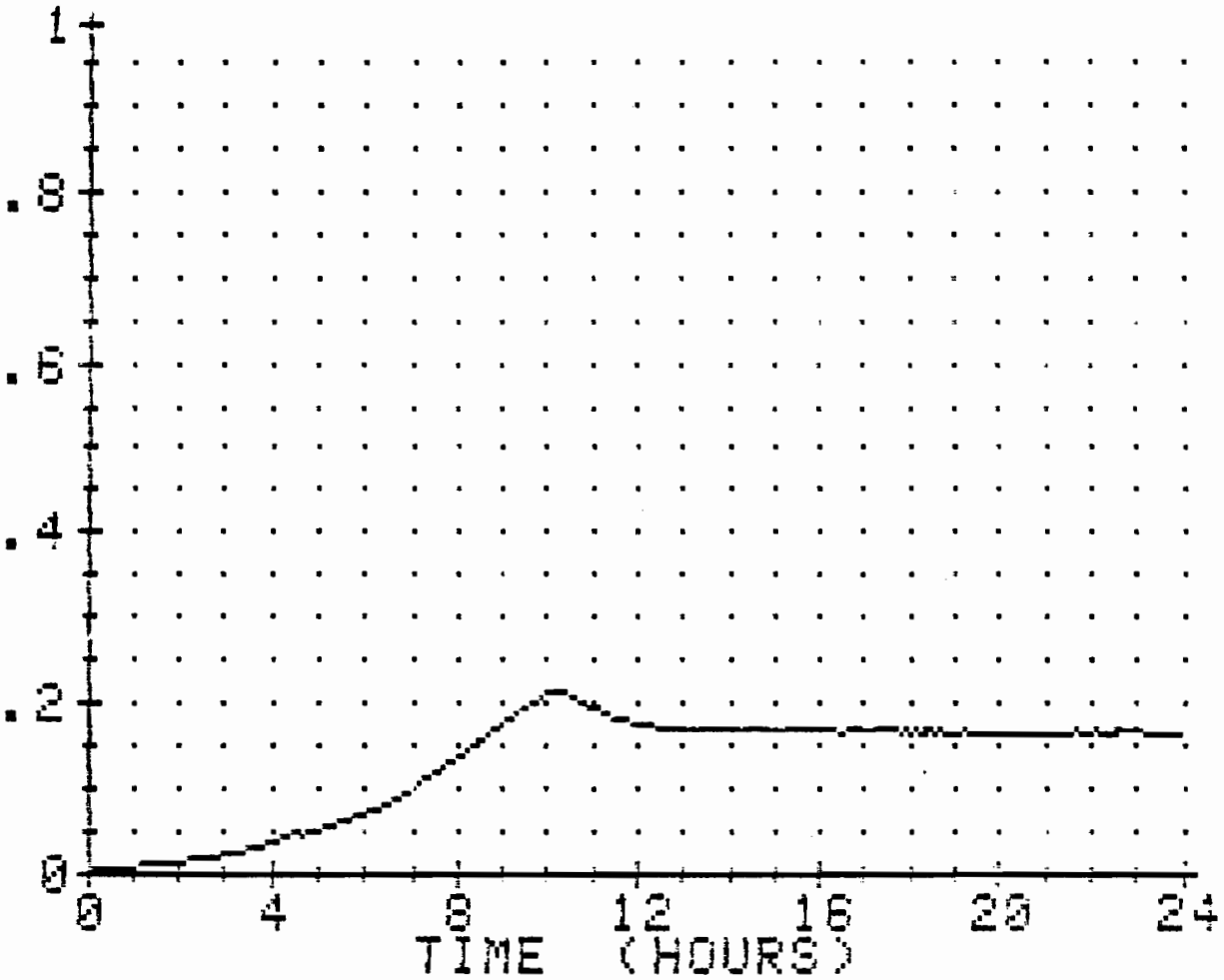

Figure 9

Growth curve for WT E.coli cultured in MSS containing 0.0035M glucose and methyl-alpha-glucose.

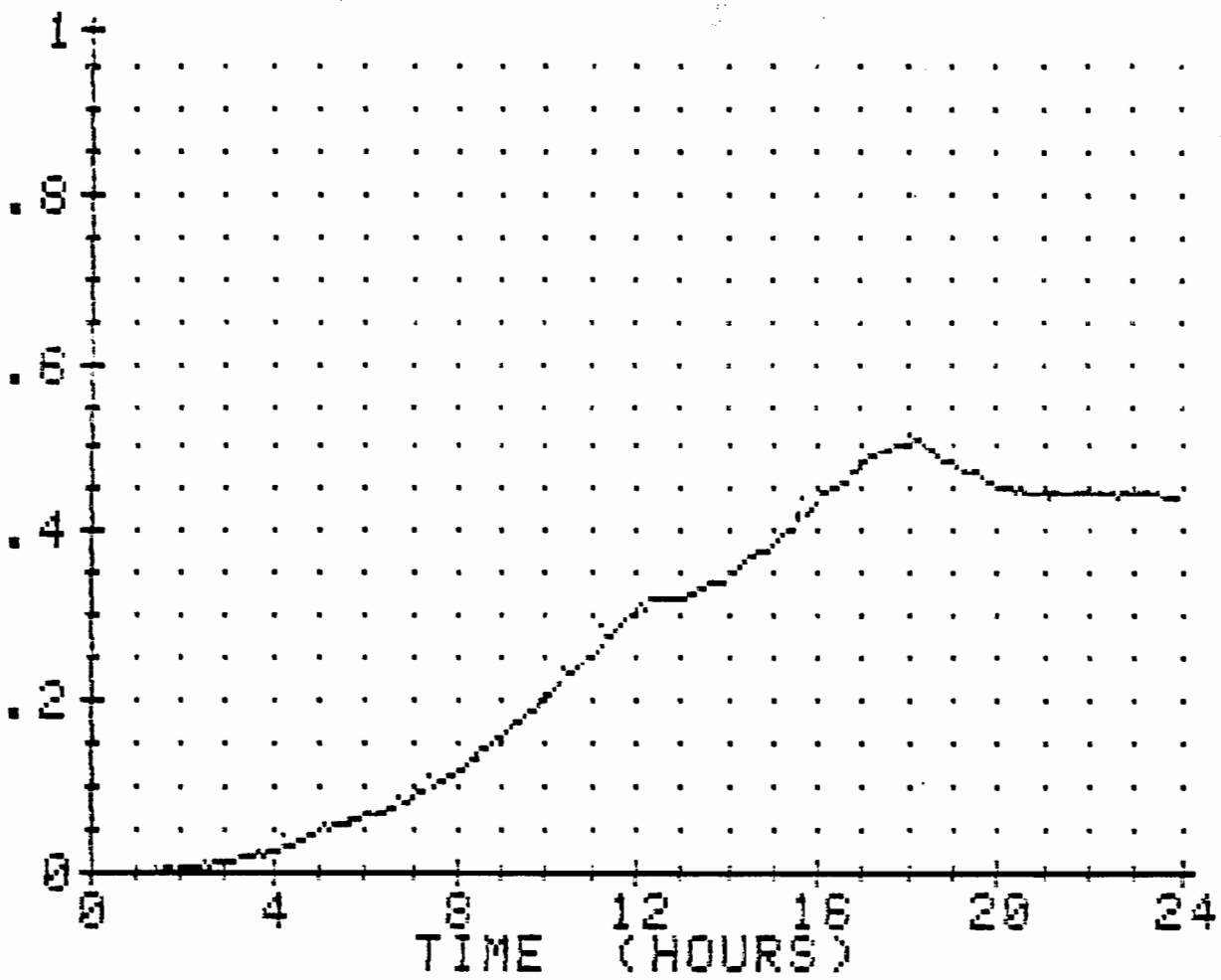

Figure 10

Growth curve for WT E.coli cultured in MSS containing 0.0035M glucose and lactose, and 0.007M (2X) methyl-alpha-glucose.

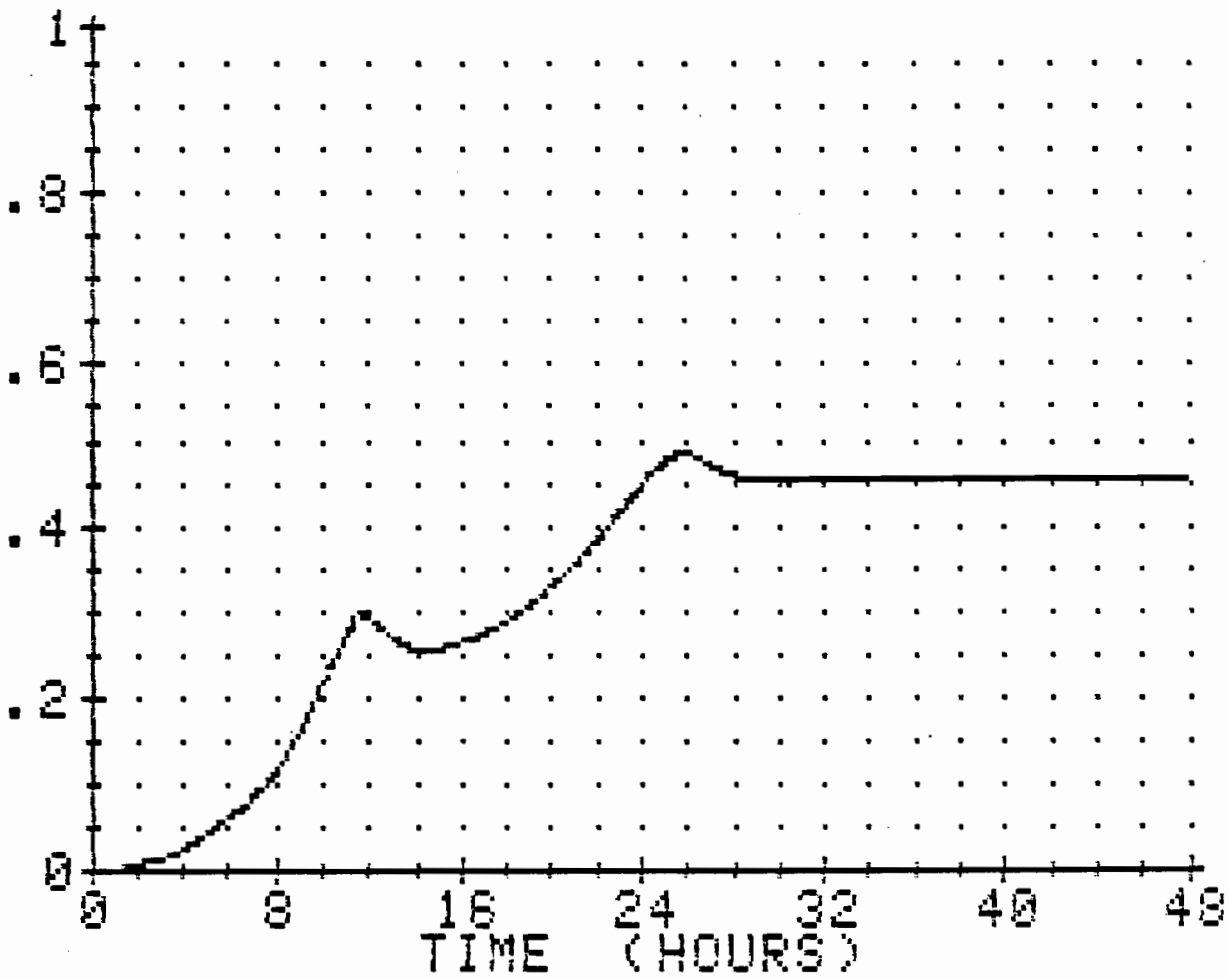

Figure 11

Growth curve for WT E.coli cultured in MSS containing 0.0035M glucose and lactose, and 0.00175M (0.5X) methyl-alpha-glucose.

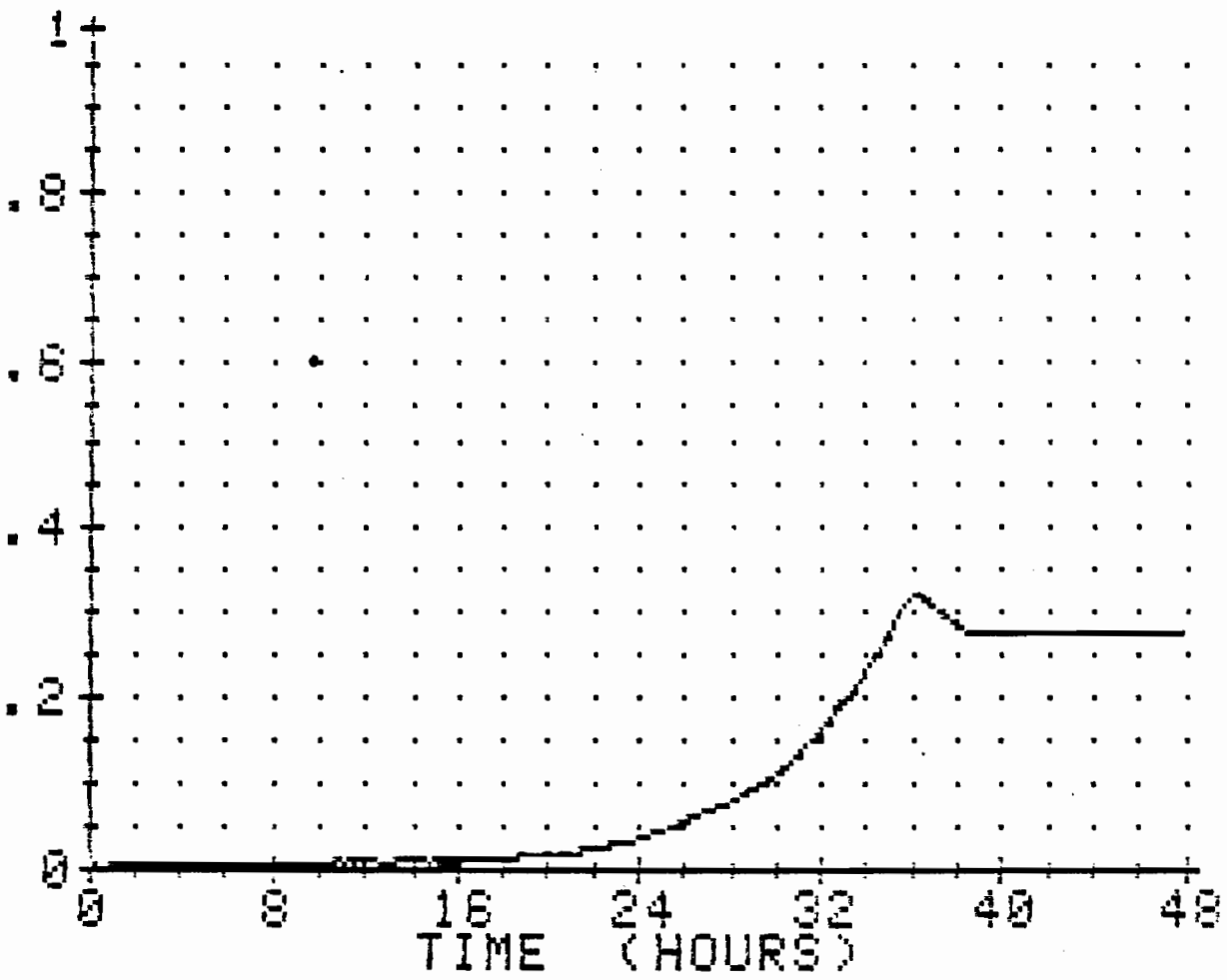

Figure 12

Growth curve for WT E.coli cultured in MSS containing 0.0035M lactose and methyl-alpha-glucose.

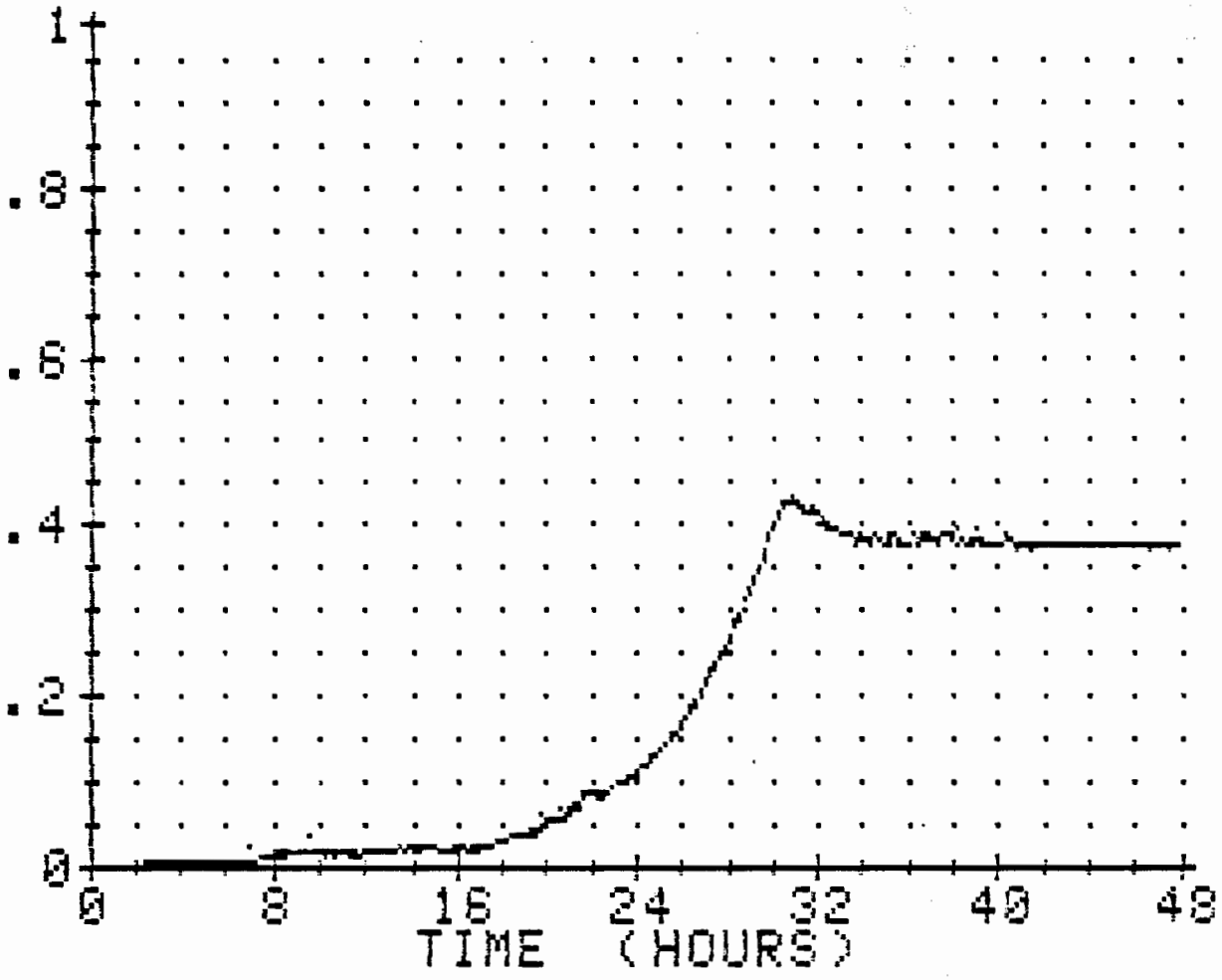

Figure 13

Growth curve for WT E.coli cultured in MSS containing 0.0035M lactose and 0.007M (2X) methyl-alpha-glucose.

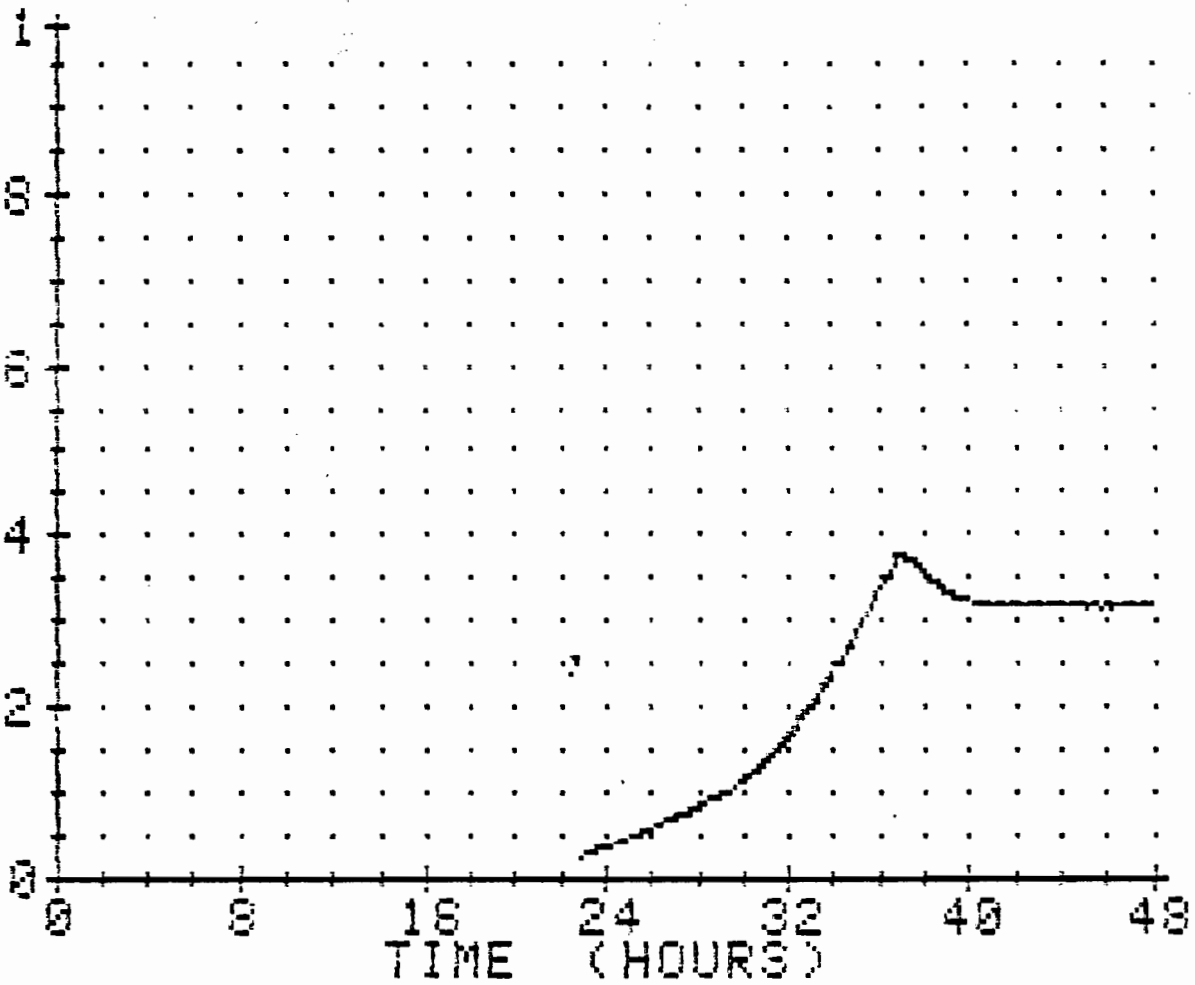

Figure 14

Growth curve for WT E.coli cultured in MSS containing 0.0035M lactose and 0.00175M (0.5X) methyl-alpha-glucose.

Table 1 Results of Biochemical Tests

Bacterium	Mal	Mann	Sor	Inos	Rh	Arab	Glu	Lac	Lys	Orn	Arg	H_2S in TSI	Indole	Methyl Red	Voges Proskauer	Citrate
E. coli WT	-	AG	AG	-	AG	AG	AG	AG	+	+	-	-	+	+	-	-
E. coli FIA	-	AG	AG	-	AG	AG	AG	AG	+	+	-	-	+	+	-	-
E. coli FIB	-	AG	AG	-	AG	AG	AG	AG	+	+	-	-	+	+	-	-
E. coli FI1	-	AG	AG	-	AG	AG	AG	AG	+	+	-	-	+	+	-	-
E. coli FI2	-	AG	AG	-	AG	AG	AG	AG	+	+	-	-	+	+	-	-

AG: produced acid and gas in 48 hours