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EFFECT OF HEAVY METALS ON BACTERIA

Elena Vratonjic

The Effect of Heavy Metals on Bacteria

By

Elena Vratonjic

Columbus State University

The College of science

The Graduate Program In Environmental Science

Effect of Heavy Metals on Bacteria

A Thesis in

Environmental Science

By

Elena Vratonjic

Submitted in Partial Fulfillment
of the requirements
for the Degree of

Master of Science

December 2003

@2003 by Elena Vratonjic

I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science

Dec 11th, 2003
Date

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We approve the thesis of Elena Vratonjic as presented here.

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ABSTRACT

Heavy metals have become devastating environmental contaminants due to their widespread use in the manufacture of electronics, plastics, batteries and dyes. The discharge of heavy metals into the environment has caused concern about their effects on the ecosystem. Many metals are essential for growth in small amounts. However, at higher concentrations heavy metals are toxic because they bind to organic compounds. This accounts for their effects on some important parts of the cell structure, like their ability to denature protein molecules. The initial objective of this study was to find out the growth rate of *E.coli* and *M.roseus* in the presence of 1mM and 10 μM concentrations of cadmium and lead. *E.coli* and *M.roseus* were used due to their ability to grow in a controlled environment and ease of usage. The growth rates of the bacteria and viable counts were obtained via light scattering. Bacteria were grown on rich media (LB broth) as well as mineral M9 media supplemented with and without casamino acids and growth rates were compared. Another objective in this study was to identify genes in *E. coli* mutants that were more resistant or sensitive to cadmium. *E.coli* was mutagenized with a transposon to identify mutants with altered sensitivities to Cd. The growth rates of the mutants and wild type were obtained with and without Cd. Results suggest that Cd^{+2}, at 10μM and 1mM concentrations as well as Pb^{+2} at 1mM concentration, had a significant effect on *E.coli* and *M.roseus* in all media types. Insignificant effects were found in *M.roseus* and *E.coli* growth rates when exposed to 10μM Pb. The responses of bacteria to cadmium salts were 100 times more toxic in comparison to lead salts, suggesting slightly different biological effect of these heavy metals. The isolation of mutants resistant to specific toxic agents such as Tc and Rif antibiotics was not a very useful genetic approach in this study. It was unfortunate that it cannot be proven that real mutants were created. Instead, the donor was already cadmium sensitive, and the Rif^r donor appeared to be even more sensitive to cadmium and therefore behaved like a mutant. UV-light exposure, % survival, growth rates, and appearance of plasmids confirmed exactly the same behavior of Rif resistant donor and suspected mutant. Therefore it seemed like our donor strain was already a "mutant" that has very high cadmium sensitivity. A different approach was taken In order to identify *E. coli* genes involved with cadmium resistance, by simply increasing concentrations of Cd^{+2}. *E.coli* strain was found that is resistant to 4.5 mM Cd^{+2}.

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INTRODUCTION

The effect of heavy metals on bacteria

Heavy metals have become significant environmental contaminants due to their widespread use in the manufacture of electronics, plastics, batteries and dyes. The discharge of heavy metals into the environment has caused concern about their effects on the ecosystem (Shapiro and Keasling, 1996). Though many metals are essential for growth in small amounts, at higher concentrations heavy metals are toxic because they bind to organic compounds. This accounts for their effects on some important parts of the cell structure, like their ability to denature protein molecules (Shapiro and Keasling, 1996, Morozzi et al., 1982). Because of their intrinsically persistent nature and toxicity of the metals to living systems, the mechanisms of resistance and repair of damage caused by heavy metals is an important and unresolved problem (Pazirandeh, 1996). Some of the earliest attempts to control microorganisms by chemical means were based on the use of heavy metals. For example, copper sulphate was used as a plant fungicide, and mercury salts were used in the treatment of certain infectious diseases (Sadler and Trudinger, 1967).

Data from numerous studies indicates that the presence of high concentrations of metals provides a selective medium favoring metal resistant bacteria (Mietz and Sjogren, 1982). Some bacteria are resistant to concentrations of heavy metals that are toxic to higher organisms. These bacteria may concentrate or change the valence of heavy metals and remove hem from the environment. Each species may be affected in different ways, so that a particular concentration of a metal may exert no, little, or a dramatic effect, depending on the microorganism's characteristics (Gadd, 1990).

Most bacteria exhibit a biphasic response to a number of heavy metals. At low concentrations of the metal there is a stimulation of growth, but as the metal concentration is increased growth becomes progressively inhibited and finally ceases. The "cross-over" zone between stimulation and inhibition by metals is generally over a very narrow range. The actual concentrations at which the responses occur depend upon the organism, the form of the metal, and the chemical and physical composition of the medium in which the responses are determined (Gadd, 1990). An understanding of the mechanisms by which microbes survive heavy metal exposure is critical for understanding heavy metal toxicity in all organisms and for the use of such organisms for the removal of metals from the environment (Shapiro and Keasling, 1996). Furthermore, the biological effects of such pollutants on microorganisms and the frequency of appearance of heavy metal resistant bacteria can be useful as an indicator in bioassay systems (Gelmi et al., 1994).

An increasing frequency of appearance of bacteria resistant to toxic metals such as cadmium seems to be correlated with increasing loads of metals in the environment (Gelmi et al., 1994). However, the interaction of inorganic lead (Pb) and cadmium (Cd) with well-studied bacteria such as *Escherichia coli* has received little attention. One reason for this may be because *E. coli* is not a normal member of soil flora, so it is usually not isolated from metal contaminated sites. Another reason for the lack of study of effects of heavy metals on *E. coli* may be that phosphate found in most standard media combine with many metal ions to form an insoluble precipitate. Metal solubility is critical for uptake into the bacterial cell. Avoidance of precipitation by the phosphate found in most standard media is necessary prior to analysis of growth inhibition. Metals reduce growth if a modified medium containing glycerol-2-phosphate is used to lower the concentration of precipitate (LaRossa et al., 1994). Chelating compounds, such as citrate and EDTA have been shown to slightly increase the toxicity of heavy metals (Gadd and Griffiths, 1978).

The extent to which a metallic cation interacts in soil environments with organic and inorganic surfaces determines the concentration of metal in solution. Bacteria have a high surface area to volume ratio and a high capacity for sorbing metals from solution. Many studies have shown that large quantities of metallic cations are complexed by bacteria (Mullen et al., 1989). Previous studies concluded that cell walls of gram-positive bacteria such as *B. subtilis* bind larger quantities of several metals than cell envelopes of the *E. coli* (gram-negative bacterium) (Beverage and Fyfe, 1985).

The objective of a microbe study by Mullen et al. (1989) was to determine the metal binding capacities of whole cells of two-gram positive bacteria and two-gram negative bacteria. Concentrations of 1μM - 1mM Ag^{+}, Cd^{2+}, Cu^{2+}, and La^{3+} were used as metal salt solutions. Examinations of the values of Cd sorption showed gram-negative *E. coli* and *P. aeruginosa* were more efficient at

Cd sorption than gram-positive bacteria. On the other hand, *B. subtilis* removed the most Cu^{2+} at an equilibrium concentration of 1μM. High equilibrium concentrations affected gram-negative bacteria the most and gram-positive bacteria the least. This research evaluated the sorption capabilities of selected bacteria (Mullen et al., 1989).

Traditionally, adsorption, chemisorption, and ion exchange have been used to describe the removal of various cations. When using intact bacteria cells we should also consider other processes like active uptake of the metal into the cytoplasm through non-specific cation transport systems. Evidence suggests that the role of bacteria is not limited to short term metal immobilization and that bacteria cells are capable of binding large quantities of metal cations (Mullen et al., 1989). There is evidence suggesting that microbial biomasses have high heavy metal uptake capacities. One possible method of concentration may be due to the production of certain metal-binding proteins such as metallothioneins. These proteins are cysteine rich and bind heavy metals, such as Cd, with very high affinity (Pazirandeh, 1996). Pazirandeh (1996) suggests further that *E. coli* does not have any gene encoding metallothionein-like proteins. Even without a metallothionein gene, *E.coli* demonstrates considerable heavy metal resistance (Gelmi et al., 1994). The study of this resistance could identify the genes that will give *E. coli* improved ability to sequester heavy metals (Pazirandeh, 1996).

Cenci et al. (1985) used total dehydrogenese activity (TDH) as a means of measuring metal toxicity in *E. coli*. TDH was used since it is a measure of bacterial activity closely related to energetic metabolic systems and beta-galactosidase as a typical inducible enzyme in *E. coli.* From the results it was possible to define the order of metal toxicity. The 50 percent inhibition of TDH-activity was: 7.4, 10, 30, and 50 μM, respectively for Hg, Zn, Cd, and Cu. In the presence of the metals incubation time of over 60 minutes consistently lead to the inhibition of TDH activity. Experiments were carried out to define some characteristics of the biological damage to the total cell population. Hg and Cu markedly inhibited growth in Trypticase soy agar (TSYA) medium, while the viability of cells treated with Cd and Zn remained constant in the same medium. This indicated that treatment with Cd and Zn, unlike that observed for Hg and Cu, did not cause metabolic injury to *E. coli* (Cenci et al., 1985).

Continued pollution of the biosphere with toxic heavy metals makes it clear that microbe-based technologies will have an important role in environmental remediation (Gadd, 1990). Technological applications of microbial metal concentration may depend on the ease of metal recovery either by biomass regeneration or by reclamation. If inexpensive biomass is used to reclaim valuable metals, then destructive recovery (incineration or dissolution in acids or alkalis) may be economically feasible. Microorganisms with high capacity for metal uptake removal such as *Staphylococcus aureus, Bacillus sphaericus, Bacillus licheniformis* and *Arthrobacter species* can be used as both biological monitors of environmental contamination and for remediation (El-Bestawy et al., 1998). These species were isolated from water heavily polluted with heavy metals. Mutagenesis, both physical and chemical, and gradual increase in concentration of heavy metals significantly increased bacterial resistance against a wide range of metals and led to high efficiencies for their removal from soil. Physical mutation was induced by u.v light (254 nm) at 30 cm height, and at the dose that resulted in 90% mortality. Agar well diffusion technique was used for chemical mutation that was induced by 1% ethidium bromide. In this study, the highest removal efficiency achieved by the different selected mutants was Cd (89.9-100%), Cr (87.3-99.7%) and Pb (40.2-51%) (El-Bestawy et al., 1998).

Microbes are generally the first organisms exposed to heavy metals present in the environment. Although the presence of heavy metals is detrimental to them, toxic metals select resistant variants that confer the ability to tolerate higher levels of the toxic compounds (Cervantes et al., 1994). Heavy metal resistance has been shown to be plasmid mediated in some cases, and the genetic determinants responsible for heavy metal resistance often reside on plasmids that also mediate antibiotic resistance (Mietz and Sjogren, 1982).

Transposons are useful for making mutations since they can be helpful in gene identification. Transposons are specific DNA segments with the ability to move as a unit in a more or less random fashion from one genetic point to another. Complete loss of gene function often results upon insertion of a transposon within a gene. Therefore, transposons can be used as a mutagenic agent, if a replicon that carries the transposon can be efficiently introduced into the organism to be mutagenized. Selecting for transposon-mediated antibiotic resistance can easily isolate transposition events. Molecular cloning of prokaryotic genes and manipulation and genetic analysis of bacteria have been greatly facilitated by the use of antibiotic-resistance transposons (Simon et al., 1983).

Genetic manipulation of bacteria may allow the development of new strains with better growth rates and improved resistance to toxic metals. Therefore, manipulation of wild type, mutant, and individual or mixed cultures may be a useful tool for decontamination of polluted effluents by metal bioremediation (Brierley et al., 1982).

Bacterial growth

The simplest measure of the effect of heavy metals on bacteria is obtained by examining their effect on growth rate. Bacterial cultures are exposed to different concentrations of various heavy metals and the growth rate is determined (Mandelstam and Mc Quillen, 1968).

When bacterial cells are placed in a nutritionally favorable environment they will grow and divide. At some point due to exhaustion of nutrients, depletion of oxygen, and/or accumulation of toxic products, the environment will become unfavorable for growth. Until this occurs, the bacteria grow in an unhindered manner, and each cell will grow, on the average, at the same rate as its predecessors. This is a geometric doubling called logarithmic growth (Ford and Mitchell, 1992).

Growth parameters most often used to determine growth rate are those that can be easily and accurately measured. One parameter easily measured is turbidity of a liquid culture, which depends on the amount of light that is scattered by the organisms and is related to the bacterial mass (Collins and Stotzky, 1992). Measuring living or viable cells is also a useful method for determining growth rates. The number of viable bacteria is determined by colony count, which is performed by placing an appropriate dilution of the culture on or in a solid medium where the organisms are known to grow. Since colonies arise from single cells, the number of colonies multiplied by the dilution factor equals the number of viable bacteria originally present. Standard curves are used to relate the viable cells to the turbidity (Mandelstam and Mc Quillen, 1968).

E. coli grows well in chemically defined media. Some of the media are very simple, containing only mineral salts and a single organic component as a source of carbon and energy. When glucose is the organic component the bacterial mass may double every 45-60 minutes. Growth is slower when other substances such as acetate or succinate are used as the carbon energy and source. The growth rate is increased considerably by the addition of amino acids, purines and pyrimidines, and vitamins. These are best provided by use of a complex medium containing these substances (Ford and Mitchell, 1992). Since *E. coli* can take up sugars, proteins and amino acids from the complex medium and need not synthesize them *de novo*, growth is much more rapid than on mineral medium. Many different types of culture media have been employed in the study of bacteria, and some common laboratory species will grow in complex medium. Complex media is generally a solution composed of peptones, concentrated meat or yeast extract, and salt. Extracts are composed of the water-soluble constituents of meat or yeast, which will diffuse out of the meat or yeast on standing in water, and it supplements the peptone and helps the growth of bacterial species (Ford and Mitchell, 1992).

The growth rate of bacteria can also be manipulated by altering the temperature. With *E. coli* at 37°C the growth rate ranges from 18-20 minutes in a complex medium to about 2 hours in media where the sole carbon and energy source is one of the several sugars which are metabolized inefficiently (Sadler and Trudinger, 1967).

If *E. coli* is inoculated into an appropriate growth medium in the evening, by the following morning there will be signs of considerable growth. In order to obtain growing bacteria in log phase, a portion of this overnight culture must be sub-cultured into fresh medium. Growth will not begin immediately, but will start slowly, reaching the maximum growth rate in a gradual manner. This period prior to the maximum growth rate is called the lag phase. The duration of this period depends on many considerations, including the organism, the medium, the conditions of previous cultivation, the degree of aeration, and the way in which bacterial growth is measured. At the end of the lag phase, the cells are in a physiological steady state, undergoing balanced growth as previously described. This state ceases more or less gradually when the cells begin to exhaust essential nutrients or when they accumulate toxic products (See Figure 1). The time at which the log phase ends is entirely dependent on the species of organism and the actual conditions of cultivation. When either the carbon or the nitrogen source is limiting in a synthetic medium, growth of *E. coli* ends very abruptly (Mandelstam and Mc Quillen, 1968).

Heavy metals such as Cd^{+2}, Cr^{+6}, Hg^{+2}, Pb^{+2}, and Zn^{+2}, produce a decrease of *E. coli* growth rate, and reduced total biomass. All the concentrations of toxicants tested in the work done by Mariscal et al. (from 0.3 μM to 5 μM) produced at least a partial inhibition of *E. coli* growth rate. This inhibition was reflected in the decrease of the respective values of growth rate, in comparison with those of controls. Comparison of the values revealed that, Cr^{+6}, Hg^{+2}, Cd^{+2}, Pb^{+2}, and Zn^{+2} in that order, were the most to least toxic to *E. coli* (Mariscal et al., 1995).

Studies have resulted from the reduction of population growth of several microorganisms in bodies of water from industrial effluents containing heavy metals. Heavy metals probably bound with the cellular membrane of the bacteria by impairing certain physiological and biochemical parameters. The results suggested that the degree of growth population of *E. coli* varied among metal ions in the aquatic environment. Effects of different concentrations of metals on the population growth of *E. coli* after 7 and 28 days were analyzed in the treated and untreated experimental sets. A gradual decline in population growth of *E. coli* was noted with increasing incubation time and metal concentration. The harmful effects of the metals were found to be highest in Cd, then Pb, Cu, As, Hg, and lowest in Cr (Jana and Bhattacharya, 1987).

In a study by Pickett and Dean (1979), the effect of different concentrations of Cd^{+2} and Zn^{+2} on *Bacillus subtilis* subsp. *niger* and

Pseudonomas fluorescens were investigated. Organisms in this study that were cultured many generations in liquid medium at concentrations between 0.12 and 0.20 mM of Cd^{+2} or Zn^{+2} all survived on agar plates containing metal ions at that concentration, but at higher concentrations the viability decreased sharply. The minimum Cd^{+2} concentrations completely inhibiting the growth *of Bacillus subtilis* subsp. *niger* and *Pseudonomas fluorescens* were 0.62 and 0.13 mM respectively, suggesting that *Pseudonomas sp.* is a more sensitive strain. The same trend was seen for Zn^{+2}, which inhibited these strains at 1.53 and 0.12 mM respectively. As with many other toxic agents, the lag increased as the inhibitor concentration increased. There was also a distinct anomaly in the form of a plateau, after the lag phase, which occurred at Cd^{+2} concentrations between 0.12 and 0.2 mM. Evidence suggests that well-defined sites such as the cell walls on the organisms were initially attacked by metal ions. The degree of inhibition of growth rates was proportional to the concentration of metal ions present in the medium. When the cell walls were saturated with metal ions, small increases in concentration had no effect, and a relatively high concentration was required before other less accessible sites were affected. In liquid medium the lag and the mean generation time of *Bacillus subtilis* supsp. *niger* increased with increasing Cd^{+2} or Zn^{+2} concentrations, whereas only the total biomass was affected in *Pseudonomas fluorescens*. Nevertheless, the responses of both species indicted a specific action at low concentration and a more general toxic action at high concentrations (Pickett and Dean, 1979).

In a study by Morozzi et al. (1982) the effect of Hg^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Cr^{2+}, and Pb^{2+} on *E.coli* were studied. The growth curves of *E. coli* in cultures containing sub-lethal concentrations of metal ions (ranging from 0.01 μM to 100 μM) show that there is no effect on the lag phase time until the threshold concentration is reached. The metals studied, and the corresponding values of threshold concentration that were derived from the graph, were as follow: 0.005 μM for Hg^{2+}, 0.5 μM for Cd^{2+}, 5 μM for Cu^{2+}, 23 μM for Cu^{2+}, 30 μM for Zn^{2+} and 45 μM for Pb^{2+}. In order to evaluate the persistence of the tolerance to metal ions, *E. coli* previously exposed to metals were subcultured into fresh media and then re-inoculated into media plus metal at different times (0-12 hours). Previous exposure to the single metals diminished the toxicity of Hg^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, and Cr^{2+}. The mid-log phase times of primary sub-cultures, after four, five, and six hours respectively, came near the values of the controls without metals. The acquired tolerance is lost in a very short time if bacteria grow in the absence of metal. In this experiment, the lag phase began to lengthen again when bacteria without the metal were sub-cultured into medium containing the corresponding ion. Loss of viability was only observed for microorganisms tested after the exposure to lead. The reduction of metallic ions indicated a physiological adaptation of microbes rather than the selection of mutants. In a study by Morozzi et al. (1982) cells that have become adapted to the presence of metal ions lost this adaptation after growing in a metal-free medium. In conclusion, metal ions Hg^{2+}, Cd^{2+}, Cu^{2+},

Cr^{2+}, Zn^{2+} did not cause selection of metal-resistant mutants of *E. coli* (Morozzi et al., 1982).

OBJECTIVES:

The initial objective of this study is to find out the growth rate of *E. coli* and *M. roseus* in the presence of 1mM and 10 µM concentrations of cadmium and lead. *E. coli* and *M. roseus* will be used due to their ability to grow in a controlled environment and ease of usage. Growth rates of *Escherichia coli* (gram-negative bacteria) and *Micrococus roseus* (gram positive bacteria) will be observed before and after exposure to lead or cadmium. The growth rates of the bacteria will be measured via light scattering, and viable counts will be obtained. Bacteria will be grown on rich media (LB broth) as well as mineral M9 media supplemented with and without casamino acids and growth rates will be compared.

Another objective in this study is to identify genes in *E. coli* mutants that will be more resistant or sensitive to cadmium. *E. coli* will be mutagenized with a transposon to identify mutants with altered sensitivities to Cd. The growth rates of the mutants and wild type will be obtained with and without Cd. The null hypothesis in this study states, "Mutagenesis will have no effect in mean growth rates between wild type and mutant".

MATERIAL AND METHODS

Chemicals and instruments utilized were available in the Departments of Biology and Chemistry and Geology Department, College of Science, Columbus State University. Rifamycin (Rf) and tetracycline (Tc) were purchased from Sigma.

Bacterial strains and growth conditions

E. coli was supplied from Bactrol discs (ATCC 25922), and *M.roseus* from Microbank (29). For transposon mutagenesis *E.coli* S17 mini Tn5-Tc (Tc^r, Rif ^{s}) (de Lorenzo et al., 1990) was the plasmid donor. *E. coli* (ATCC 25922) was grown on Rif plates and surviving colonies were used as the recipient (Tc^s, Rif ^{r}). Selection for antibiotic resistance was performed on LA plates supplemented with Tc or Rf to final concentrations of 15μg/ml and 50μg/ml, respectively from sterile stock solutions. Antibiotic stock solutions were prepared by adding 50 mg of Rif per ml of Dimethyl Formamide (DMF), and 15 mg of Tc per ml of 70% ethanol followed by filter sterilization.

Escherichia coli and *Micrococus roseus* were initially grown in minimal medium containing 4.85μg Na_2HPO_4, 3.0g NaCl, 1.0g NH_4Cl, 0.27g $MgSO_4$, and 100μL of 1.0M $CaCl_2$ per Liter of distilled water at pH 7.0. When necessary, 5.0g of casamino acids and 5g of glucose or lactose were added. Agar powder was added to 15 g/L to prepare solid medium. Bacteria were also grown in LB broth (Difco Laboratories, Detroit, MI) prepared according to manufacturer's instructions, always supplemented with 2g/L of glucose. When necessary, Tc and Rif were added to LB. All media were sterilized before addition of antibiotics, by autoclaving at 121°C for 15 minutes.

Filter sterilized stock solutions of 0.1M of $CdCl_2$, $PbNO_3$, and EDTA were prepared in de-ionized water and metal solutions were simultaneously introduced to EDTA. Solutions were then introduced at final concentrations of 10^{-3} and 10^{-5} M.

Measurement of growth rates

Growth rates were monitored using a Milton Roy Spectronic 20D spectrophotometer (St. Petesburg, FL) set at a wavelength of 650 nm. Measurements were plotted against time to determine growth rate for each species.

Viable count procedure

Viable cell counts were performed by pour plate technique of tenfold serial dilutions of control and 1 mM Cd and Pb containing samples. 1 ml of culture dilutions was pipetted into a sterile petri plate, adding 20 ml of 45°C Tryptic Soy Agar and gently swirling the plate on the tabletop. Plates that have between 30 and 300 colonies were counted. Three replicate plates of each dilution were prepared.

Experimental protocol for preparing different media

Bacteria were inoculated into M9 broth media (with 5% glucose or lactose) with and without casamino acids and LB broth media (supplemented with 2 % glucose) and grown overnight. These bacteria were then used to inoculate standing tubes for the experiment. The experimental tubes included M9 media containing lactose only, glucose only, lactose with severely limited glucose, M9 media without casamino acids and LB broth. Cultures were inoculated on ice to prevent growth of the bacteria, and then transferred to a 37°C water bath for standing incubation. Turbidity was measured every 30 minutes with spectrophotometer at 650 nm.

Testing for sensitivity to Cd and Pb

After control readings of the four media types above were completed, ten-milliliter test tubes of differently supplemented broths were inoculated with bacteria using a sterile, plastic inoculating loop. Identical procedures were used as the control, but medium contained the following:

1. Glucose with casamino acid and cadmium
2. Glucose with casamino acid and lead
3. Lactose with casamino acid and cadmium
4. Lactose with casamino acid and lead
5. Lactose with casamino acid, limited glucose, and cadmium

6. Lactose with casamino acid, limited glucose, and lead
7. Glucose with cadmium
8. Glucose with lead
9. LB broth with glucose and cadmium
10. LB broth with glucose and lead

Controls were repeated at this stage. All tubes were incubated in a water bath at 37°C, and the absorbance was read every 30 minutes until bacterial growth was well into stationary phase. Three independent repetitions of each experiment were performed in this study.

ANOVA and Duncan's multiple test

Analysis of Variance (ANOVA) was used for analyzing data in this study. ANOVA is used to uncover the overall effects of independent variables on an interval dependent variable. The key statistic in ANOVA is the F-test of difference of group means, testing if the means of the groups formed by values of the independent variable are different enough not to have occurred by chance. If the group means do not differ significantly then it is inferred that the independent variables did not have an effect on the dependent variable. If the F test shows that overall the independent variables are related to dependent variables, then multiple comparisons tests of significance are used (Krebs, 1999). In this study, once it was determined that a significant difference exists within the level means of a variable, it was necessary to detect the location of significant differences. Duncan's Multiple Test was used to distinguish exactly where the differences were significant for *E.coli* and *M.roseus*. Duncan's Multiple Test involves three steps consisting of calculating standard error (S_x), arraying or ranking the means for the treatment or interaction in question, and calculating confidence intervals and comparing them (Steel and Torrie, 1960).

Mutagenesis

Overnight cultures of donor and recipient strains (0.1 ml) were transferred into 10 ml of LB liquid cultures with appropriate antibiotics, and grown to log phase. 0.1 ml of each strain was centrifuged in separate Eppendorf tubes for 1 minute. The cell pellets were washed twice in 0.1 ml of LB and re-suspended in 0.1 ml of LB. The two strains were mixed together, centrifuged for 1 minute, the liquid discarded, and the cell pellets re-suspended in 20 μl of LB broth. The mating mixture was spotted on LA plates and incubated overnight at 30°C. The overnight growth was scraped off the plate and suspended in 1 ml of LB, and 0.1 ml samples were then spread on LA plates with Tc and Rif. Controls of donor and recipient only were treated identically. Approximately 1000 colonies that acquired the transposon antibiotic resistance were obtained on these plates. Tc^r, Rif^r colonies were picked and replica-plated on LA/Tc/Rf, and LA/Tc/Rf/$CdCl_2$ plates. Putative mutants were cultured in liquid LB + 1 mM $CdCl_2$ to confirm Cd sensitivity. Mutants exhibiting abnormal growth were selected for further analysis.

Determination of minimal inhibitory concentration

Suspected mutants that didn't grow on plates in the presence of 1 mM of Cd were tested to find their minimal inhibitory concentration. Mutants were grown overnight at 37°C in LB medium + Tc + Rf +10 μM Cd^{+2}. Growth was observed after 24 hours, suggesting that the minimal inhibitory concentration was higher than 10 μM. Concentrations were incrementally increased until no cell growth was observed.

***E. coli* plasmid mini-prep**

1-2 ml of cell culture was centrifuged for 1 minute in a microfuge, the pellet was collected and the culture fluid was discarded. The cell pellet was resuspended in 0.1 ml of TE buffer. Lysis solution (0.2 ml) was added and mixed thoroughly by inversion. Potassium acetate (3M) 0.15 ml was added and mixed thoroughly by inversion until a white precipitate formed. The solution was centrifuged for 5 minutes in a microfuge. The cleared lysate was transferred to a new tube, avoiding the white precipitate that was on the side and bottom of the tube. Cold 95-100% (0.8 ml) was added to the lysate and mixed. The solution was centrifuged for 5 minutes in microfuge. The liquid was discarded and the pellet was saved. The liquid was completely removed with a pipetteman. The pellet was washed with 0.5 ml of cold 70% ethanol, dried, and resuspended in 20-50 μl water or TE buffer. Instructions outlined in the Laboratory Manual by Sambrook et al. (1989) were used to prepare alkaline lysis buffers.

Preparation of DNA electrophoresis gel

Plasmid preparations were made for potential mutants, wild type, and donor cell (Tc^r). Pellets from the plasmid preps were dissolved in 30 µL of TE buffer and divided into a control portion and a portion to be digested. These plasmid preps were then digested with restriction enzyme Pst I (Roche Diagnostic Corporation, Indianapolis, IN) according to manufacturer's instructions. Instructions for preparing 1% agarose gel and 10X loading buffer were extracted from the Labaratory Manual by Sambrook et al. (1989). Prior to electrophoresis, 2 µL of 10X loading buffer was added to each tube. DNA Molecular Marker IV (Roche Diagnostic Corporation, Indianapolis, IN) was also run on the gel with all of the samples. After electrophoresis, the gel was stained in 10µg/ml ethidium bromide solution for 10 minutes. The gel was transferred to distilled water for five minutes, and then visualized by Foto//Phoreisis I apparatus by Fotodyne at wavelength of 312 nm. Photography of these gels was taken afterwards.

Selection of a Cd^{+2} resistant *E. coli* strain

E. coli was grown on LA plates that were prepared with different concentrations of Cd^{+2}. The minimum starting concentration was 500 µM. Flourishing colonies were selected and grown in LB with the same concentration of Cd^{+2}. Once the bacteria reached log phase, a loopful was spread on LA plates prepared with a higher concentration of Cd^{+2}. LA plates were started with very low cadmium concentration of 0.5mM, followed by the intermediate concentrations of 1mM, 1.5mM, 2mM, 2.5mM, 3mM, 3.5mM, and 4mM. This process was replicated until suspected mutant reached its maximal growth with Cd^{+2} of 4.5 mM. At the end, wild type *E. coli* and mutant that was Cd^{+2} resistant were tested for Cd^{+2} sensitivity. They were grown in LB with and without maximum concentration of Cd^{+2} that mutant could grow on and results were recorded.

UV exposure

Wild type (Rif ^{r} recipient), Tc ^{r} donor, Rif ^{r} donor, and suspected mutant were grown overnight in LB at 37°C. Once grown to same OD of 0.35, an equal volume of culture was placed on LA plates along five lines. Each line was exposed to UV light in five seconds increments ranging from 0 to 20 seconds. Uv mineralized lamp (UVS-54) was set five inches above the plates at a wavelength of 254 nm. The plates were incubated for 24 hours at 37°C, and then examined for growth.

The survival of bacterial cells exposed to 10 seconds of UV-light was then compared to controls that were not exposed to the UV-light. Wild type (Rif ^{r} recipient), Rif ^{r} donor, Tc ^{r} donor, and suspected mutant were grown to mid-log phase, and samples of grown culture were diluted 10^1 to 10^7 times with sterile phosphate buffer (PBS). Spread plating on a series of LA plates was done by 100 µl of diluted samples. After 24-hour incubation at 37°C, the number of colonies formed before and after the UV-light exposure was counted and % survival was determined. Only plates that contained between 30 to 300 colonies were counted. Three replicate plates of each dilution were prepared.

RESULTS

E. coli and _M. roseus_ growth rates with glucose and lactose at $1x10^{-3}$ M cadmium and lead.

Doubling times for *E. coli* and *M. roseus* were initially derived from absorbance vs. time. Two-Factor ANOVA with replication showed that there was a significant change in absorbance observed with increasing concentration of cadmium and lead (P-value < 0.001). Duncan's Multiple Test was used to distinguish where the differences are significant between *E. coli* and *M. roseus* when supplemented with glucose and lactose and exposed to Cd and Pb. Therefore there is no significant difference in doubling times between *M. roseus* and *E. coli* grown in M9 mineral medium supplemented with glucose and lactose. There were significant differences noted in *M. roseus* doubling times when exposed to Cd and Pb, but not between the heavy metals alone. All interactions between the heavy metals and control were significant in *E. coli* doubling times.

Doubling times were also found in *E. coli* and *M. roseus* from cell growth vs. time and cell growth vs. absorbance. Two-Factor ANOVA with replication showed that increasing concentration of cadmium and lead (P-value < 0.001) caused a significant reduction in growth rates. Duncan's Multiple Test replicates previously mentioned results for both *E. coli* and *M. roseus*. Therefore, only media with glucose was used since there was no significant difference observed between media supplemented with glucose or lactose at 95% confidence level.

M. roseus growth rates in different media at $1x10^{-3}$ M and $1x10^{-5}$ M cadmium and lead.

Graphs of absorbance vs. time for *M. roseus* illustrate growth rates in M9 mineral and rich media (LB) when exposed to $1x10^{-3}$ M and $1x10^{-5}$ M of Cd and Pb. Two-Factor ANOVA with replication showed that there was a significant decrease in growth rates observed with increasing concentration of cadmium and lead (P-value < 0.001). Duncan's Multiple Test indicated that the differences between $1x10^{-3}$M and $1x10^{-5}$M Cd and Pb were significant, while interactions between $1x10^{-3}$M Pb and $1x10^{-5}$M Cd, and $1*10^{-5}$M Pb and control (bacteria that hasn't been exposed to either Pb or Cd) were not significant. Also, there is no significant difference between *M. roseus* in M9 mineral and rich media.

E. coli growth rates in different media at $1x10^{-3}$ M and $1x10^{-5}$ M cadmium and lead.

Since *M. roseus* couldn't grow in M9 media without casamino acid, growth rates of *E. coli* were compared in M9 medium with and without casamino acid and LB, at $1x10^{-3}$ M and $1x10^{-5}$ M Cd and Pb. Two-Factor ANOVA with replication showed that there was a significant decrease in growth rates observed with increasing concentration of cadmium and lead (P-value < 0.001).

It can be observed from Duncan's Multiple Test that there is no significant difference between $1*10^{-5}$ M of Pb and control. All of the other interactions of growth rates in between the heavy metals, and heavy metals and controls were significant, as well as all of the growth rates of *E. coli* that were grown in M9 medium with and without casamino acid and LB at 95% confidence level.

FIGURES A-1 through A-125 in APPENDIX A, and FIGURES B-1 through B-99 in APPENDIX B, as well as TABLES 1-1 through 5-4 present all of the above raw data of *E.coli* and *M.roseus* and their complete analysis.

Mutagenesis

Approximately 1000 Tc^r, Rf^r colonies that acquired the transposon antibiotic resistance were obtained on LA plates. Colonies were replica-plated on LA/Tc/Rf, and LA/Tc/Rf/$CdCl_2$ plates. The effect of Tc^r, Rf^r colonies with and without Cd was different. Their appearance varied from small and dry to large and slimy in the presence of Cd. Eight different mutants were found that didn't grow at all on plates in the presence of Cd. Putative mutants, wild type *E. coli*, Rif ^{r} recipient, Rif ^{r} donor, and Tc ^{r} donor were grown in LB, LB/Rif/Tc, and LB/Rif/Tc and 50µM, 100µM, and 300µM of Cd. Observed growth rates are presented in APPENDIX C, FIGURES C-1 to C-32, and in summary TABLE C-1. Rif ^{r} donor, Tc ^{r} donor, and suspected mutant demonstrated the same growth rates. Minimal Inhibitory concentration (MIC) was found to be 300 µM in all mutants. It was possible that this Rif^r, Tc^r phenotype would arise from a Tc^r mutant or from Rif^r donor. If that were true suspected mutant would have Tc donor plasmid that has never entered the chromosome. Therefore donor and recipient strains and their Cd sensitivity were tested and compared to the "mutants". Cd sensitivities in wild type, recipient, donor, and "mutant" strains in summary TABLE C-1 suggest the possibility of picking Rif resistant donor that was naturally more Cd sensitive than the recipient strain. Wild type, recipient, donor, and "mutant" strains were prepared and run on DNA electrophoresis gel. Figures C-33 to C-36 show the presence of tranposons in the "mutant" strains.

To confirm our results, UV exposure was done on wild type *E. coli*, Rif ^{r} recipient, Rif ^{r} donor, Tc ^{r} donor, and suspected mutant. UV-light exposure was necessary to see if suspected mutant behaved like the donor or wild type. After identical incubation the LA plates were inspected. Wild type *E. coli* and Rif ^{r} recipient demonstrated the same UV resistance. Suspected mutant was sensitive to 5 seconds exposure to UV light. Tc ^{r} donor was sensitive to longer exposure of 10 seconds to UV mineral light. Rif ^{r} donor behaved in the same manner as suspected mutant illustrating the same 5 seconds sensitivity to UV light.

Since Uv-light exposure couldn't tell us the exact number of cells that were affected, it was decided to validate the results by exposing bacteria to UV-light to determine % survival. Viable counts were compared to non-exposed samples of the same culture. The control invariably contained 100 ±10 colonies. Percent survival of wild type (Rif ^{r} recipient), Tc ^{r} donor, Rif ^{r} donor, and mutant was 78.7%, 13.5%, 6.2%, and 4.2 %, respectively. Single Factor

ANOVA showed that there was a significant change in % survival among tested bacteria (P-value < 0.001). The calculated F-value of 287 and F-critical value of 4 indicated that means of % survival are significantly different for two degrees of freedom. Analysis of Variance and Duncan's Multiple Test were used to distinguish exactly where the differences are significant. Analysis of Variance Test shows overlapping of confidence intervals between Rif ^{r} donor and mutant. Therefore, both tests presented and supported that there was no significant difference in % survival between Rif ^{r} donor and mutant. All of the other interactions in between the wild type, Tc ^{r} donor, Rif ^{r} donor, and mutant were significant at 95% confidence level. TABLES 5-5 through 6-1 and FIGURE 3-7 present all of the above analysis.

Wild type *E. coli* was exposed to different concentrations of Cd and strain that is cadmium resistant was discovered. *E. coli* strain that is cadmium resistant could grow at the maximum Cd concentration of 4.5 mM. Wild type *E. coli* and Cd resistant mutant were tested for Cd sensitivity. They were grown in LB with and without 4 mM Cd. Wild type *E. coli* grew in LB with no Cd, but demonstrated no growth in LB supplemented with 4 mM Cd. Cadmium resistant *E. coli* strain grew in both, LB with and without Cd.

Table 1-1. Doubling times for *E. coli* absorbance vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control	Avg
Ec-glu	150	90	60	100
	150	90	60	100
	150	90	60	100
Ec-lac	150	90	30	90
	150	90	30	90
	150	90	60	100

Variable 1: Bacteria and nutrients (glucose/lactose)
Ec- *E.coli*

Variable 2: Treatment

Table 1-2. Anova: Two-Factor With Replication for for *E. coli* absorbance vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

SUMMARY	1x10-3M	1x10-3M	Control	Total
Ec-glu				
Count	3	3	3	9
Sum	450	270	180	900
Average	150	90	60	100
Variance	0	0	0	1575
Ec-lac				
Count	3	3	3	9
Sum	450	270	120	840
Average	150	90	40	93.3333
Variance	0	0	300	2350
Total				
Count	6	6	6	
Sum	900	540	300	
Average	150	90	50	
Variance	0	0	240	

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	200	1	200	4	0.0687	4.7472
Columns	30400	2	15200	304	5E-11	3.8853
Interaction	400	2	200	4	0.0467	3.8853
Within	600	12	50			
Total	31600	17				

Table 1-3. Duncan's Multiple Range Test for *E. coli* absorbance vs. time grown in M9 media with glucose or lactose exposed to $1x10^{-3}$M Cd and Pb

Treatment				Sw	
df=2	Value of p	2	3	50	EMS
	SSR	6.09	6.09	6	k
	LSR	17.6	17.6	8.3	EMS/k
	Rp=LSR			2.9	Sx

			Sw	
Media			50	EMS
df=1	Value of p	2	9	k
	SSR	18.0	5.6	EMS/k
	LSR	42.4	2.4	Sx

p=number of means for range being tested
Protection level = 0.05

$$Sx = \sqrt{\frac{EMS}{k}}$$

LSR=Sx*S

Table 1-4. Mean Comparisons and Rp Comparisons for *E. coli* absorbance vs. time grown in M9 media with glucose or lactose exposed to $1x10^{-3}$M Cd and Pb

High Cd	High Pb	Control
150.0	90.0	50.0

Ec-glu	Ec-lac
100	93.3

	Difference	LSR		
HC-HP	60.0	17.6	42.4	sig
HC-Co	100.0	17.6	82.4	sig
HP-Co	40.0	17.6	22.4	sig

	Difference	LSR		
Ec-glu-Ec-lac	6.7	42.4	-35.7	not sig

Table 1-5. Summary of doubling times for *E. coli* absorbance vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
150.0	90.0	50.0

Ec-glu	100
Ec-lac	93.3

	$1x10^{-3}$M Cd	$1*x0^{-3}$M Pb	Control
Ec-glu	150	90	60
Ec-lac	150	90	40

Variable 1: Bacteria and nutrients (glucose/lactose)
Ec- *E.coli*

Variable 2: Treatment

Figure 1-1. Average doubling times of *E. coli* vs. Cd and Pb from absorbance vs. time

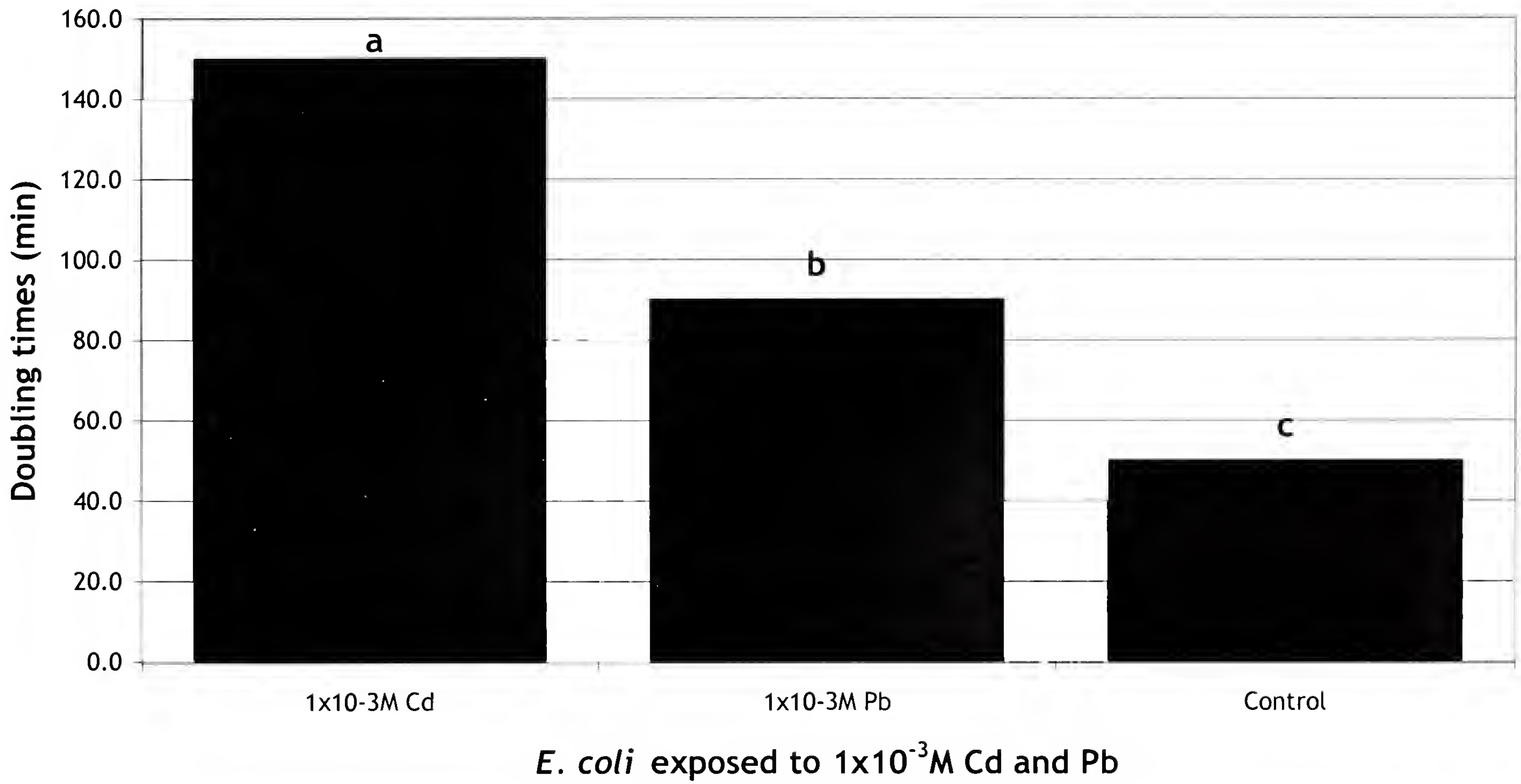


Figure 1-2. Average doubling times of *E. coli* vs. glucose or lactose from absorbance vs.time

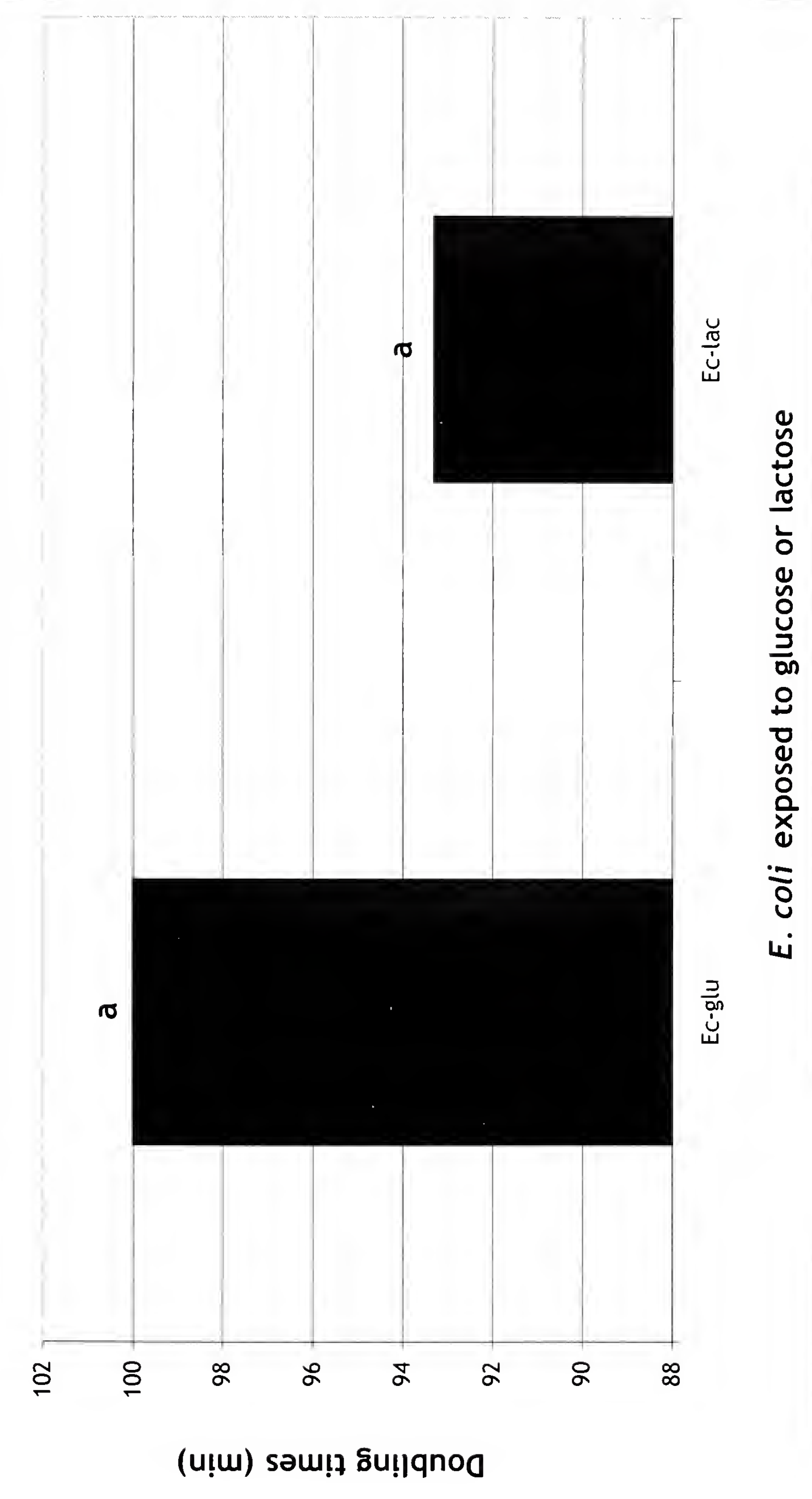


Figure 1-3. Summary of average doubling times of *E. coli* from absorbance vs. time

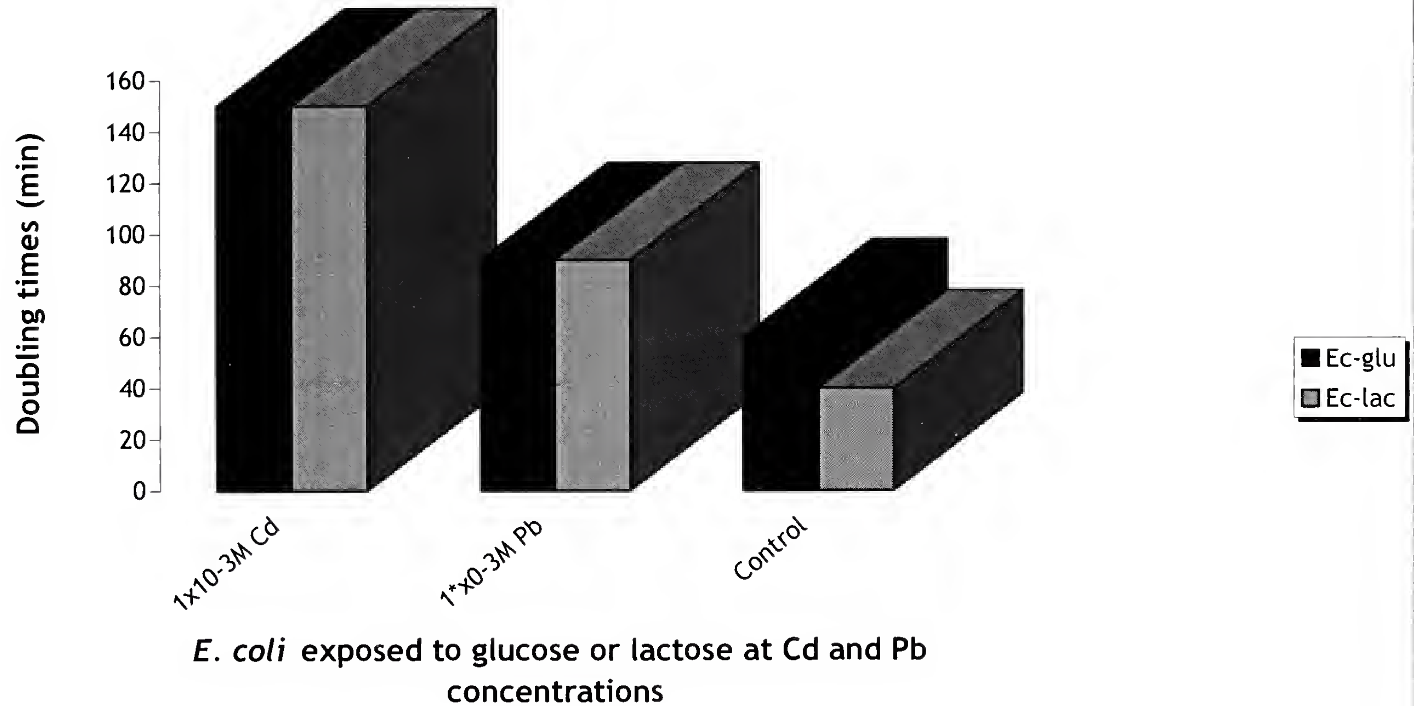


Table 1-6. Doubling times for *M. roseus* absorbance vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control	Avg
Mr-glu	240	210	150	200
	240	210	150	200
	240	210	150	200
Mr-lac	240	240	120	200
	240	210	120	190
	270	210	150	210
Avg	245	215	140	

Variable 1: Bacteria and nutrients (glucose/lactose)
Mr - *M.roseus*

Variable 2: Treatment

Table 1-7. Anova: Two-Factor With Replication for *M. roseus* absorbance vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

SUMMARY	1x10-3M	1x10-3M	Control	Total
Mr-glu				
Count	3	3	3	9
Sum	720	630	450	1800
Average	240	210	150	200
Variance	0	0	0	1575
Mr-lac				
Count	3	3	3	9
Sum	750	600	390	1740
Average	250	200	130	193.333
Variance	300	300	300	2950
Total				
Count	6	6	6	
Sum	1470	1230	840	
Average	245	205	140	
Variance	150	150	240	

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	200	1	200	1.3333	0.2707	4.7472
Columns	33700	2	16850	112.33	2E-08	3.8853
Interaction	700	2	350	2.3333	0.1393	3.8853
Within	1800	12	150			
Total	36400	17				

Table 1-8. Duncan's Multiple Range Test for *M. roseus* absorbance vs. time grown in M9 media with glucose and lactose exposed to $1x10^{-3}M$ Cd and Pb

Treatment				Sw	
df=2	Value of p	2	3	150	EMS
	SSR	6.09	6.09	6	k
	LSR	30.5	30.5	25.0	EMS/k
	Rp=LSR			5.0	Sx

			Sw	
Media			150	EMS
df=1	Value of p	2	9	k
	SSR	18.0	16.7	EMS/k
	LSR	73.5	4.1	Sx

p=number of means for range being tested
Protection level = 0.05

$$Sx = \sqrt{\frac{EMS}{k}}$$

LSR=Sx*SSR

Table 1-9. Mean Comparisons and Rp Comparisons for *M. roseus* absorbance vs. time grown in M9 media with glucose and lactose exposed to $1x10^{-3}M$ Cd and Pb

High Cd	High Pb	Control
245.0	215.0	140.0

Mr-glu	Mr-lac
200	200

	Difference	LSR		
HC-HP	30.0	30.5	-0.4	not sig
HC-Co	105.0	30.5	74.5	sig
HP-Co	75.0	30.5	44.5	sig

	Difference	LSR		
Mr-glu-Mr-lac	0.0	73.5	-73.5	not sig

Table 2-1. Summary of doubling times for *M. roseus* absorbance vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
245.0	215.0	140.0

Mr-glu	200
Mr-lac	200

	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
Mr-glu	240	210	150
Mr-lac	250	200	130

Variable 1: Bacteria and nutrients (glucose/lactose)
Mr- *M.roseus*

Variable 2: Treatment

Figure 1-4. Average doubling times of *M. roseus* vs. Cd and Pb from absorbance vs. time

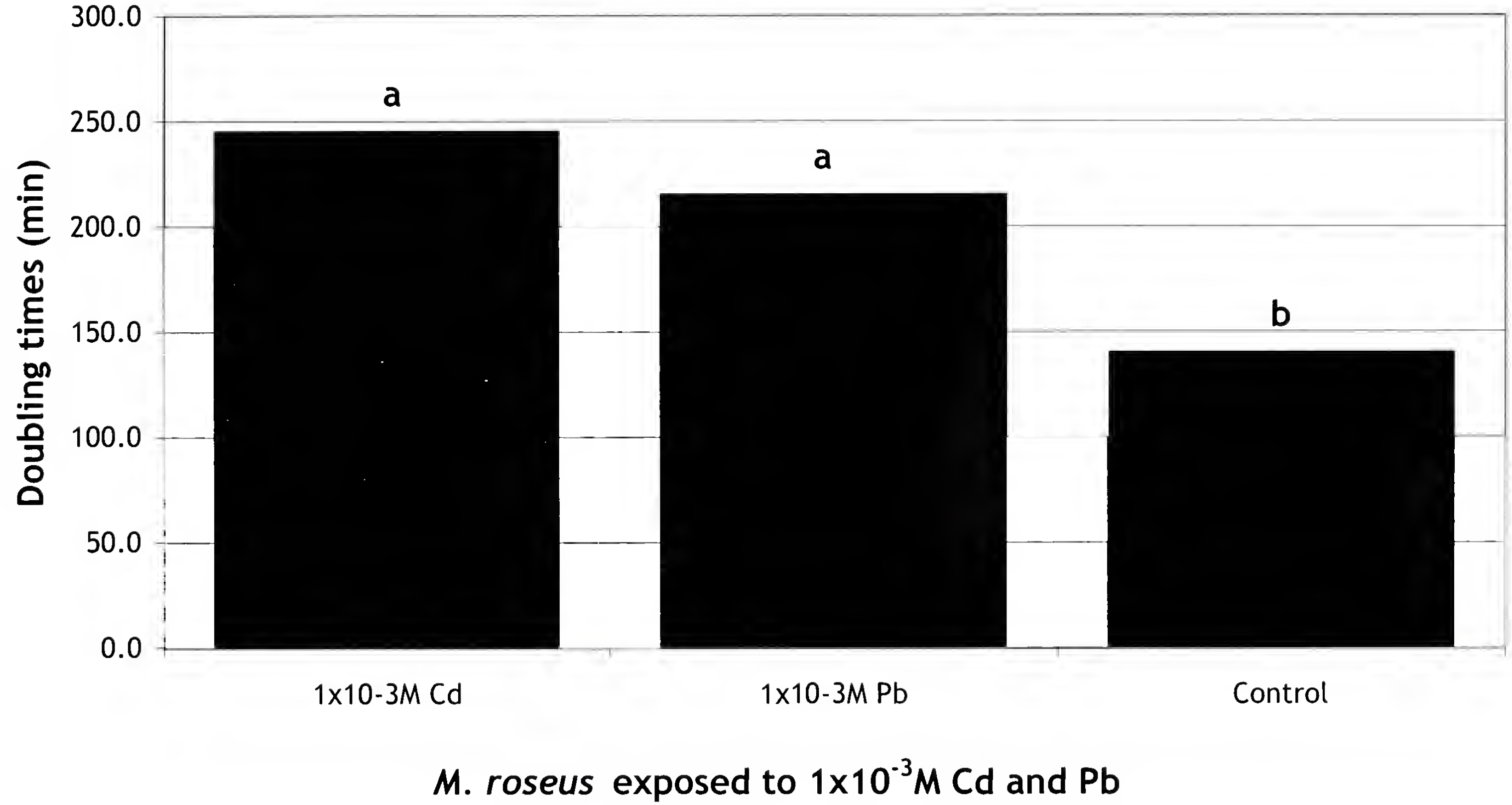


Figure 1-5. Average doubling times *M. roseus* vs. glucose or lactose from absorbance vs. time

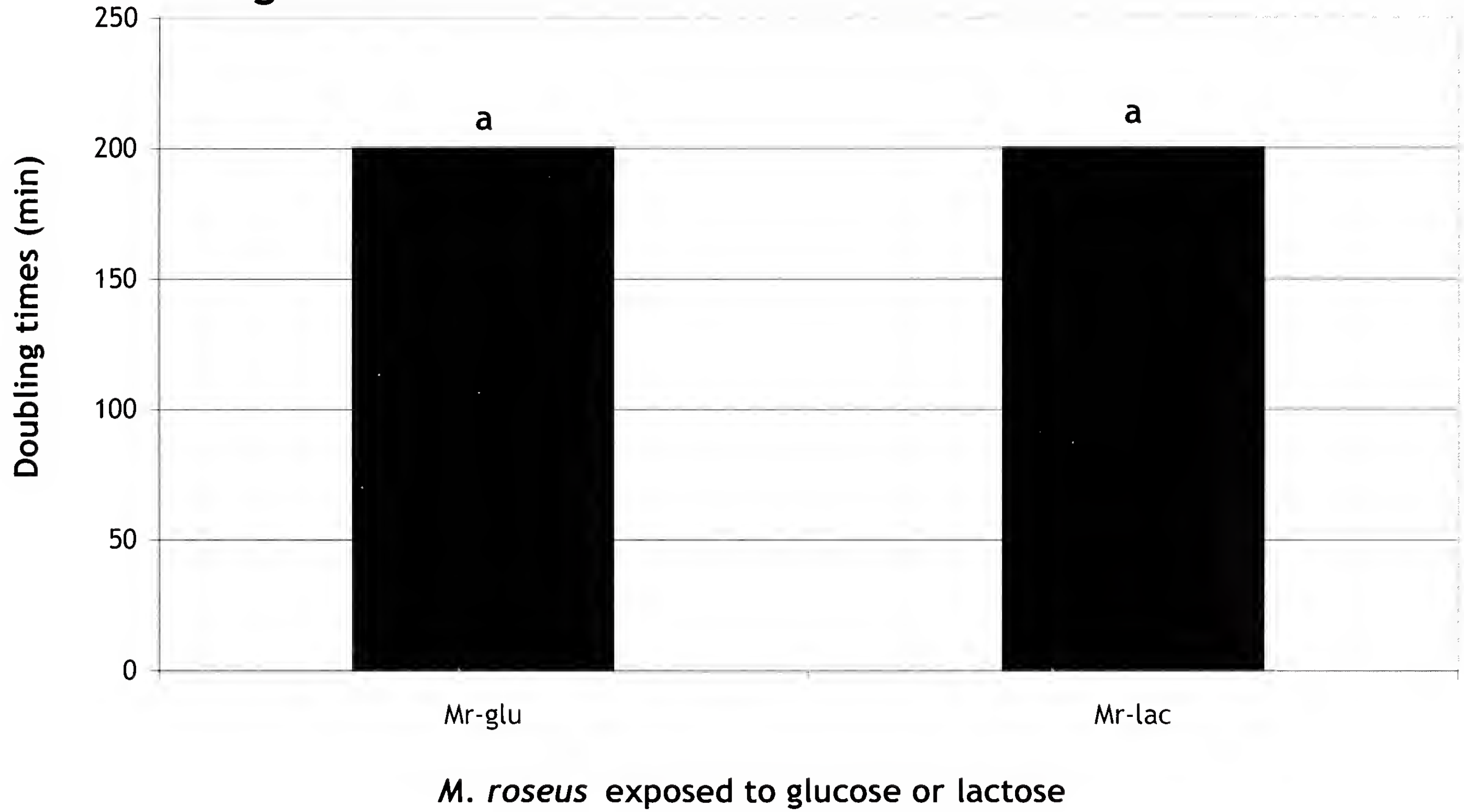
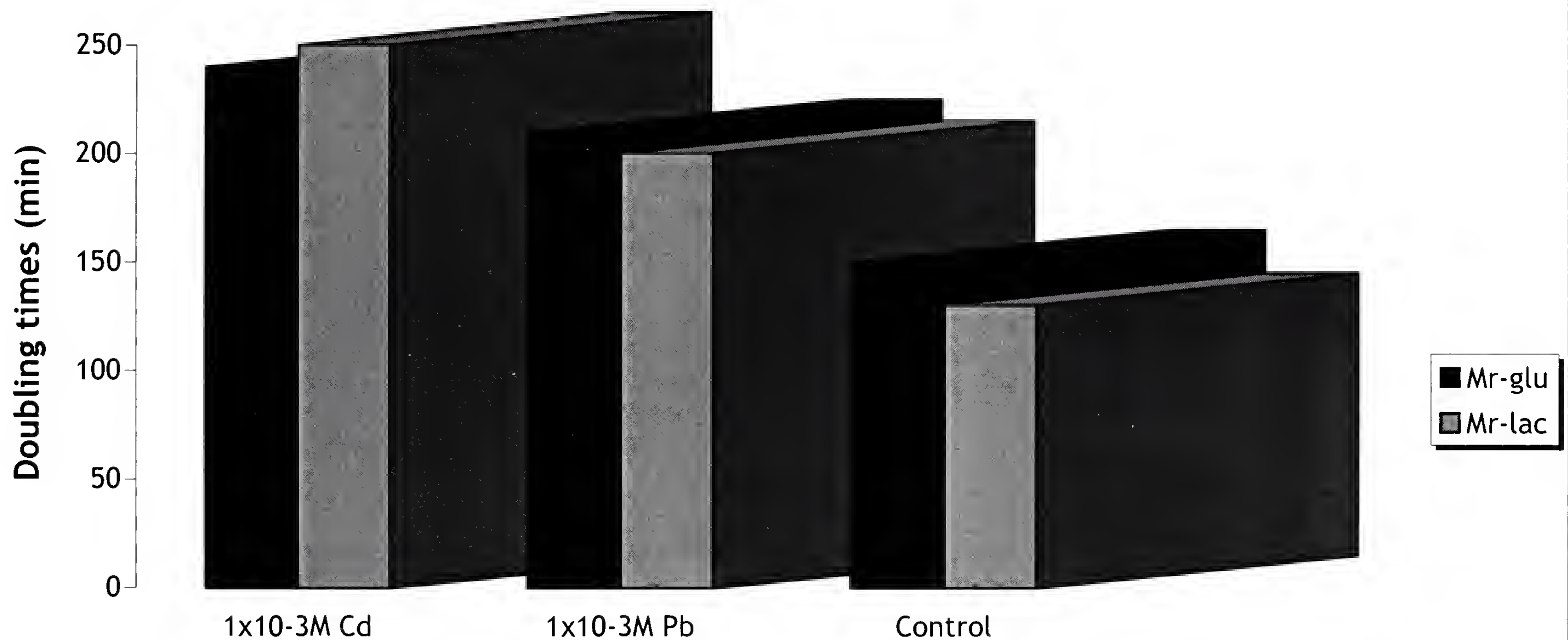


Figure 1-6. Summary of average doubling times of *M. roseus* from absorbance vs. time



M. roseus exposed to glucose or lactose at Cd and Pb concentrations

Table 2-2. Doubling times for *E. coli* cell count vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}M$ Cd and Pb

	Control	$1x10^{-3}M$ Cd	$1x10^{-3}M$ Pb	AVG
Lactose	60	180	120	120.0
	30	210	120	
	30	210	120	
Glucose	90	210	120	173.3
	60	300	210	
	60	300	210	
AVG	55.0	235.0	150.0	

Variable 1: Bacteria and nutrients (glucose/lactose)
Ec- *E.coli*

Variable 2: Treatment

Table 2-3. Two-Factor ANOVA With Replication for *E. coli* cell count vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$M Cd and Pb

Anova: Two-Factor With Replication

SUMMARY	Control	Cd	Pb	Total
Lactose				
Count	3	3	3	9
Sum	120	600	360	1080
Average	40	200	120	120
Variance	300	300	0	4950
Glucose				
Count	3	3	3	9
Sum	210	810	540	1560
Average	70	270	180	173.333
Variance	300	2700	2700	8950
Total				
Count	6	6	6	
Sum	330	1410	900	
Average	55	235	150	
Variance	510	2670	2160	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	12800	1	12800	12.1905	0.00445	4.74722
Columns	97300	2	48650	46.3333	2.3E-06	3.88529
Interaction	1300	2	650	0.61905	0.5548	3.88529
Within	12600	12	1050			
Total	124000	17				

Table 2-4. Duncan's Multiple Range Test for *E. coli* cell count vs. time grown in M9 media with glucose and lactose exposed to $1x10^{-3}$M Cd and Pb

Treatment					Sw	
df=2	Value of p	2	3		1050	EMS
	SSR	6.09	6.09		6	k
	LSR	80.4	80.4		175.0	EMS/k
	Rp=LSR				13.2	Sx

			Sw	
Nutrient			1050	EMS
df=1	Value of p	2	9	k
	SSR	18.0	116.7	EMS/k
	LSR	194.4	10.8	Sx

LSR=Sx*SSR

$$Sx = \sqrt{\frac{EMS}{k}}$$

p=number of means for range being tested
Protection level = 0.05

Table 2-5. Mean Comparisons and Rp Comparisons for *E. coli* cell count vs. time grown in M9 media with glucose and lactose exposed to $1x10^{-3}$M Cd and Pb

Cd	Pb	Control
235	150	55

Glucose	Lactose
173.3	120

	Difference	LSR		
Cd-Pb	85.0	80.4	4.6	sig
Pb-Control	95.0	80.4	14.6	sig
Cd-Control	180.0	80.4	99.6	sig

	Difference	LSR		
Glucose-Lactose	53.3	194.4	-141.1	not sig

Table 2-6. Summary of doubling times for *E. coli* cell count vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$M Cd and Pb

$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
235.0	150.0	55.0

Glucose	120
Lactose	173.3

	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
Lactose	200	120	40
Glucose	270	180	70

Variable 1: Bacteria and nutrients (glucose/lactose)
Ec- *E.coli*

Variable 2: Treatment

Figure 1-7. Average doubling times of *E. coli* vs. Cd and Pb from cell count vs. time

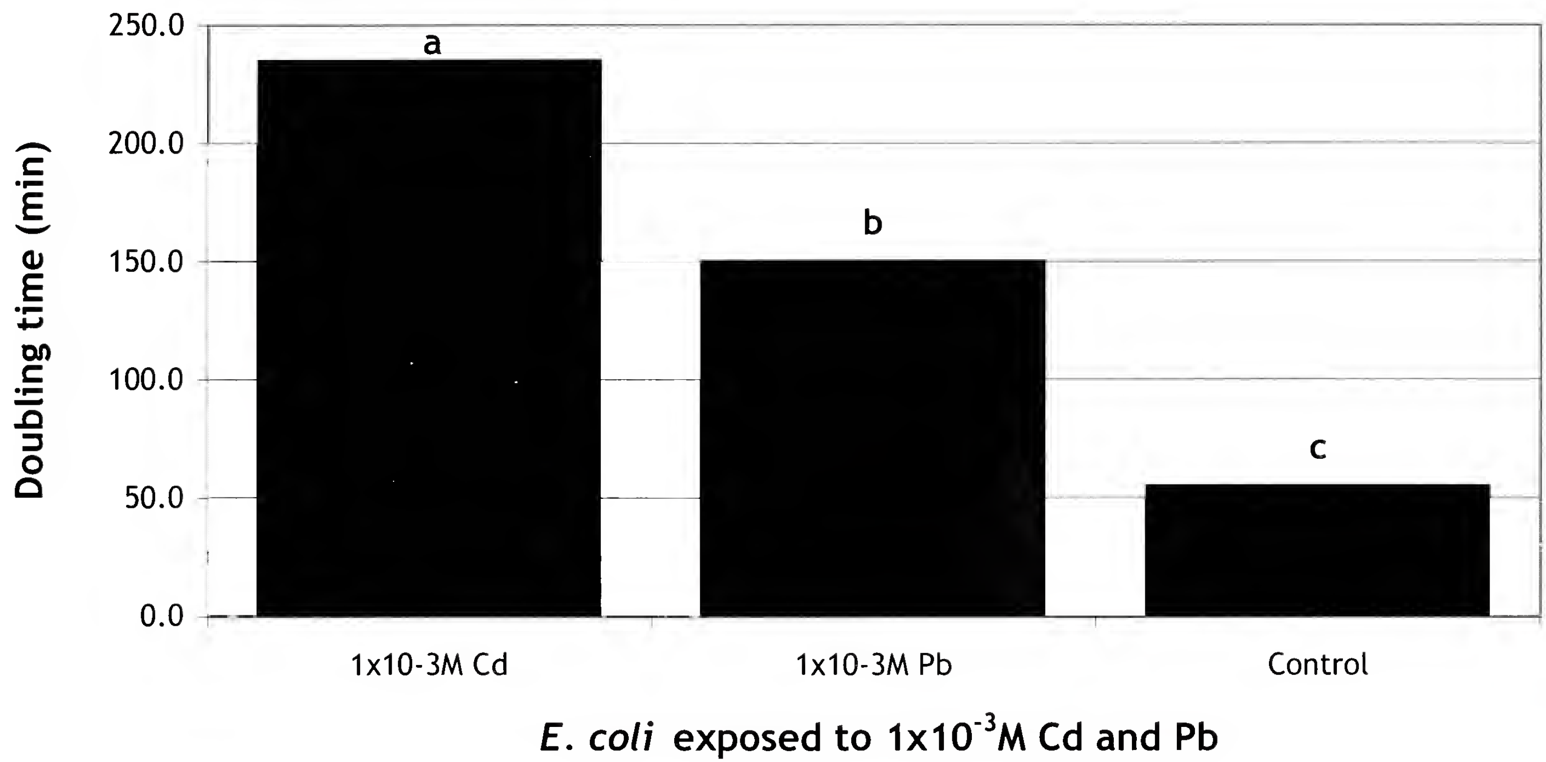


Figure 1-8. Average doubling times of *E.coli* vs. glucose or lactose from cell count vs. time

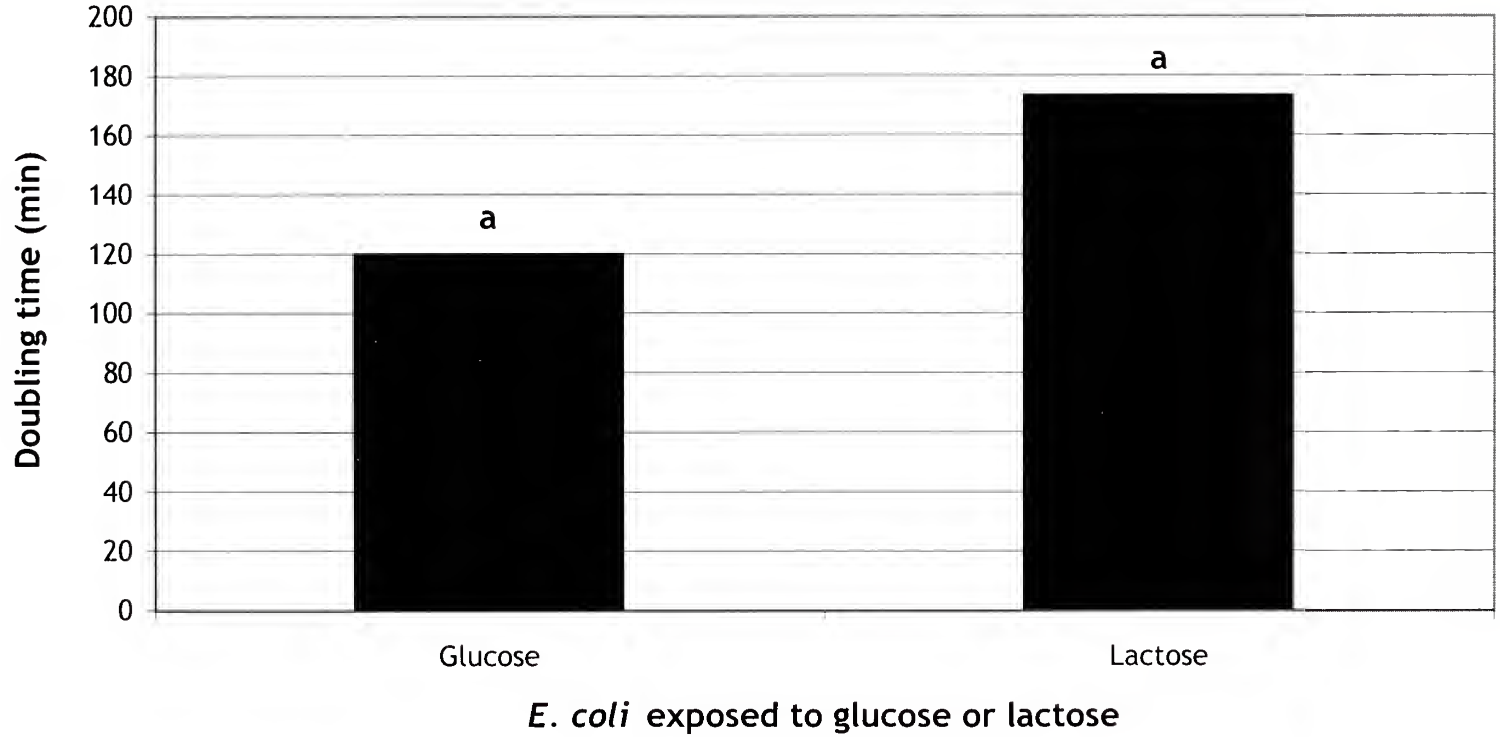


Figure 1-9. Summary of average doubling times of *E. coli* from cell count vs. time

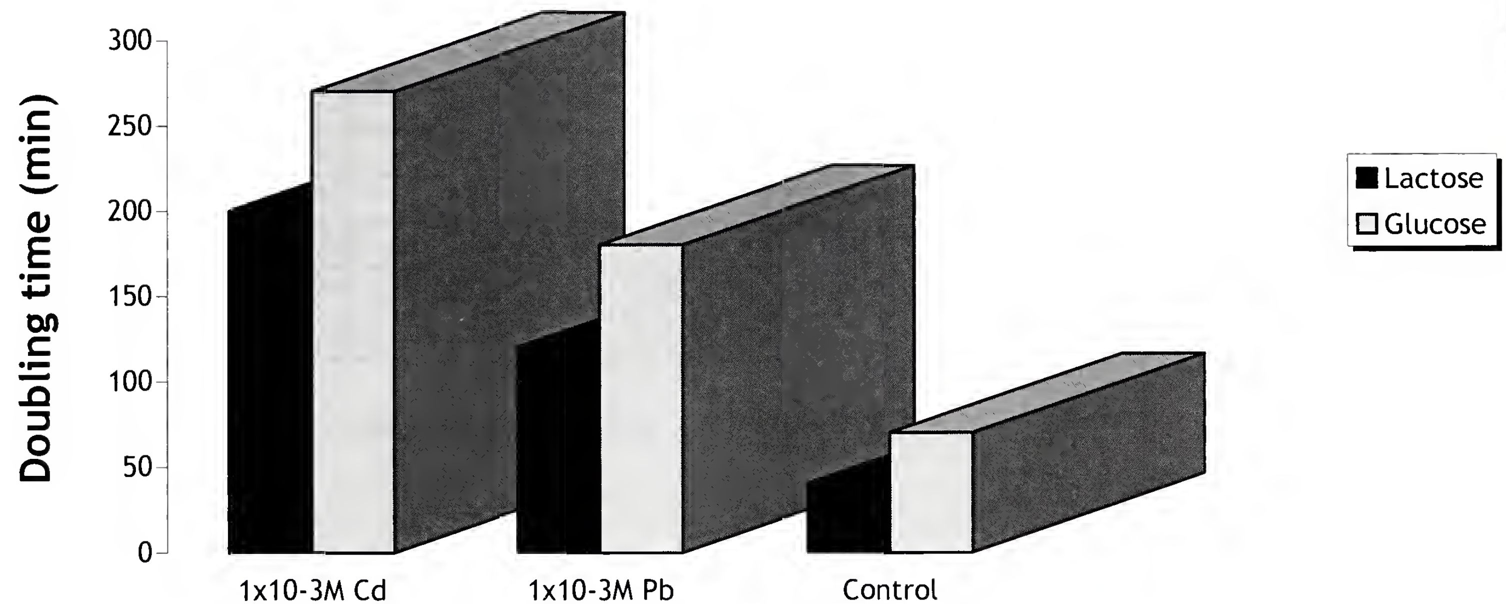


Table 2-7. Doubling times for *E. coli* cell count vs. absorbance in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

	Control	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	AVG
Lactose	0.077	0.029	0.044	0.050
	0.074	0.022	0.037	
	0.091	0.026	0.046	
Glucose	0.099	0.016	0.048	0.062
	0.109	0.023	0.051	
	0.124	0.027	0.063	
AVG	0.096	0.024	0.048	

Variable 1: Bacteria and nutrients (glucose/lactose)
Ec- *E.coli*

Variable 2: Treatment

Table 2-8. Two-Factor ANOVA With Replication for *E. coli* cell count vs. absorbance in M9 media with glucose or lactose exposed to 1×10^{-3} M Cd and Pb

Anova: Two-Factor With Replication

SUMMARY	Control	Cd	Pb	Total
Lactose				
Count	3	3	3	9
Sum	0.242	0.077	0.127	0.446
Average	0.08067	0.02567	0.04233	0.04956
Variance	8.2E-05	1.2E-05	2.2E-05	0.00063
Glucose				
Count	3	3	3	9
Sum	0.332	0.066	0.162	0.56
Average	0.11067	0.022	0.054	0.06222
Variance	0.00016	3.1E-05	6.3E-05	0.00158
Total				
Count	6	6	6	
Sum	0.574	0.143	0.289	
Average	0.09567	0.02383	0.04817	
Variance	0.00037	2.1E-05	7.5E-05	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.00072	1	0.00072	11.7292	0.00503	4.74722
Columns	0.01602	2	0.00801	130.1	7.3E-09	3.88529
Interaction	0.00085	2	0.00043	6.92329	0.01002	3.88529
Within	0.00074	12	6.2E-05			
Total	0.01833	17				

Table 2-9. Duncan's Multiple Range Test for *E. coli* cell count vs. absorbance grown in M9 media with glucose or lactose exposed to $1x10^{-3}$M Cd and Pb

Treatment					Sw	
df=2	Value of p	2	3		0.0000616	EMS
	SSR	6.09	6.09		6	k
	LSR	0.01951	0.01951		0.0000103	EMS/k
	Rp=LSR				0.003204	Sx

			Sw	
Nutrient			0.0000616	EMS
df=1	Value of p	2	9	k
	SSR	18.0	0.0000068	EMS/k
	LSR	0.0471	0.002616	Sx

$$Sx = \sqrt{\frac{EMS}{k}}$$

LSR=Sx*SSR

p=number of means for range being tested
Protection level = 0.05

Table 3-1. Mean Comparisons and Rp Comparisons for *E. coli* cell count vs. absorbance grown in M9 media with glucose or lactose exposed to $1x10^{-3}$M Cd and Pb

Cd	Pb	Control
0.024	0.048	0.096

Glucose	Lactose
0.062	0.05

	Difference	LSR		
Cd-Pb	0.02400	0.01951	0.00449	sig
Pb-Control	0.04800	0.01951	0.02849	sig
Cd-Control	0.03400	0.01951	0.01449	sig

	Difference	LSR		
Glucose-Lactose	0.0120	0.0471	-0.035	not sig

Table 3-2. Summary of doubling times for *E. coli* cell count vs. absorbance in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
0.024	0.048	0.0960

Glucose	0.062
Lactose	0.050

	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
Lactose	0.026	0.042	0.081
Glucose	0.017	0.054	0.111

Variable 1: Bacteria and nutrients (glucose/lactose)
Ec- *E.coli*

Variable 2: Treatment

Figure 2-1. Average absorbance of *E. coli* vs. Cd and Pb from cell count vs. absorbance

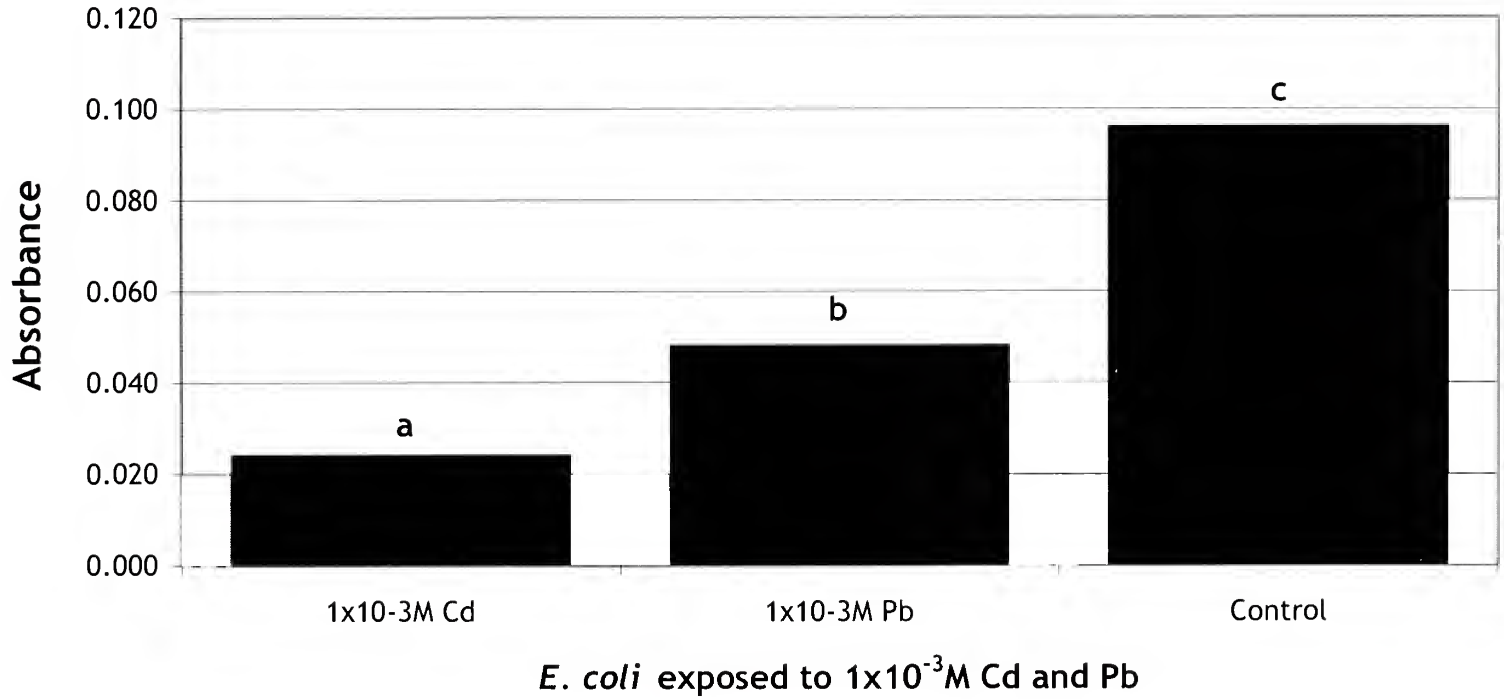


Figure 2-2. Average absorbance of *E. coli* vs. glucose or lactose from cell count vs. absorbance

Figure 2-3. Summary of average absorbance's of *E. coli* from cell count vs. absorbance

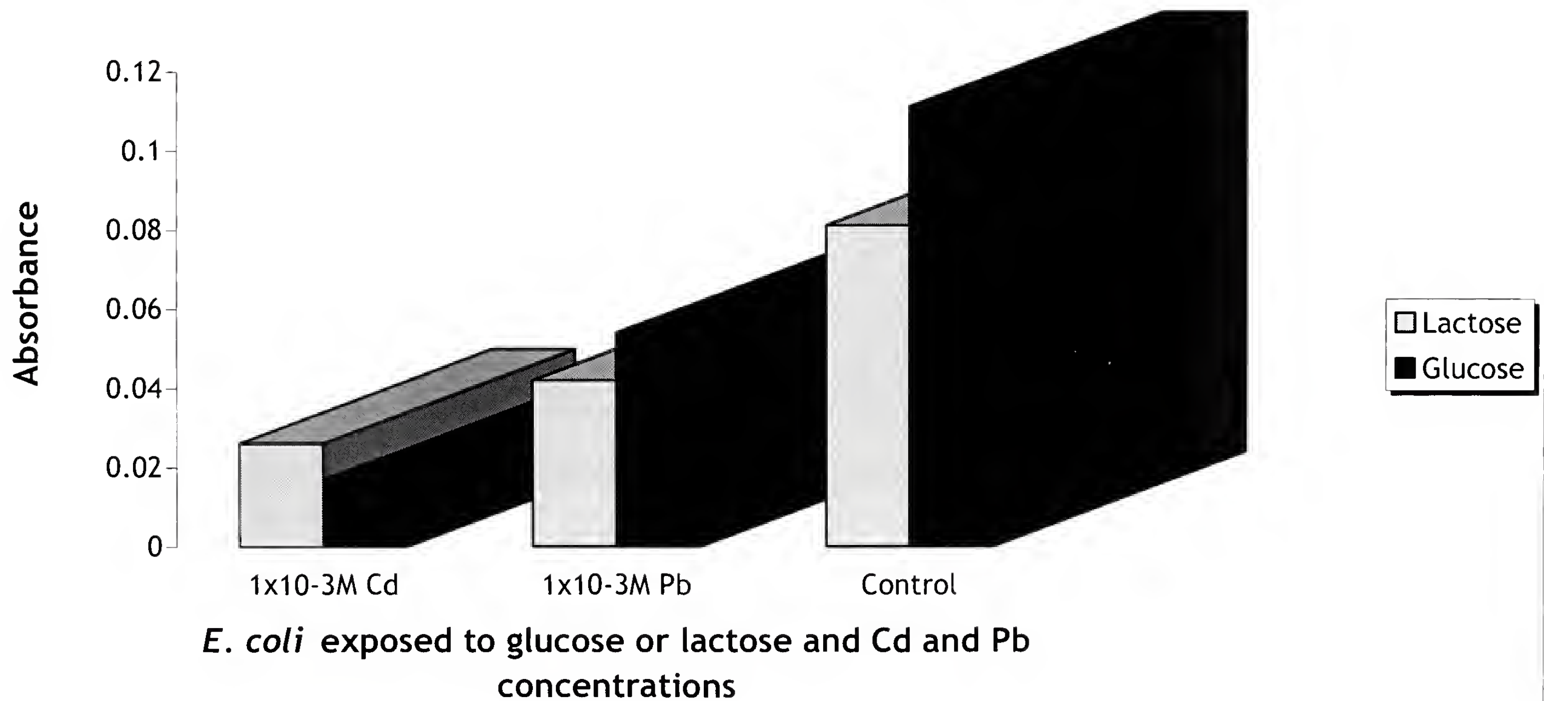


Table 3-3. Doubling times for *M. roseus* cell count vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

	Control	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	AVG
Lactose	60	300	180	190.0
	60	300	180	
	120	180	180	
	120	420	180	
Glucose	120	660	660	410.0
	60	540	600	
	60	360	300	
	180	720	660	
AVG	97.5	435.0	367.5	

Variable 1: Bacteria and nutrients (glucose/lactose)
Mr- *M. roseus*

Variable 2: Treatment

Table 3-4. Two-Factor ANOVA With Replication for *M. roseus* cell count vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$M Cd and Pb

Anova: Two-Factor With Replication

SUMMARY	Control	Cd	Pb	Total
Lactose				
Count	4	4	4	12
Sum	360	1200	720	2280
Average	90	300	180	190
Variance	1200	9600	0	11018.2
Glucose				
Count	4	4	4	12
Sum	420	2280	2220	4920
Average	105	570	555	410
Variance	3300	25200	29700	66654.5
Total				
Count	8	8	8	
Sum	780	3480	2940	
Average	97.5	435	367.5	
Variance	1992.857	35742.9	52907.1	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	290400	1	290400	25.2522	8.8E-05	4.41386
Columns	510300	2	255150	22.187	1.4E-05	3.55456
Interaction	137100	2	68550	5.96087	0.01032	3.55456
Within	207000	18	11500			
Total	1144800	23				

Table 3-5. Duncan's Multiple Range Test for *M. roseus* cell count vs. time grown in M9 media with glucose and lactose exposed to $1x10^{-3}$M Cd and Pb

Treatment					Sw	
df=2	Value of p	2	3		11500	EMS
	SSR	6.09	6.09		9	k
	LSR	217.4	217.4		1277.8	EMS/k
	Rp=LSR				35.7	Sx

			Sw	
Nutrient			11500	EMS
df=1	Value of p	2	12	k
	SSR	18.0	958.3	EMS/k
	LSR	558.0	31.0	Sx

p=number of means for range being tested
Protection level = 0.05

$$Sx = \sqrt{\frac{EMS}{k}}$$

LSR=Sx*SSR

Table 3-6. Mean Comparisons and Rp Comparisons for *M. roseus* cell count vs. time grown in M9 media with glucose and lactose exposed to $1x10^{-3}$M Cd and Pb

Cd	Pb	Control
435.00	367.50	97.50

Glucose	Lactose
410	190

	Difference	LSR		
Cd-Pb	67.5	217.4	-149.9	**not sig**
Pb-Control	270.0	217.4	52.6	**sig**
Cd-Control	337.5	121.8	215.7	**sig**

	Difference	LSR		
Glucose-Lactose	220	558.0	-338.00	**not sig**

Table 3-7. Summary of doubling times for *M. roseus* cell count vs. time in M9 media with glucose or lactose exposed to $1x10^{-3}$M Cd and Pb

$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
435.00	367.50	97.50

Glucose	410
Lactose	190

	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
Lactose	300	180	90
Glucose	570	555	105

Variable 1: Bacteria and nutrients (glucose/lactose)
Mr- *M. roseus*

Variable 2: Treatment

Figure 2-4. Average doubling times of *M. roseus* vs. Cd and Pb from cell count vs. time

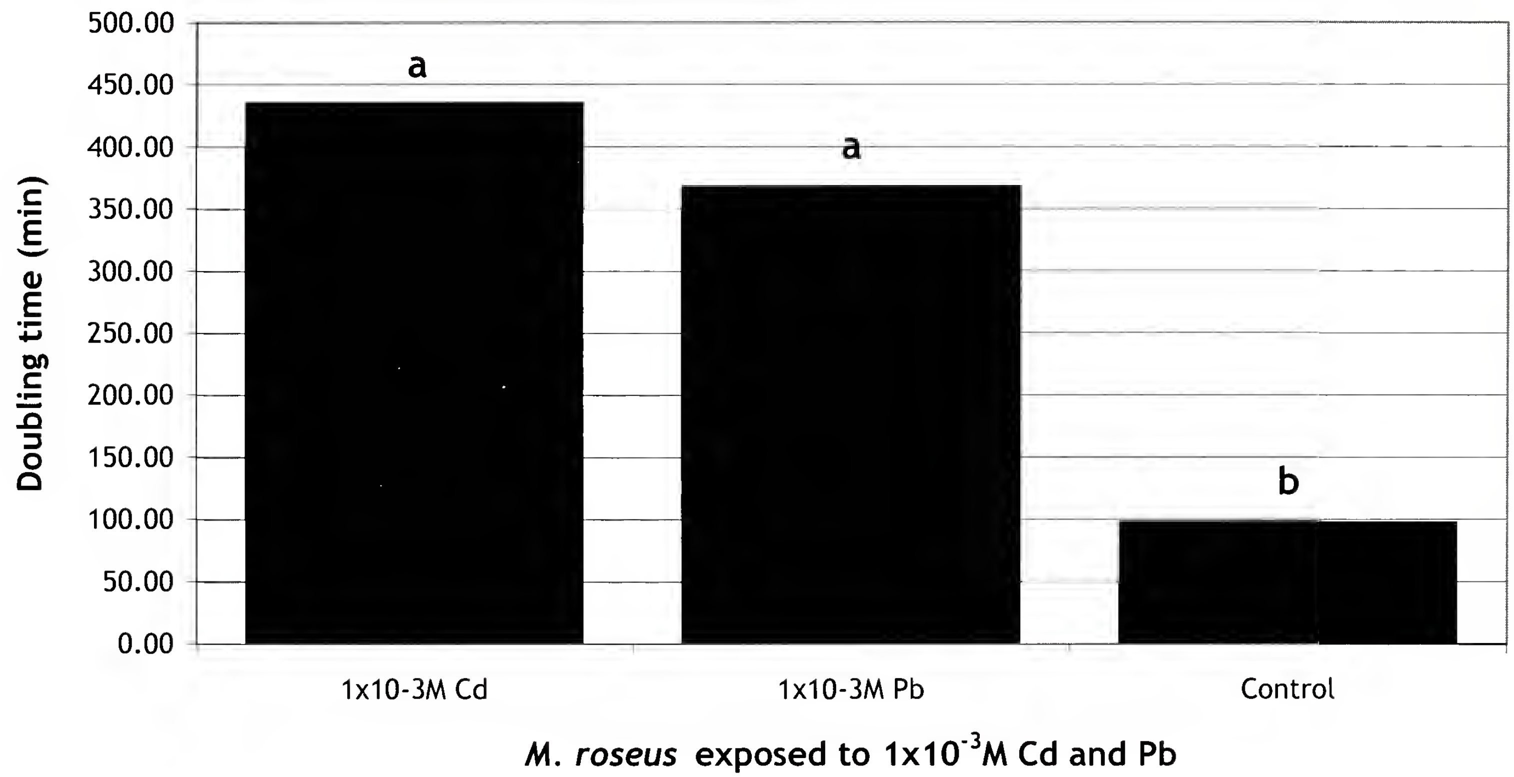


Figure 2-5. Average doubling times of *M. roseus* vs. glucose or lactose from cell count vs. time

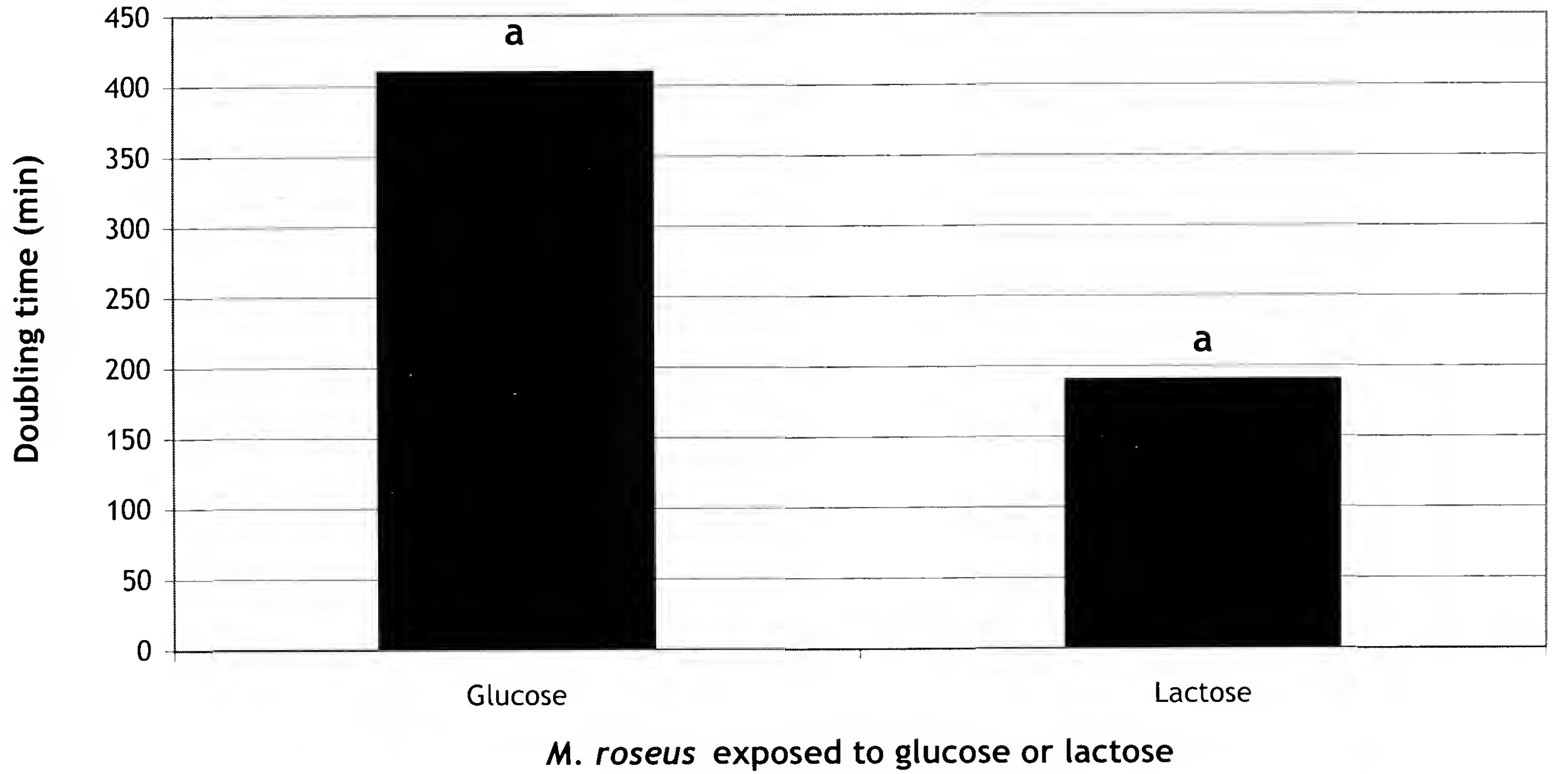


Figure 2-6. Summary of average doubling times of *M. roseus* from cell count vs. time

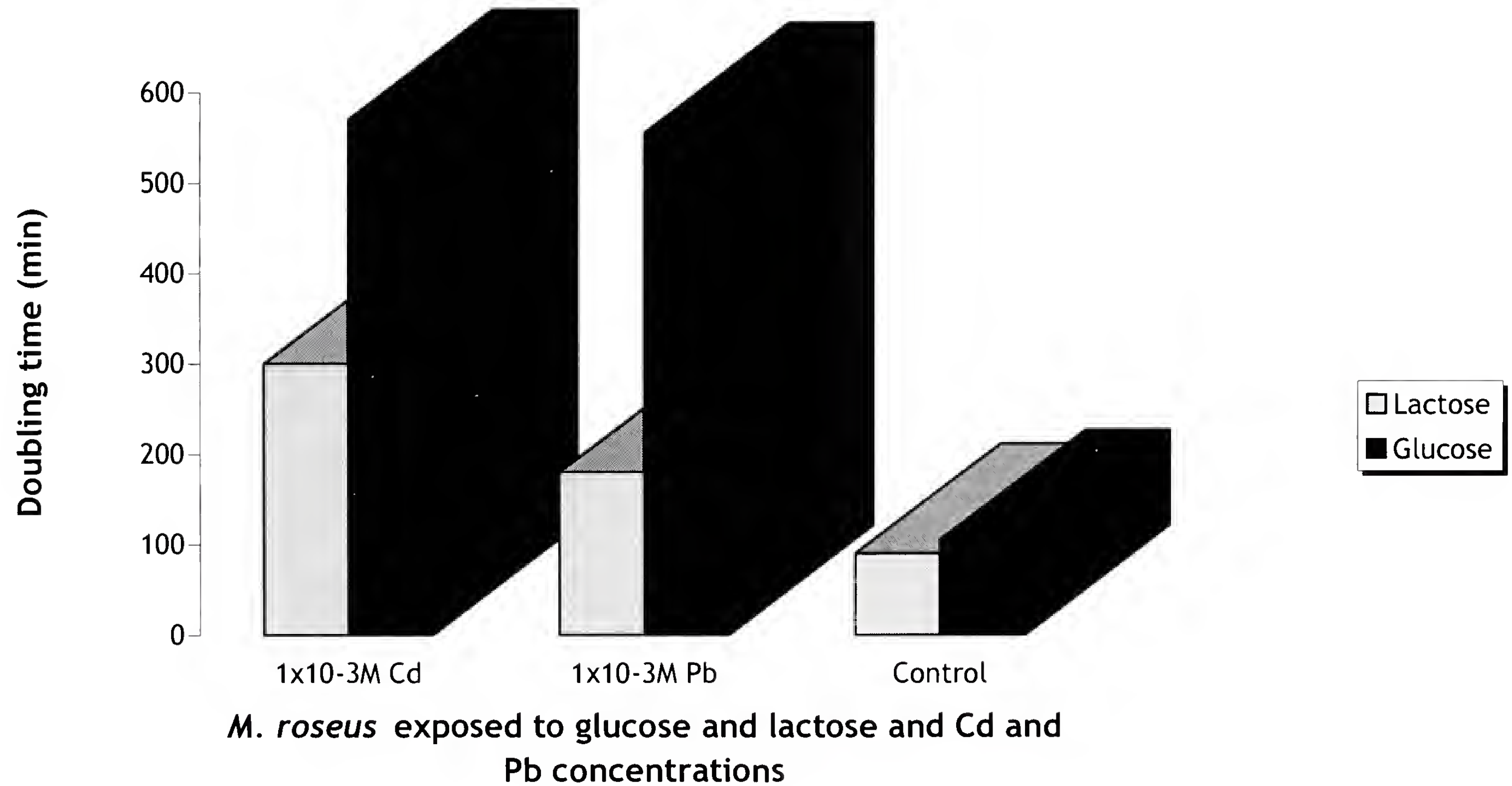


Table 3-8. Doubling times for *M. roseus* cell count vs. absorbance in M9 media with glucose or lactose exposed to $1x10^{-3}$ M Cd and Pb

	Control	$1*10^{-3}$M Cd	$1*10^{-3}$M Pb	AVG
Lactose	0.138	0.047	0.057	0.080
	0.104	0.05	0.079	
	0.104	0.06	0.077	
Glucose	0.078	0.069	0.053	0.060
	0.077	0.04	0.058	
	0.071	0.038	0.057	
AVG	0.095	0.051	0.064	

Variable 1: Bacteria and nutrients (glucose/lactose)
Mr- *M. roseus*

Variable 2: Treatment

Table 3-9. Two-Factor ANOVA With Replication for *M. roseus* cell count vs. absorbance in M9 media with glucose or lactose exposed to 1x10^{-3} M Cd and Pb

Anova: Two-Factor With Replication

SUMMARY	Control	Cd	Pb	Total
Lactose				
Count	3	3	3	9
Sum	0.346	0.157	0.213	0.716
Average	0.11533	0.05233	0.071	0.07956
Variance	0.00039	4.6E-05	0.00015	0.00093
Glucose				
Count	3	3	3	9
Sum	0.226	0.147	0.168	0.541
Average	0.07533	0.049	0.056	0.06011
Variance	1.4E-05	0.0003	7E-06	0.00022
Total				
Count	6	6	6	
Sum	0.572	0.304	0.381	
Average	0.09533	0.05067	0.0635	
Variance	0.00064	0.00014	0.00013	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.0017	1	0.0017	11.3174	0.005631	4.74722
Columns	0.00635	2	0.00317	21.1075	0.000156	3.88529
Interaction	0.00105	2	0.00053	3.50148	0.06341	3.88529
Within	0.0018	12	0.00015			
Total	0.0109	17				

Table 4-1. Duncan's Multiple Range Test for *M. roseus* cell count vs. absorbance grown in M9 media with glucose or lactose exposed to $1x10^{-3}$M Cd and Pb

Treatment					Sw	
df=2	Value of p	2	3		0.00015	EMS
	SSR	6.09	6.09		6	k
	LSR	0.03048	0.03048		0.00002505	EMS/k
	Rp=LSR				0.005005	Sx

			Sw	
Nutrient			0.00015	EMS
df=1	Value of p	2	9	k
	SSR	18.0	0.00002	EMS/k
	LSR	0.0736	0.00409	Sx

$$Sx = \sqrt{\frac{EMS}{k}}$$

LSR=Sx*SSR

p=number of means for range being tested
Protection level = 0.05

Table 4-2. Mean Comparisons and Rp Comparisons for *M. roseus* cell count vs. absorbance grown in M9 media with glucose or lactose exposed to 1×10^{-3}M Cd and Pb

Cd	Pb	Control
0.051	0.064	0.0950

Glucose	Lactose
0.06	0.08

	Difference	LSR		
Cd-Pb	0.01300	0.03048	-0.01748	not sig
Pb-Control	0.03100	0.03048	0.00052	sig
Cd-Control	0.04400	0.03048	0.01352	sig

	Difference	LSR		
Glucose-Lactose	0.0200	0.0736	-0.054	not sig

Table 4-3. Summary of doubling times for *M. roseus* cell count vs. absorbance in M9 media with glucose or lactose exposed to 1x10-3 M Cd and Pb

$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
0.051	0.064	0.0950

Glucose	0.06
Lactose	0.080

	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	Control
Lactose	0.052	0.071	0.115
Glucose	0.049	0.056	0.075

Variable 1: Bacteria and nutrients (glucose/lactose)
Mr- *M. roseus*

Variable 2: Treatment

Figure 2-7. Average absorbances of *M. roseus* vs. Cd and Pb from cell count vs. absorbance

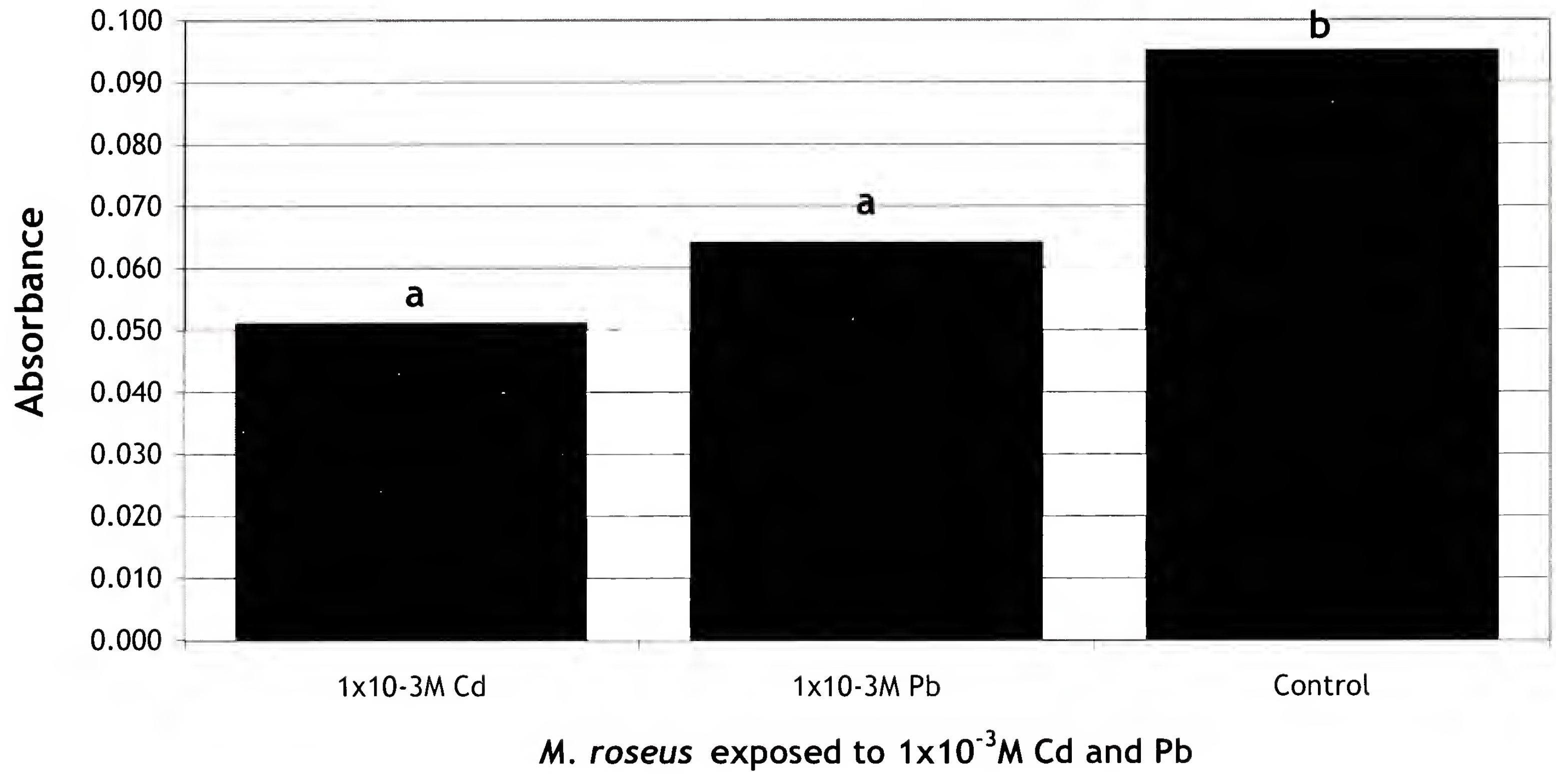


Figure 2-8. Average absorbances of *M. roseus* vs. glucose or lactose from cell count vs. absorbance

Figure 2-9. Summary of average absorbance's of *M.roseus* from cell count vs. absorbance

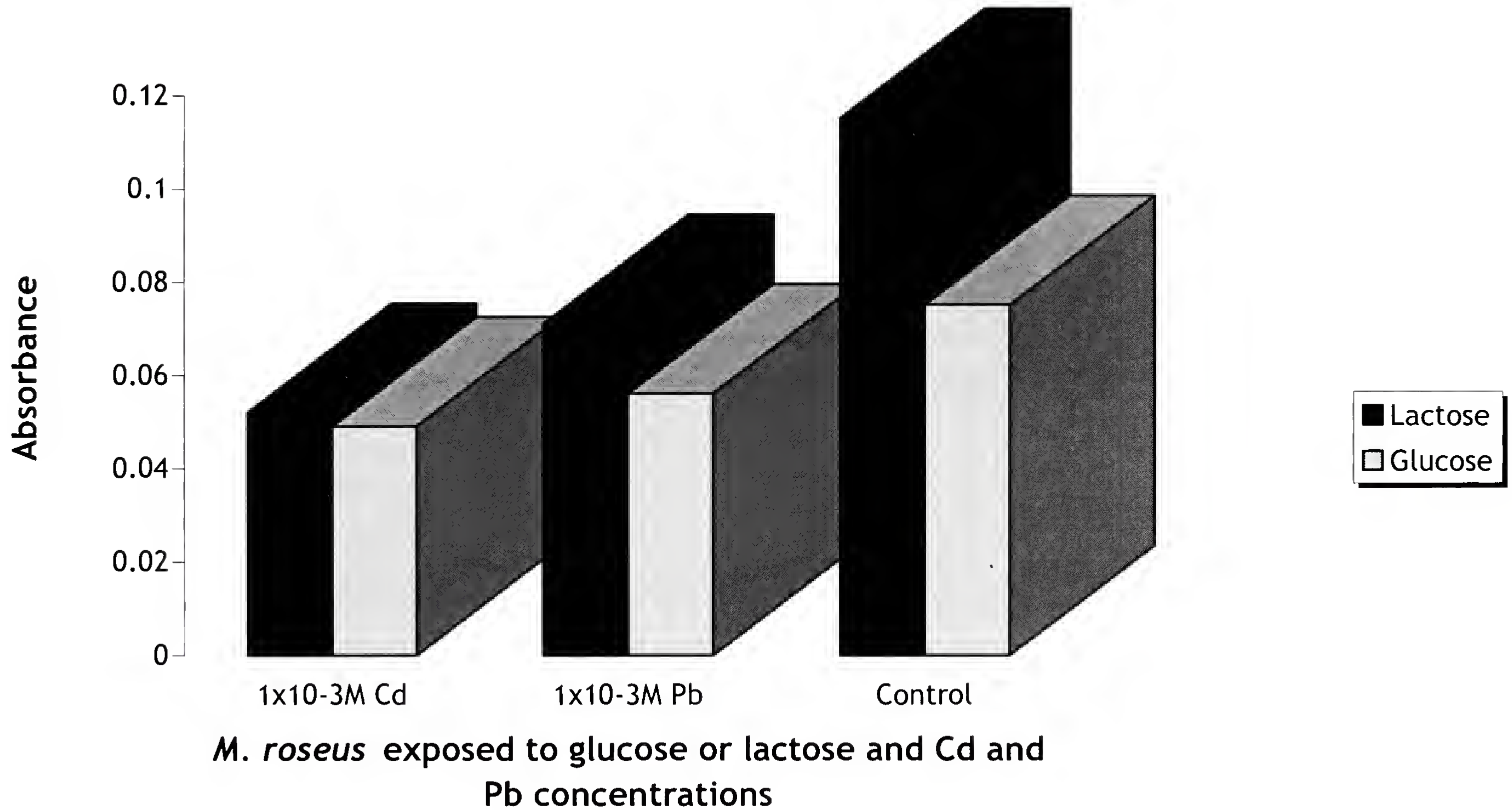


Table 4-4. Doubling times for *M. roseus* absorbance vs. time in M9 and LB media exposed to $1x10^{-3}M$ and $1x10^{-5}M$ Cd and Pb

	$1x10^{-3}M$ Cd	$1x10^{-3}M$ Pb	$1x10^{-5}M$ Cd	$1x10^{-5}M$ Pb	Control	Avg
Mr-M1	240	210	180	150	150	186
	240	210	180	150	150	186
	240	210	210	180	150	198
Mr-M2	240	240	180	120	120	180
	240	180	180	120	120	168
	300	240	210	180	120	210
Avg	250	215	190	150	135	

Variable 1: Bacteria and Media
M1= Control medium M9 media
M2= Control rich LB media

Variable 2: Treatment

Table 4-5. Anova: Two-Factor With Replication for for *M. roseus* absorbance vs. time in M9 and LB media exposed to $1x10^{-3}M$ and $1x10^{-5}M$ Cd and Pb

SUMMARY	1*10-3M (	1*10-3M I	1*10-5M (	1*10-5M I	Control	Total
Mr-M1						
Count	3	3	3	3	3	15
Sum	720	630	570	480	450	2850
Average	240	210	190	160	150	190
Variance	0	0	300	300	0	1242.86
Mr-M2						
Count	3	3	3	3	3	15
Sum	780	660	570	420	360	2790
Average	260	220	190	140	120	186
Variance	1200	1200	300	1200	0	3368.57
Total						
Count	6	6	6	6	6	
Sum	1500	1290	1140	900	810	
Average	250	215	190	150	135	
Variance	600	510	240	720	270	

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	120	1	120	0.2667	0.6112	4.3513
Columns	52980	4	13245	29.433	4E-08	2.8661
Interaction	2580	4	645	1.4333	0.2596	2.8661
Within	9000	20	450			
Total	64680	29				

Table 4-6. Duncan's Multiple Range Test for *M. roseus* absorbance vs. time grown in M9 and LB media exposed to $1x10^{-3}$M and $1x10^{-5}$M Cd and Pb

Treatment						Sw	
df=4	Value of p	2	3	4	5	450	EMS
	SSR	3.9	4.0	4.0	4.0	6	k
	LSR	34.0	34.7	34.8	34.8	75.0	EMS/k
	Rp=LSR					8.66	Sx

			Sw	
Bacteria Type and Media			450	EMS
df=1	Value of p	2	15	k
	SSR	18.0	30.0	EMS/k
	LSR	98.6	5.5	Sx

p=number of means for range being tested
Protection level = 0.05

$Sx = \sqrt{EMS / k}$

LSR=Sx*SSR

Table 4-7. Mean Comparisons and Rp Comparisons for *M. roseus* absorbance vs. time grown in M9 and LB media exposed to $1x10^{-3}M$ and $1x10^{-5}M$ Cd and Pb

High Cd	High Pb	Low Cd	Low Pb	Control
250.0	215.0	190.0	150.0	135.0

Mr-M1	Mr-M2
190	190

	Difference	LSR		
HC-HP	35.0	34.0	1.0	sig
HC-LC	60.0	34.7	25.3	sig
HC-LP	100.0	34.8	65.2	sig
HC-Co	115.0	34.8	80.2	sig
HP-LC	25.0	34.0	-9.0	not sig
HP-LP	65.0	34.7	30.3	sig
HP-Co	80.0	34.8	45.2	sig
LC-LP	40.0	34.0	6.0	sig
LC-Co	55.0	34.7	20.3	sig
LP-Co	15.0	34.0	-19.0	not sig

	Difference	LSR		
Mr-M1-Mr-M2	0	98.6	-98.6	not sig

Table 4-8. Summary of doubling times for *M. roseus* absorbance vs. time in M9 and LB media exposed to $1x10^{-3}$M and $1x10^{-5}$M Cd and Pb

$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	$1x10^{-5}$M Cd	$1x10^{-5}$M Pb	Control
250.0	215.0	190.0	150.0	135.0

Mr-M1	190
Mr-M2	190

	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	$1x10^{-5}$M Cd	$1x10^{-5}$M Pb	Control
Mr-M1	240	210	190	160	150
Mr-M2	260	220	190	160	120

Variable 1: Bacteria and Media

M1= Control medium M9 media

M2= Control rich LB media

Variable 2: Treatment

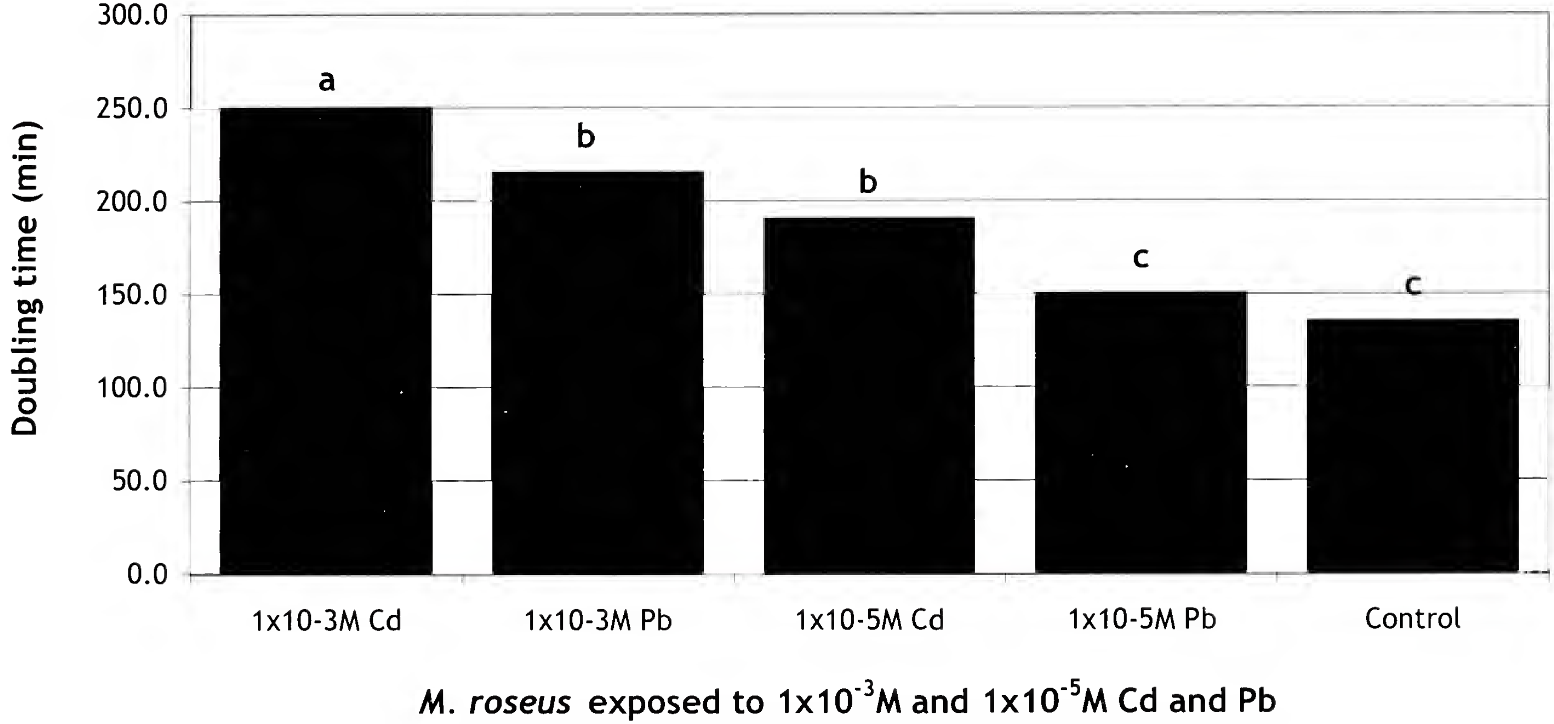


Figure 3-1. Average doubling times of *M. roseus* vs. Cd and Pb from absorbance vs. time

Figure 3-2. Average doubling times of *M. roseus* vs. different media from absorbance vs. time

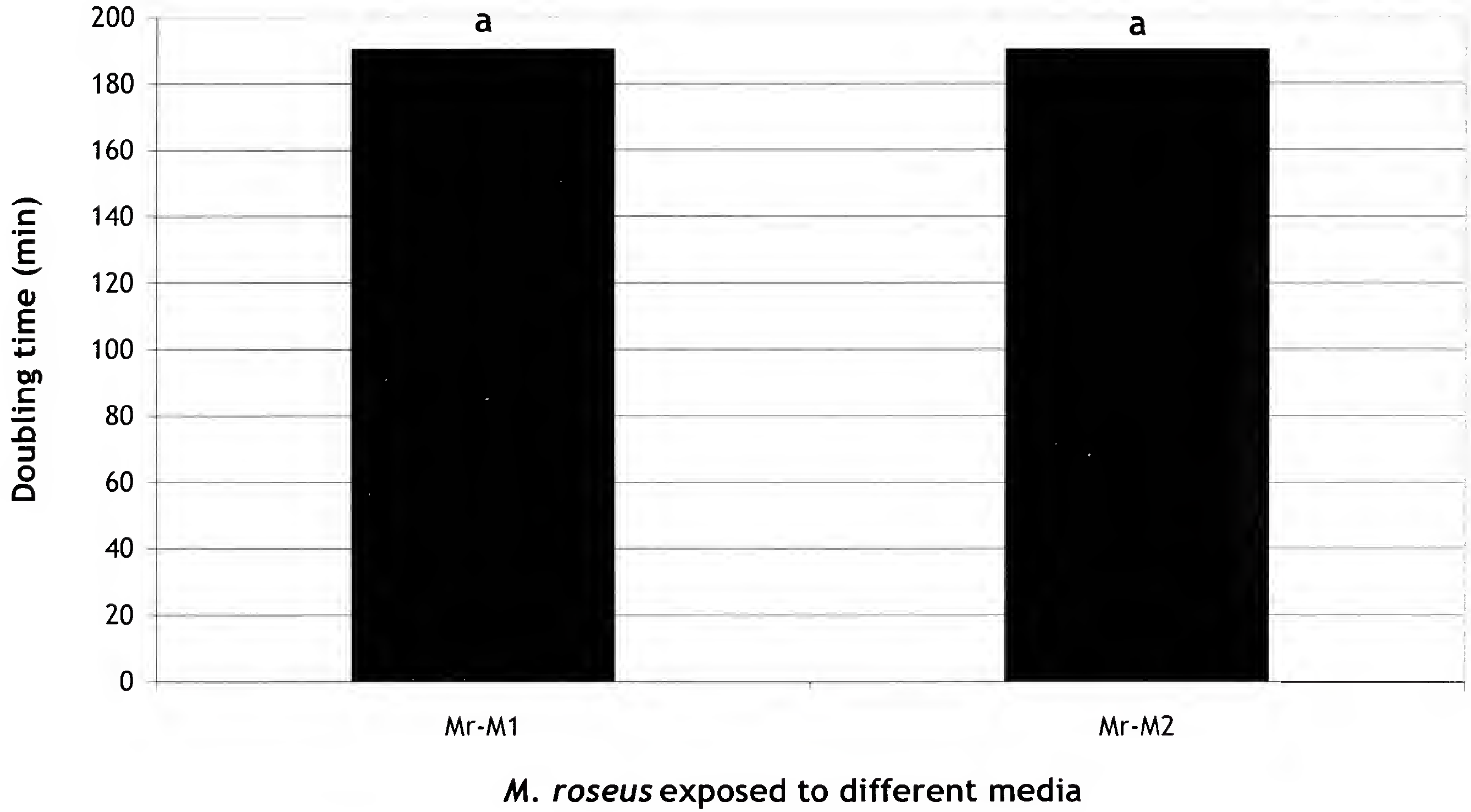


Figure 3-3. Summary of average doubling times of *M. roseus* from absorbance vs. time

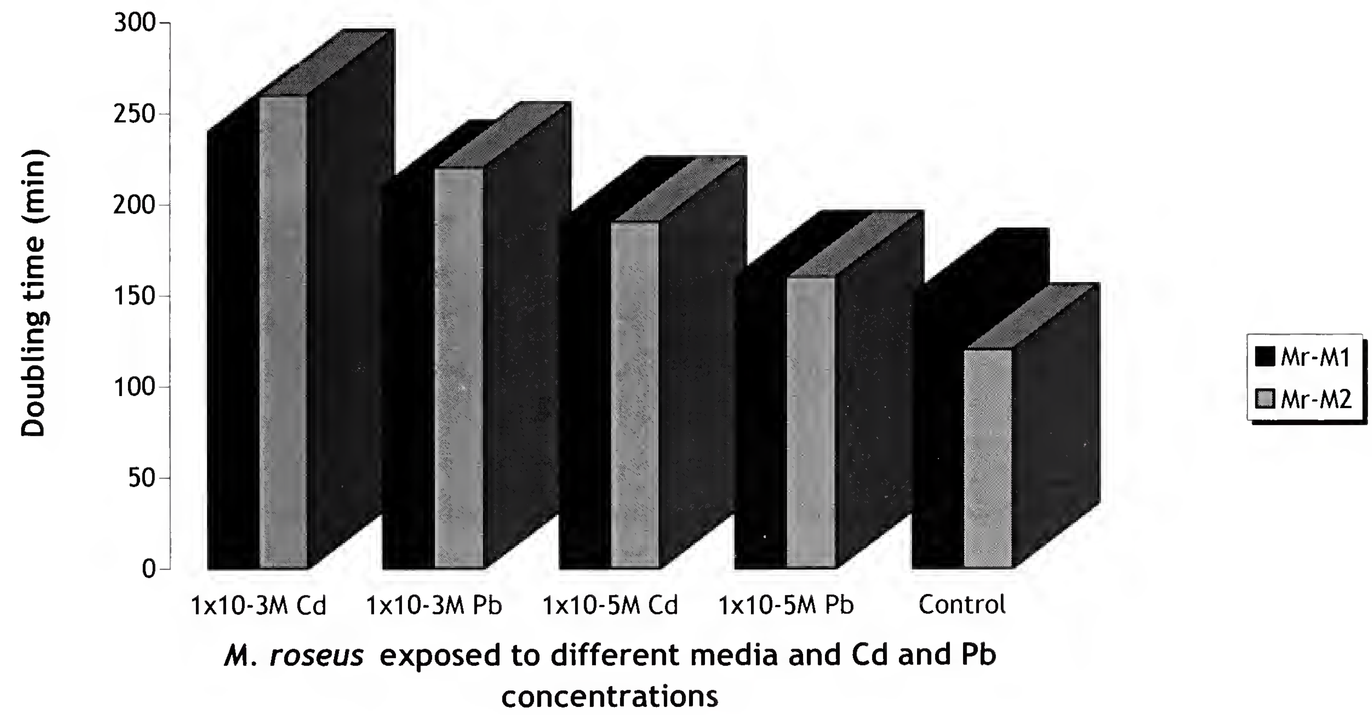


Table 4-9. Doubling times for *E. coli* absorbance vs. time in M9 with and without casamino acids and LB media exposed to $1x10^{-3}M$ and $1x10^{-5}M$ Cd and Pb

	Control	$1*10^{-5}M$ Cd	$1*10^{-3}M$ Cd	$1*10^{-5}M$ Pb	$1*10^{-3}M$ Pb	AVG
M1	120	180	210	150	210	180
	150	180	210	150	210	
	150	180	240	150	210	
M2	60	90	150	60	90	90
	60	90	150	60	90	
	60	90	150	60	90	
M3	30	60	90	30	60	56
	30	60	90	30	60	
	30	60	90	30	90	
AVG	76.7	110	153.3	80.0	123.3	

Variable = *E.coli* (only *E. coli* since *M. roseus* didn't grow in low medium)

M1= Control low media (M9 with no casamino acids)

M2= Control medium M9 media

M3= Control rich LB media

Table 5-1. Two-Factor ANOVA With Replication for *E. coli* absorbance vs. time in M9 with and without casamino acids and LB media exposed to $1x10^{-3}M$ and $1x10^{-5}M$ Cd and Pb

Anova: Two-Factor With Replication

SUMMARY	Control	Low Cd	High Cd	Low Pb	High Pb	Total
M1						
Count	3	3	3	3	3	15
Sum	420	540	660	450	630	2700
Average	140	180	220	150	210	180
Variance	300	0	300	0	0	1157.143
M2						
Count	3	3	3	3	3	15
Sum	180	270	450	180	270	1350
Average	60	90	150	60	90	90
Variance	0	0	0	0	0	1157.143
M3						
Count	3	3	3	3	3	15
Sum	90	180	270	90	210	840
Average	30	60	90	30	70	56
Variance	0	0	0	0	300	625.7143
Total						
Count	9	9	9	9	9	
Sum	690	990	1380	720	1110	
Average	76.6667	110	153.333	80	123.333	
Variance	2500	2925	3250	2925	4375	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	123160	2	61580	1026.33	2.4E-28	3.315833
Columns	36520	4	9130	152.167	1.8E-19	2.689632
Interaction	2840	8	355	5.91667	0.00014	2.266162
Within	1800	30	60			
Total	164320	44				

Table 5-2. Duncan's Multiple Range Test for *E. coli* absorbance vs. time grown in M9 with and without casamino acids and LB media at $1x10^{-3}M$ and $1x10^{-5}M$ Cd and Pb

Treatment							Sw	
df=4	Value of p	2	3	4	5		60	EMS
	SSR	3.9	4.0	4.0	4.0		9	k
	LSR	10.2	10.5	10.5	10.5		6.7	EMS/k
	Rp=LSR						2.6	Sx

				Sw	
Media				60	EMS
df=2	Value of p	2	3	15	k
	SSR	6.1	6.1	4.0	EMS/k
	LSR	12.2	12.2	2.0	Sx

$$Sx = \sqrt{\frac{EMS}{k}}$$

$$LSR = Sx * SSR$$

p=number of means for range being tested
Protection level = 0.05

Table 5-3. Mean Comparisons and Rp Comparisons for *E. coli* absorbance vs. time grown in M9 with and without casamino acids and LB media at $1x10^{-3}$M and $1x10^{-5}$M Cd and Pb

High Cd	High Pb	Low Cd	Low Pb	Control
153.3	123.3	110	80	76.7

M1	M2	M3
180	90	54

	Difference	LSR		
HC-HP	30.0	10.2	19.8	sig
HC-LC	43.3	10.5	32.8	sig
HC-LP	73.3	10.5	62.8	sig
HC-Co	76.6	10.5	66.1	sig
HP-LC	13.3	10.2	3.1	sig
HP-LP	43.3	10.5	32.8	sig
HP-Co	46.6	10.5	36.1	sig
LC-LP	30.0	10.2	19.8	sig
LC-Co	33.3	10.5	22.8	sig
LP-Co	3.3	10.2	-6.9	not sig

	Difference	LSR		
M1-M2	90	12.2	77.8	sig
M1-M3	126	12.2	113.8	sig
M2-M3	36	12.2	23.8	sig

Table 5-4. Summary of doubling times for *E. coli* absorbance vs. time in M9 with and without casamino acids and LB media exposed to $1x10^{-3}$M and $1x10^{-5}$M Cd and Pb

$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	$1x10^{-5}$M Cd	$1x10^{-5}$M Pb	Control
153.30	123.30	110.00	80.00	76.67

M1	180
M2	90
M3	56

	$1x10^{-3}$M Cd	$1x10^{-3}$M Pb	$1x10^{-5}$M Cd	$1x10^{-5}$M Pb	Control
M1	220	210	180	150	140
M2	150	90	90	60	60
M3	90	70	60	30	30

Variable = *E.coli* (only *E. coli* since *M. roseus* didn't grow in low medium)

M1= Control low media (M9 with no casamino acids)
M2= Control medium M9 media
M3= Control rich LB media

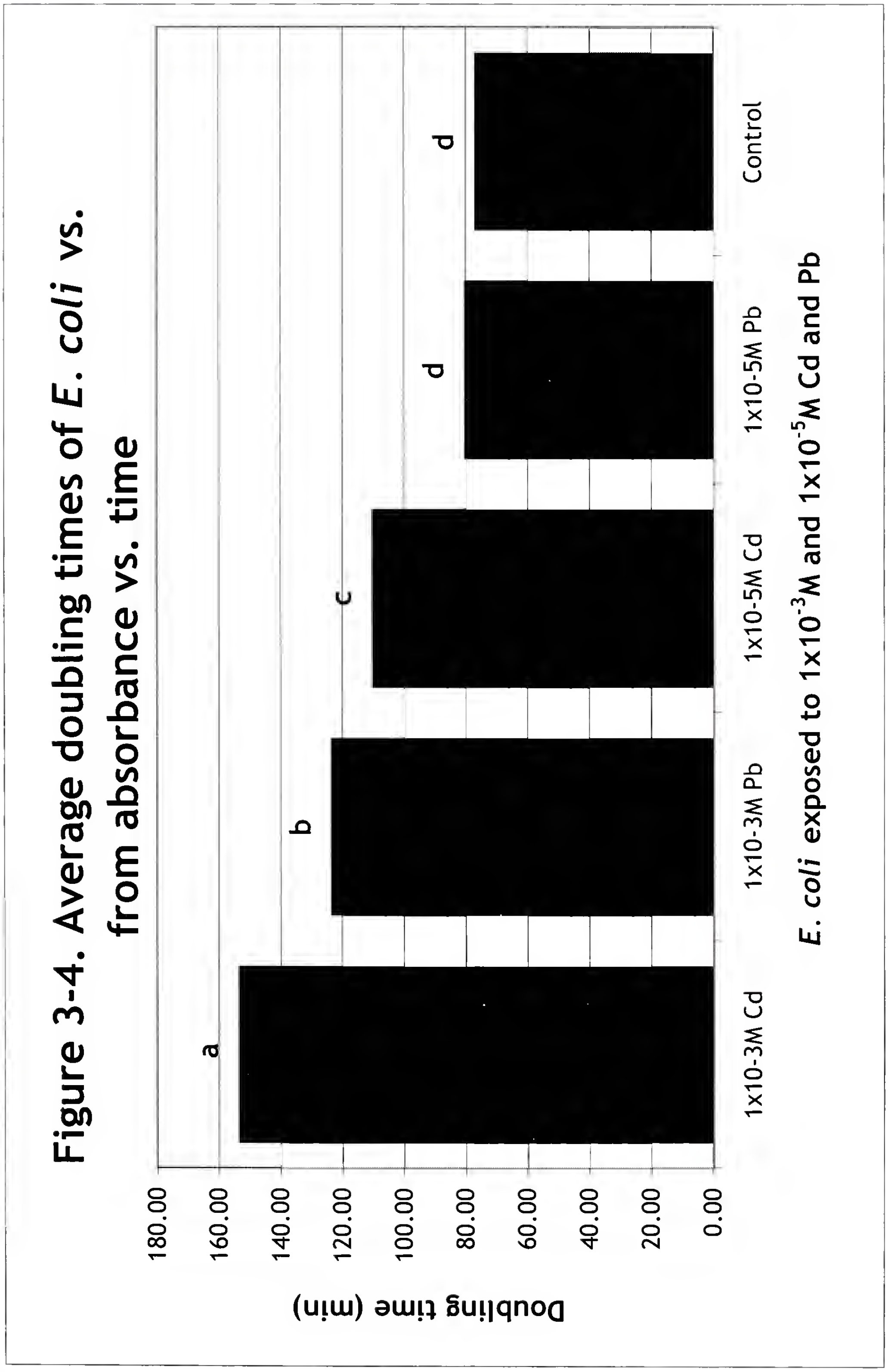


Figure 3-4. Average doubling times of *E. coli* vs. from absorbance vs. time

Figures 3-5. Average doubling times of *E. coli* vs. different media from absorbance vs. time

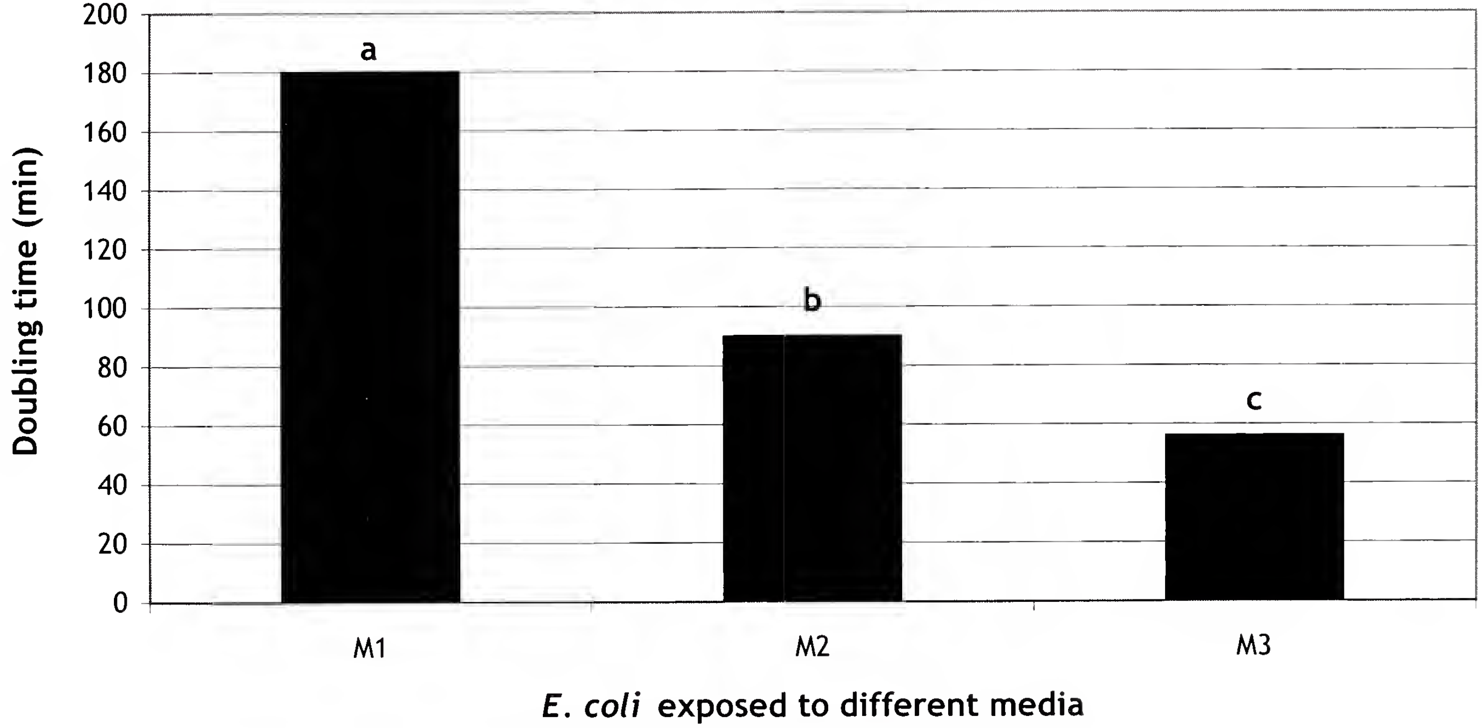
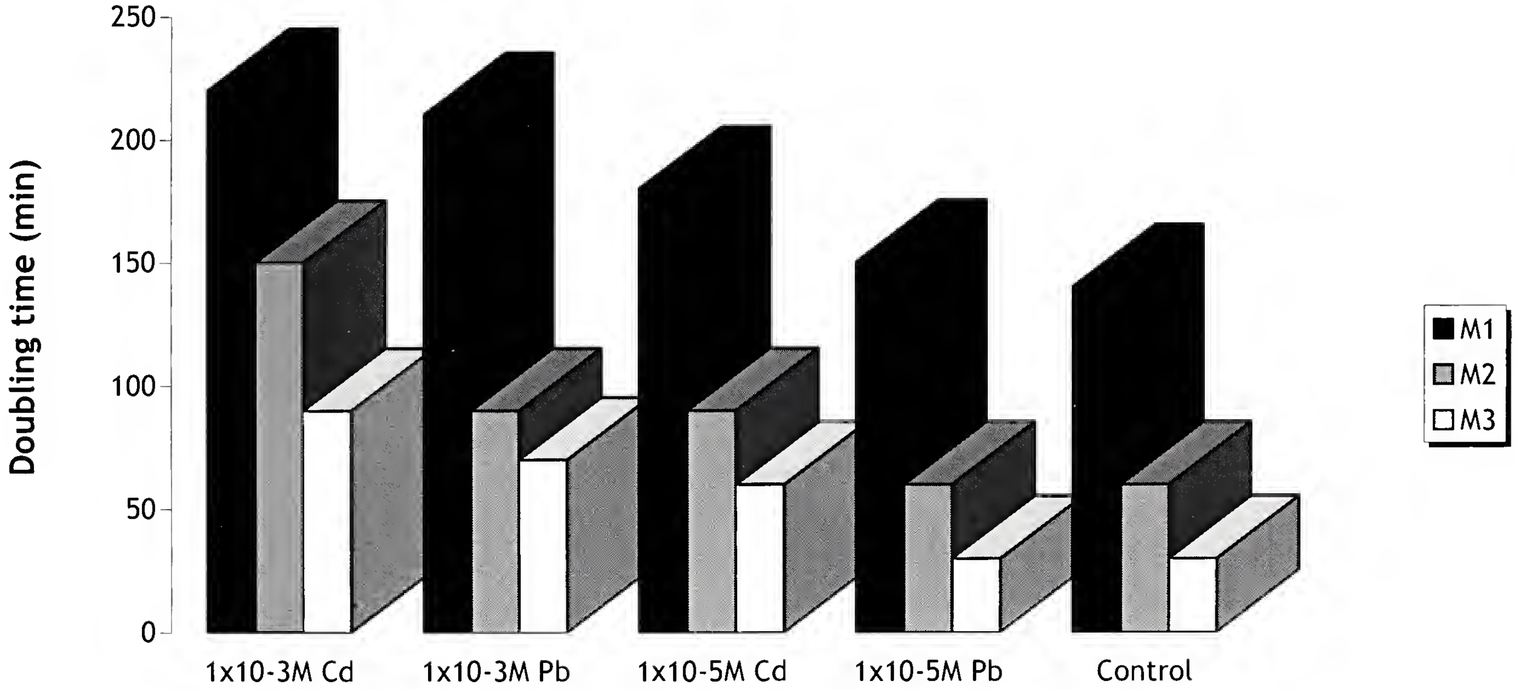


Figure 3-6. Summary of average doubling times of *E. coli* from absorbance vs. time



E. coli exposed to different media and Cd and Pb concentrations

Table 5-5. Exposure of Rf ^{r} wild type, Tc ^{r} and Rf ^{r} donor strain, and mutant to UV-light

	cell number before the UV-light exposure	cell number after the UV-light exposure
wild type	352000000	264000000
	289000000	251000000
	334000000	248000000
Tc^{r} donor	2260000	296000
	1880000	283000
	2160000	264000
Rf ^{r} donor	1960000	128000
	1790000	116000
	2190000	123000
mutant	1730000	85000
	1890000	71000
	1840000	69000
AVG	82725000	63702917

Table 5-6. % Survival of wild type, Tc^r donor, Rf^r donor and mutant strain

	wild type	Tc^r donor	Rf^r donor	mutant
% Survival Run 1	75.0	13.1	6.5	4.9
% Survival Run 1	86.9	15.1	6.5	3.8
% Survival Run 1	74.3	12.2	5.6	3.8
AVG % Survival	78.73	13.47	6.20	4.17

Table 5-7. Single Factor Anova for % survival of wild type, Tc^r donor, Rf^r donor and mutant strain

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Wild	3	236.2	78.733	50.143
Tc	3	40.4	13.467	2.203
Rf	3	18.6	6.200	0.270
mutant	3	12.5	4.167	0.403

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11418.3292	3	3806.109722	287.145207	1.76E-08	4.06618
Within Groups	106.04	8	13.255			
Total	11524.3692	11				

Table 5-8. Analysis of Variance for % survival of wild type, Tc^r donor, Rf^r donor and mutant strain

t=4.3

	s	*se*	*ci*	*ll*	*ul*	
wild type	7.081	4.088	17.580	61.154	96.313	a
Tc^r donor	1.484	0.857	3.685	9.782	17.152	b
Rf^r donor	0.520	0.300	1.290	4.910	7.490	c
mutant	0.635	0.367	1.577	2.590	5.743	c

$S = \sqrt{Variance}$

$SE = \frac{S}{\sqrt{count}}$

$CI = t * SE$

Figure 3-7. Average % survival for wild Type, Tc^r donor, Rf^r ^{d}onor, and mutant

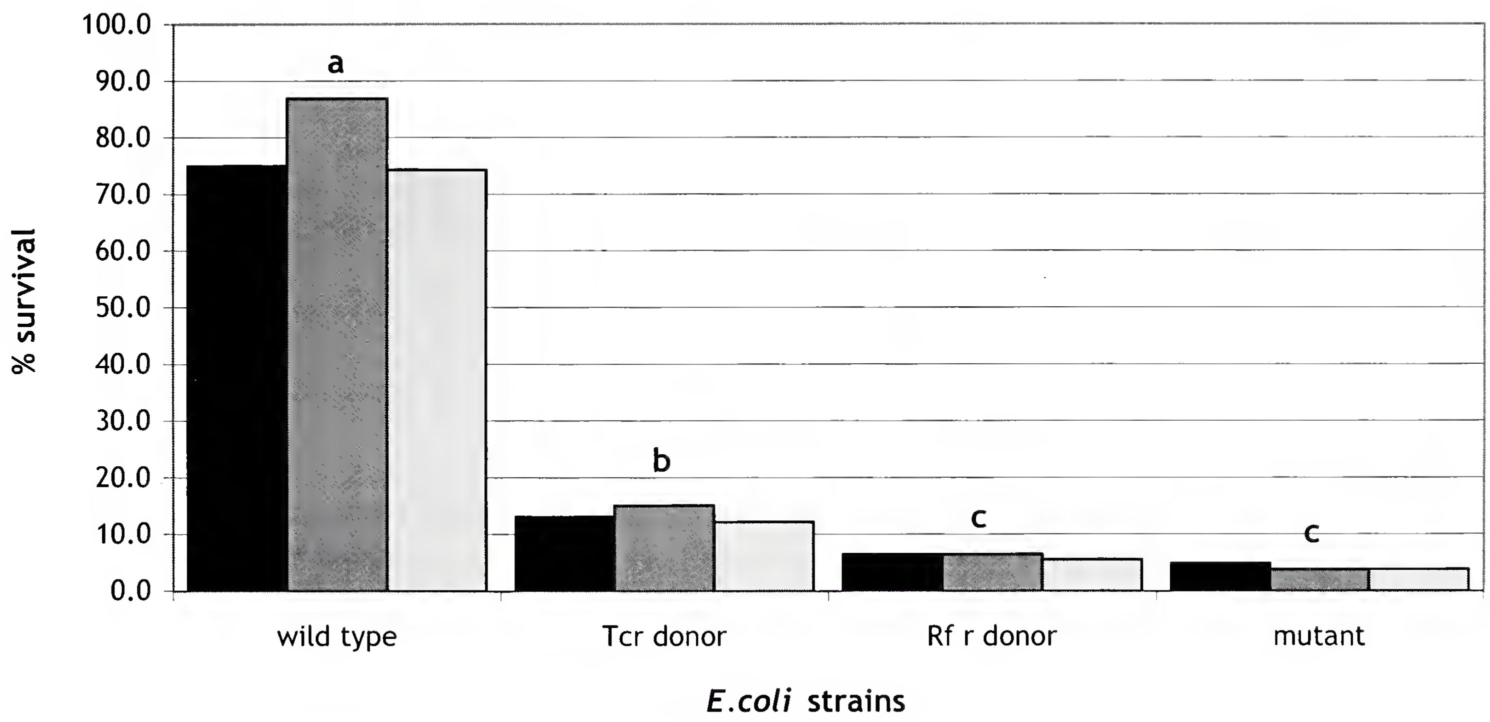


Table 5-9. Duncan's Multiple Range Test for % survival of wild type, Tc^r donor, Rf^r donor, and mutant strain

					Sw	
Media					13.255	EMS
df=3	Value of p	2	3	4	12	k
	SSR	4.5	4.5	4.5	1.1	EMS/k
	LSR	4.7	4.7	4.7	1.1	Sx

$$Sx = \sqrt{\frac{EMS}{k}}$$

p=number of means for range being tested

Protection level = 0.05

LSR=Sx*SSR

Table 6-1. Mean Comparisons and Rp Comparisons for % survival of wild type, Tc^r donor, Rf^r donor, and mutant strain

wild type	Tc^r donor	Rf^r donor	mutant
78.2	13.4	6.2	4.1

	Difference	LSR		
W-Tc	64.8	4.7	60.1	sig
W-Rf	72.0	4.7	67.3	sig
W-Mutant	74.1	4.7	69.4	sig
Tc-Rf	7.2	4.7	2.5	sig
Tc-Mutant	9.3	4.7	4.6	sig
Rf-Mutant	2.1	4.7	-2.6	not sig

DISCUSSION AND CONCLUSION

Injury to *E. coli* strains may result from environmental stress factors such as the presence of sublethal doses of heavy metals (Singh et al., 1986). Heavy metals have been introduced into the environment from industrial processes and during the mining and refining of metal ores. Some metals are essential for microbes, but at the certain level they are toxic. The exposed cells would not be necessarily killed, but physiologically damaged with the lower ability to adapt to an adverse environment (Cenci et al., 1985). The culmination of this study noted that Cd and Pb had an effect on *E. coli* and *M. roseus* doubling times. An *E. coli* strain was also identified that was more resistant to Cd.

Toxic metals Cd^{+2} and Pb^{+2} have demonstrated a significant effect on *E. coli* and *M. roseus* growth rates. Cd^{+2}, at 10μM and 1mM concentrations as well as Pb^{+2} at 1mM concentration, had a significant effect on *E. coli* in all media types. Results suggest that *E. coli* did not show any significant difference between control and growth rates exposed to 10μM Pb. However, 10μM and 1mM concentrations of both metals had a major effect on growth rates in *M. roseus* medium and rich media. Insignificant effect was found in *M. roseus* growth rates when exposed to 10μM Cd and 1mM Pb, as well as 10μM Pb and the control. Also, there was no significant difference discovered between *M. roseus* medium and rich media. The responses of *E. coli* and *M. roseus* to cadmium salts were, in general, increased in comparison to lead salts, suggesting slightly different biological effect of these heavy metals. The key to understanding distinctions may lie in the analysis of different gene induction by the two metals if they are significantly different.

Results by Mariscal et al. showed similar effects of Cd and Pb salts on *E. coli* growth rate (Mariscal et al., 1995). Cenci et al. (1985) previously discussed the complexity of metal-bacteria interaction. Their results emphasize that the binding of heavy metals to the bacterial envelope may play an important role in removing metals from environments polluted with heavy metals (Cenci et al., 1985). Higher concentrations of metals are toxic because of their ability to cause breakage in DNA (Shapiro and Keasling, 1996). Since metals play an important role in bacterial metabolism and the concentration in natural settings limits growth, bacteria have developed numerous high affinity transporters to import essential nutrients. However at high concentrations many metals ions can precipitate or bind inappropriately to macromolecules leading to toxic effects (Gaballa and Helmann, 2003).

In a study by Pazirandeh (1996), expression of the *Neurospora crassa* metallothionein gene (NCP) in *E. coli* was reported, and this recombinant *E. coli* (NCP) was able to sequester cadmium from solution rapidly and with high selectivity. It has been previously shown that NCP was capable of sequestering more Cd from solutions than control bacteria, which did not contain the metallothionein gene. The results in this study indicate that the NCP has properties that are desirable and necessary for a heavy metal biosorbent. These properties include affinity for a wide range of heavy metals, reusability and durability, it's potential to be used as non-viable biomass, and its ability to be immobilized into a matrix (Pazirandeh, 1996).

Microbes can be used to assay the toxicity and mutagenicity of heavy metals. In this study *E. coli* strains and cadmium were used to some extent to show effects in mutagenesis. The isolation of mutants resistant to specific toxic agents such as Tc and Rif antibiotics was not a very useful genetic approach in this study. It was unfortunate that it cannot be proven that real mutants were created. Instead, the donor was already cadmium sensitive, and the Rif^r donor appeared to be even more sensitive to cadmium and therefore behaved like a mutant. UV-light exposure to wild type (Rif^r recipient), Rif^r donor, Tc^r donor, and suspected mutant, and % survival among above mentioned bacteria confirmed exactly the same behavior of Rif resistant donor and suspected mutant. Growth rates of Rif resistant donor and mutant also supported our theory. Minimal inhibitory Cd concentration of 300µM was established by growing suspected mutants in LB. Cadmium sensitivity of 300µM was then tested on Tc and Rif resistant donor strain and compared with suspected mutant. Absence of growth was observed on all three strains. Therefore it seemed like our donor strain was already a "mutant" that has very high cadmium sensitivity. Further mating of the donor and recipient will be required to identify true mutant. PCR screening of these cadmium sensitive donors will be necessary to assure that the suspected mutants are valid.

In order to identify *E. coli* genes involved with cadmium resistance, wild type *E. coli* was exposed to different concentrations of Cd. Suspected mutant grew in LB with and without 4 mM of Cd and *E .coli* grew only without Cd in LB. At this time it is not known if these *E. coli* cadmium resistant strains are real mutants. They may increase the expression of some protein that allows them to grow at higher concentrations of Cd. Bacteria carrying cadmium resistance mechanisms have a selective advantage for survival in the environment. Unlike mercury and arsenic resistance systems, which are found in all bacteria studied, cadmium resistance seems to have evolved at least three times, having given rise to the ATP-dependant cadmium efflux transporters present in gram positive bacteria, the unrelated chemi-osmotic cation-proton antiporters found in gram negative bacteria, and the metallothionein system used by cyanobacteria (Schirawski et al., 2002).

Bacterial accommodation to moderate concentrations of cadmium was accompanied by transient activation of general stress proteins (Puskarova et al., 2002). Previously identified protein was identified as the product of the *E. coli yodA* ORF. Three of the four crystal forms were obtained in the presence of zinc, nickel and cadmium, suggesting that YodA may be a metal-binding protein. The increased synthesis of YodA protein during cadmium stress was found probably to be a result of transcriptional activation from one single promoter upstream of the structural *yodA* gene. Analysis of a transcriptional gene fusion, P_{yodA}-*lacZ*, demonstrated that basal expression of *yodA* is low during exponential growth, and increased 50-fold by addition of cadmium to growing cells (Puskarova et al., 2002). However, challenging cells with additional metals such as zinc, copper, cobalt and nickel did not increase the level of *yodA* expression. YodA protein was primarily detected in the cytoplasmic fraction after 45 minutes of cadmium exposure. After 150 minutes YodA protein was found in both the cytoplasmic and periplasmic compartments (Puskarova et al., 2002). The function of this protein has been identified only under conditions of bacterial stress (David et al., 2002).

P-type adenosine-triphosphatase (ATPases) is polyprotic membrane protein that catalyzes the ATP-dependent transport of cations across membranes. ATPases transporting cations has been identified in many recent studies. P-type ATPases are large and are in easily polarized form. Their physiological roles are to maintain homeostasis of the essential soft metals such as Cu (I) and to mediate resistance to toxic concentrations of Pb (II), Cd (II), Cu (I), and Ag (I) (Sharma et al., 1999). Putative soft metal P-type ATPases have been found in bacteria and can be further subdivided into two classes. The class of soft metal ATPases that are of interest to us have be shown to transport the divalent soft metals, Zn (II) including ZntA from *E. coli*, and Cd (II) including CadA from *Stahylococcus aureus*. ATPase activity was displayed by purified ZntA and specifically stimulated by Pb (II) first, then Cd (II), Zn (II), and Hg (II). This suggests that the physiological role of ZntA is to mediate resistance of these toxic metals (Sharma et al., 1999). The metal specificity study by Okkeri and Haltia shows the substrate ions Zn^{+2} and Cd^{+2} differ in their relative capability to stimulate the ATPase activity and phosphorylation. In comparison with Zn^{+2}, Cd^{+2} is more effective in prompting phosphorylation and less effective in stimulating ATP hydrolysis. These results may suggest that the enzyme has at least two different metal binding sites with slightly different metal specificities. One that activates the phosphoryl transfer from ATP and another to which the ion to be translocated ion binds (Okkeri and Haltia, 1999).

Zn (II) is a structural component of a large number of proteins in all-living systems and plays an important catalytic role in numerous enzymes. When internal concentrations of Zn (II) are high, a Zn (II) transport system ZnA is switched on. ZntA is a cation-translocating ATPase that exports Cd (II), Pb (II) and Zn (II). Purified ZntA showed the metal dependent ATP hydrolysis activity for Pb (II), Cd (II) and Zn (II) (Binet and Poole, 2000). In a study by Binet and Poole (2000) it was demonstrated that substrates of ZntA encoded exporter Cd (II) and Pb (II) are much more effective inducers of the expressions of this system than the previously considered major inducer Zn (II). From the observation in this study that a ZntA mutant exhibits a higher basal level of ZntA expression than does a wild type it can be concluded that ZntA is functional in metal ion export (Binet and Poole, 2000).

Two *Streptococcus thermophilus* genes *cadC* and *cadA* located on the chromosome of *S.thermophilus* 4134 were shown to comprise a cadmium/zinc cassette. The product of *cadA* is highly similar to P-type cadmium efflux ATPases. Electrophoretic mobility shift assays were used to demonstrate that *cadC* is a DNA binding protein that binds specifically to its own promoter region, and that this *cadC*-DNA interaction is lost in the presence of cadmium (Schirawski et al., 2002).

The metal binding sites in ZntA and CadA are yet to be discovered. It can be hypothesized that certain residues confer specificity for certain metals. Identification of the metal binding sites on ZntA and CadA may lead to understanding the basis of metal recognition and perhaps engineering of the protein to modify metal specificity (Sharma et al., 1999).

Future studies involving the identification of *E. coli* genes that are Cd resistant can provide better insight into Cd resistant mechanisms in bacteria. Additional research is required where two different bacteria should be used as donor and recipient instead of the two different strains of the same bacteria. Further potential improvements may be achieved by modification of the present study procedure for discovering mutants. Characterization of genes for wild type and donor may provide insight into the cellular targets, and the mechanisms of sensitivity to metal exposure. Therefore, it is hoped that the results described in this research thesis will be useful for further studies based on the use of this environmentally important scientific approach.

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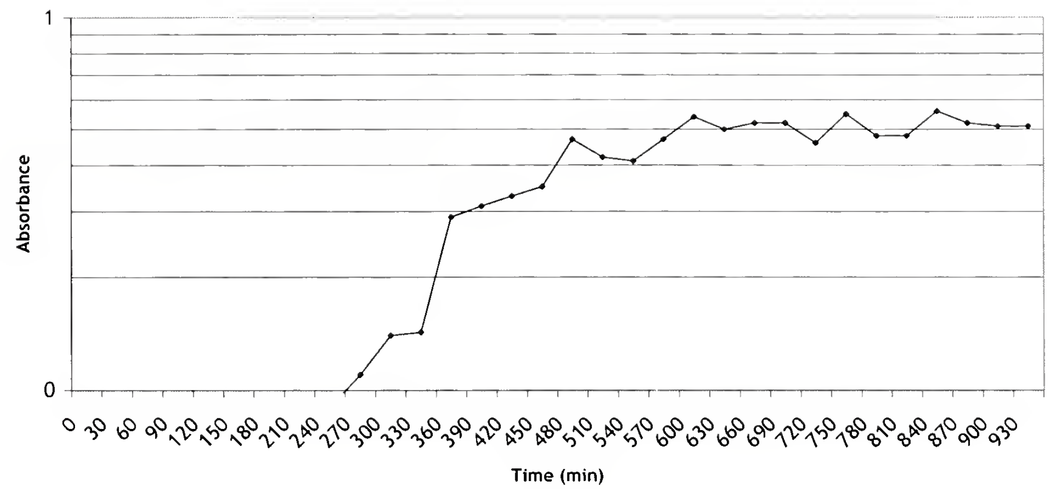
APPENDIX A

All of the doubling times for *E.coli* are presented in APPENDIX A

Data for *E.coli* from absorbance vs. time

Lactose control				
Time	Run1	Run2	Run 3	Average
0	0	0	0	0
30	0	0	0	0
60	0	0	0	0
90	0	0	0	0
120	0	0	0	0
150	0	0	0	0
180	0	0	0	0
210	0	0	0	0
240	0.09	0.11	0.10	0.10
270	0.11	0.10	0.12	0.11
300	0.14	0.12	0.14	0.13
330	0.14	0.11	0.15	0.13
360	0.29	0.22	0.29	0.27
390	0.31	0.26	0.30	0.29
420	0.33	0.29	0.31	0.31
450	0.35	0.40	0.34	0.36
480	0.47	0.39	0.45	0.44
510	0.42	0.38	0.46	0.42
540	0.41	0.42	0.47	0.43
570	0.47	0.49	0.47	0.48
600	0.54	0.41	0.54	0.50
630	0.50	0.37	0.54	0.47
660	0.52	0.52	0.55	0.53
690	0.52	0.41	0.55	0.49
720	0.46	0.47	0.54	0.49
750	0.55	0.52	0.55	0.54
780	0.48	0.54	0.53	0.52
810	0.48	0.54	0.54	0.52
840	0.56	0.54	0.54	0.55
870	0.52	0.54	0.53	0.53
900	0.51	0.50	0.52	0.51
930	0.51	0.52	0.52	0.52

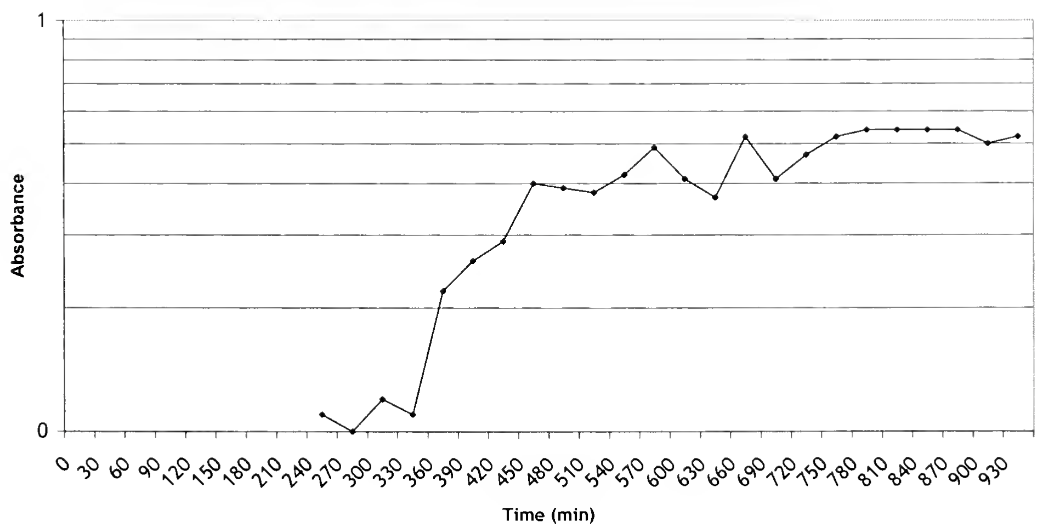
Figure A-1. Lactose Control (first set of data)



Absorbance increases from 0.15 to 0.29

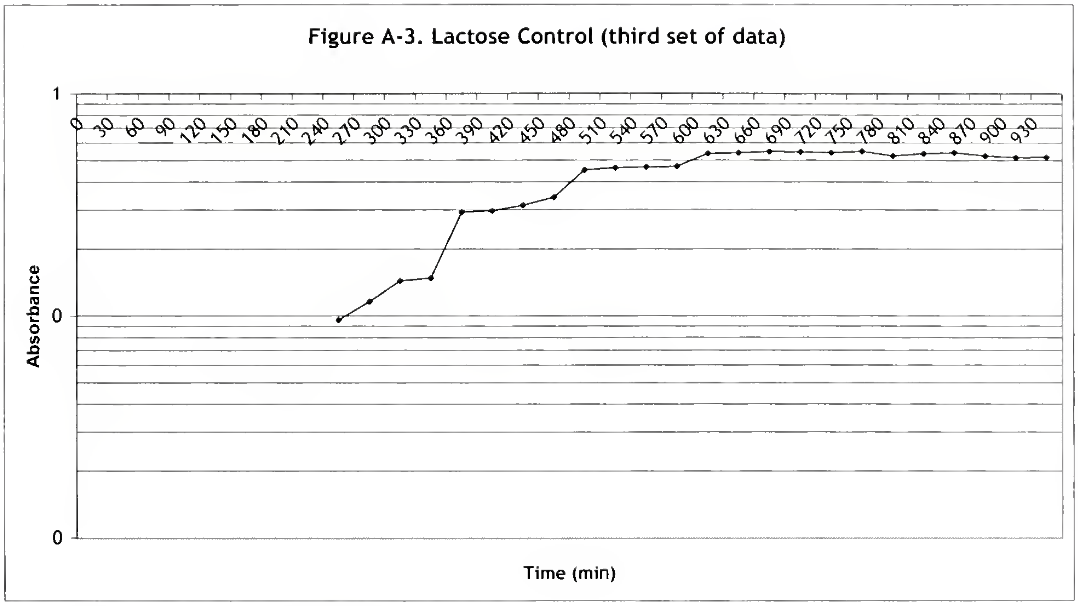
Actual grow rate (360 min -330 min) = 30 min

Figure A-2. Lactose Control (second set of data)



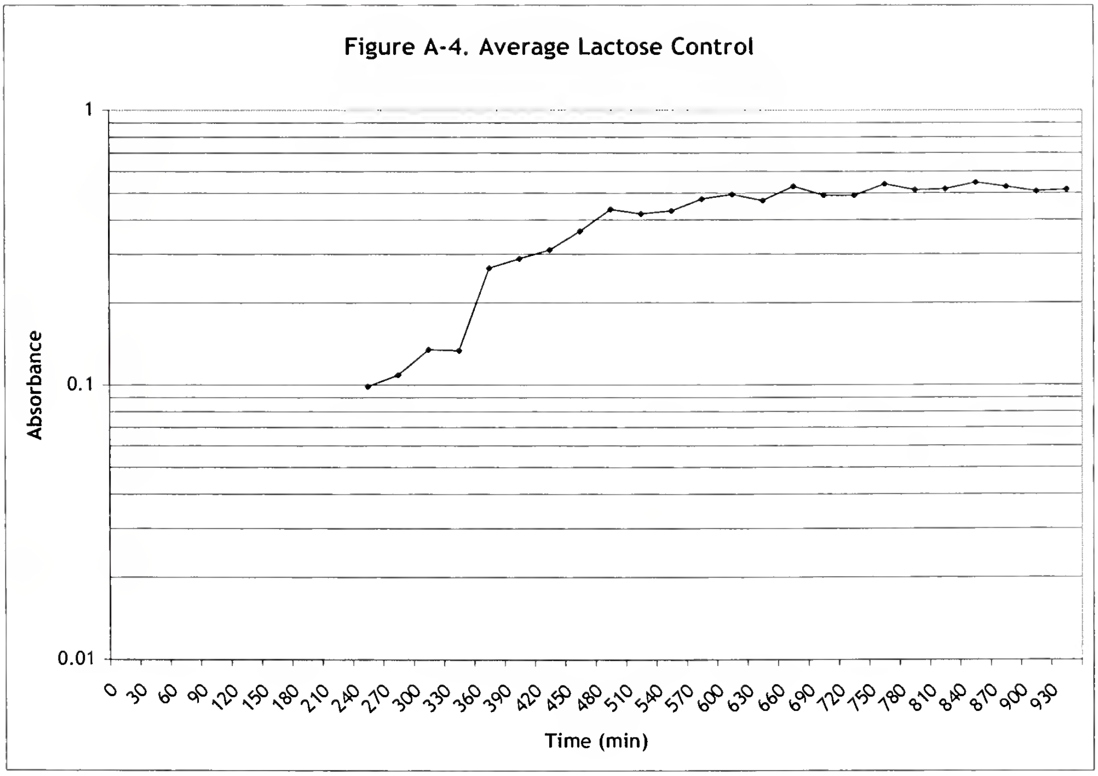
Absorbance increases from 0.11 to 0.22

Actual grow rate (360 min -330 min) = 30 min



Absorbance increases from 0.148 to 0.297

Actual grow rate (390 min -330 min) = 60 min



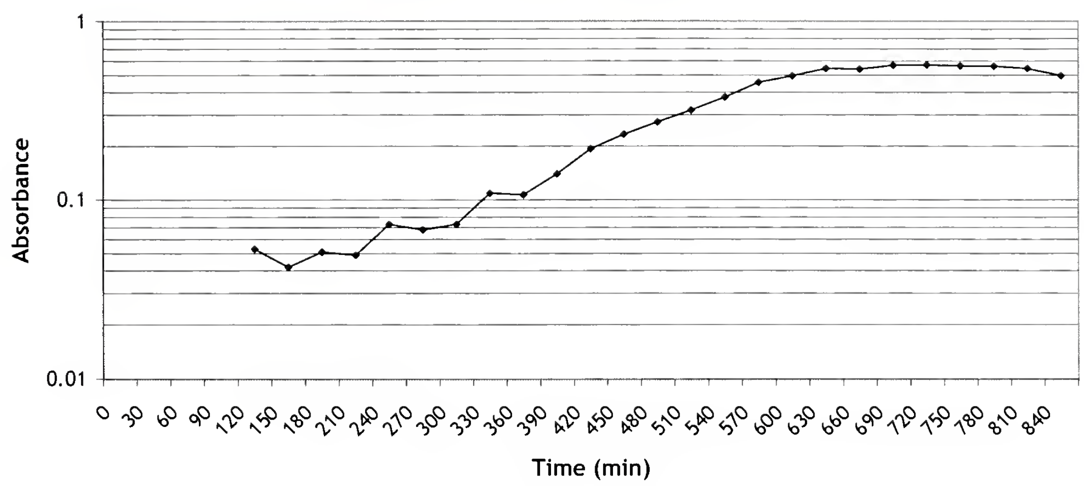
Absorbance increases from 0.134 to 0.268

Actual grow rate (360 min -330 min) = 30 min

Data for *E.coli* from absorbance vs. time

Lactose only Cadmium at $1*10^{-3}$ M					
Time (min)	Run 1	Run 2	Run 3	Run 4	Average
0	0	0	0	0	0
30	0	0	0	0	0
60	0	0	0	0	0
90	0	0	0	0	0
120	0.05	0.06	0.05	0.05	0.05
150	0.04	0.06	0.06	0.04	0.05
180	0.05	0.06	0.05	0.05	0.05
210	0.05	0.08	0.06	0.07	0.06
240	0.07	0.09	0.08	0.07	0.08
270	0.07	0.07	0.06	0.07	0.07
300	0.07	0.08	0.08	0.08	0.08
330	0.11	0.10	0.07	0.06	0.08
360	0.11	0.11	0.07	0.09	0.09
390	0.14	0.15	0.08	0.06	0.11
420	0.19	0.16	0.07	0.09	0.13
450	0.23	0.21	0.09	0.07	0.15
480	0.27	0.25	0.09	0.11	0.18
510	0.32	0.28	0.13	0.11	0.21
540	0.38	0.32	0.15	0.12	0.24
570	0.46	0.38	0.20	0.16	0.30
600	0.50	0.43	0.23	0.19	0.34
630	0.54	0.43	0.24	0.25	0.37
660	0.54	0.43	0.32	0.27	0.39
690	0.57	0.45	0.37	0.29	0.42
720	0.57	0.47	0.42	0.35	0.45
750	0.56	0.48	0.47	0.38	0.47
780	0.56	0.47	0.50	0.41	0.49
810	0.55	0.47	0.52	0.44	0.49
840	0.50	0.46	0.53	0.46	0.49

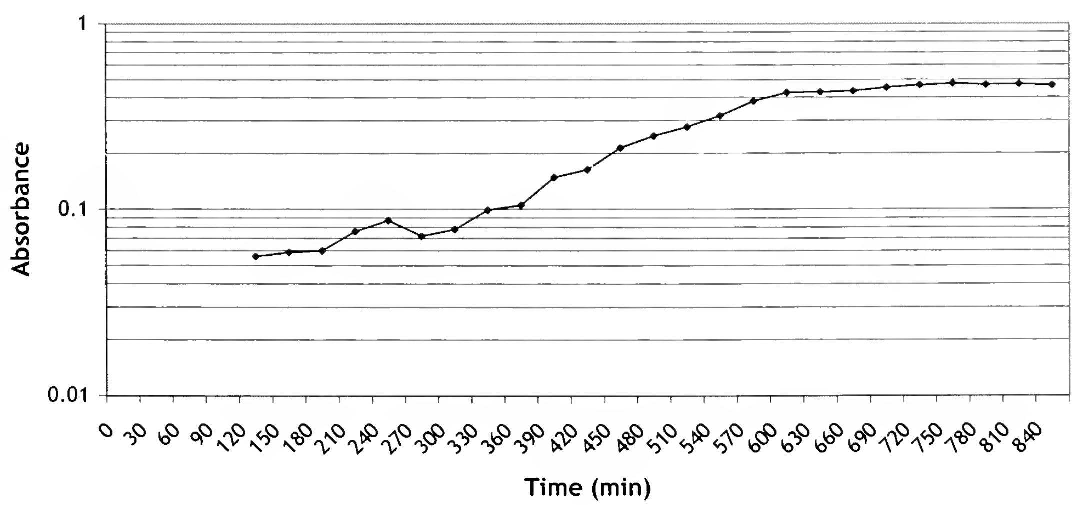
Figure A-5. Lactose With Cd 1



Absorbance increases from 0.273 to 0.544

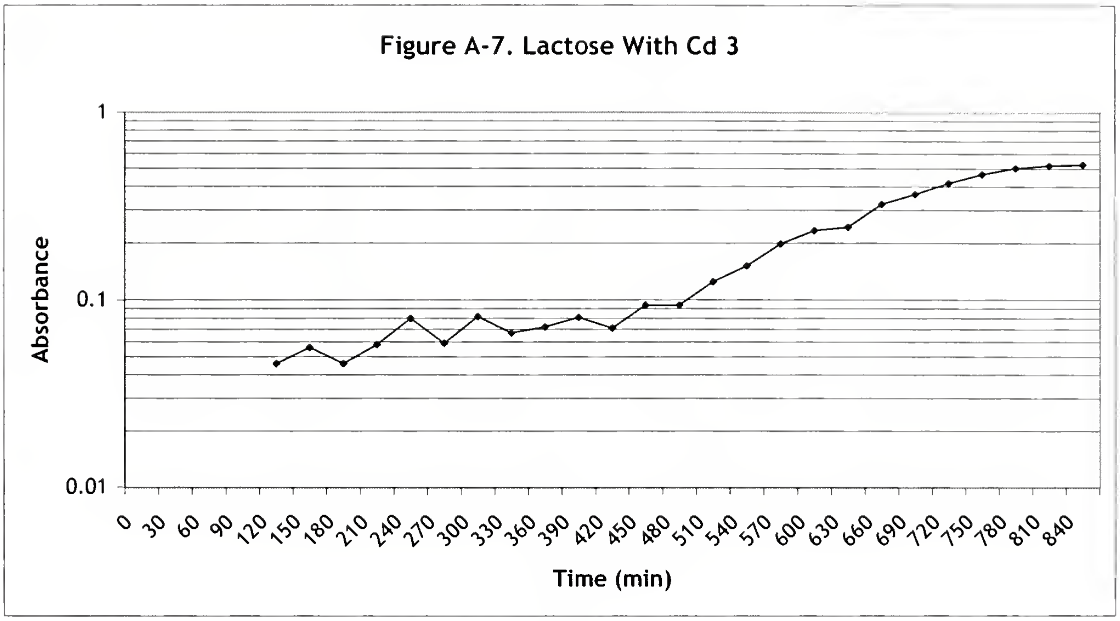
Actual growth rate = (630 min -480 min) = 150 min

Figure A-6. Lactose With Cd 2



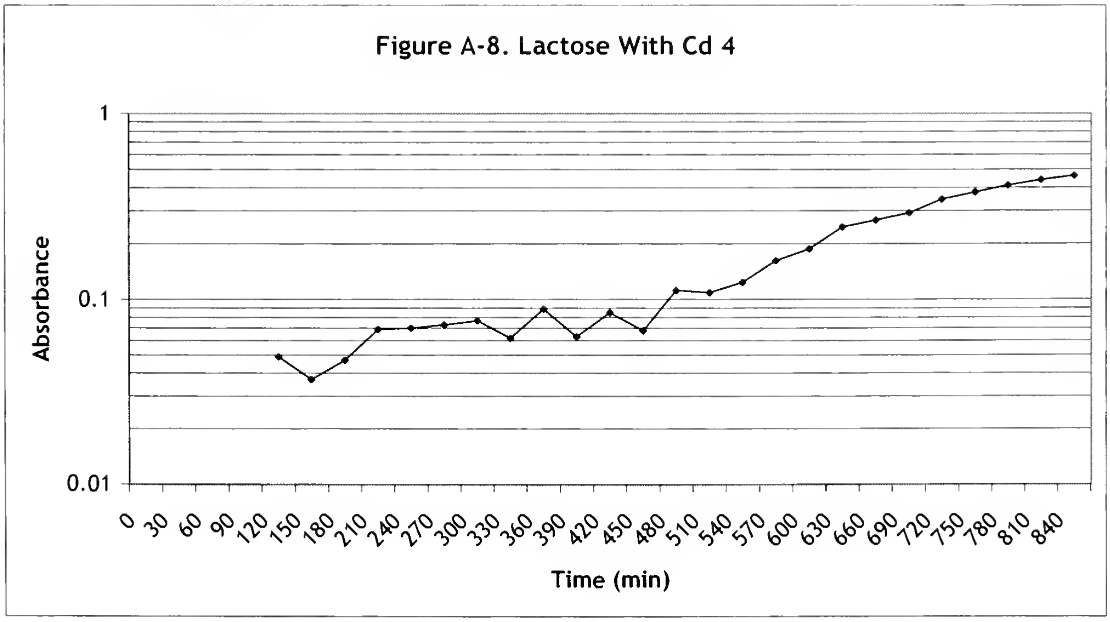
Absorbance increases from 0.214 to 0.426

Actual growth rate = (600 min -450 min) = 150 min



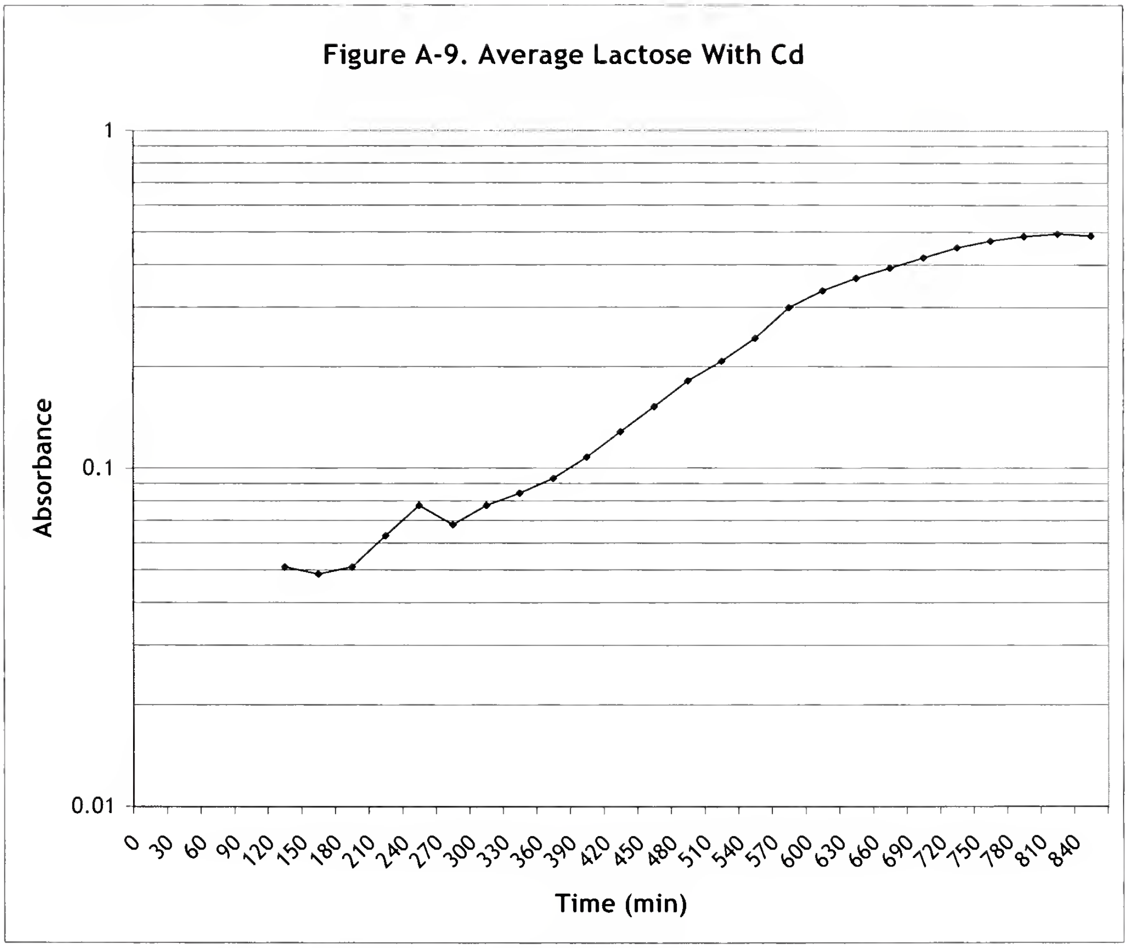
Absorbance increases from 0.234 to 0.467

Actual growth rate = (750 min -600 min) = 150 min



Absorbance increases from 0.187 to 0.378

Actual growth rate = (750 min -600 min) = 150 min

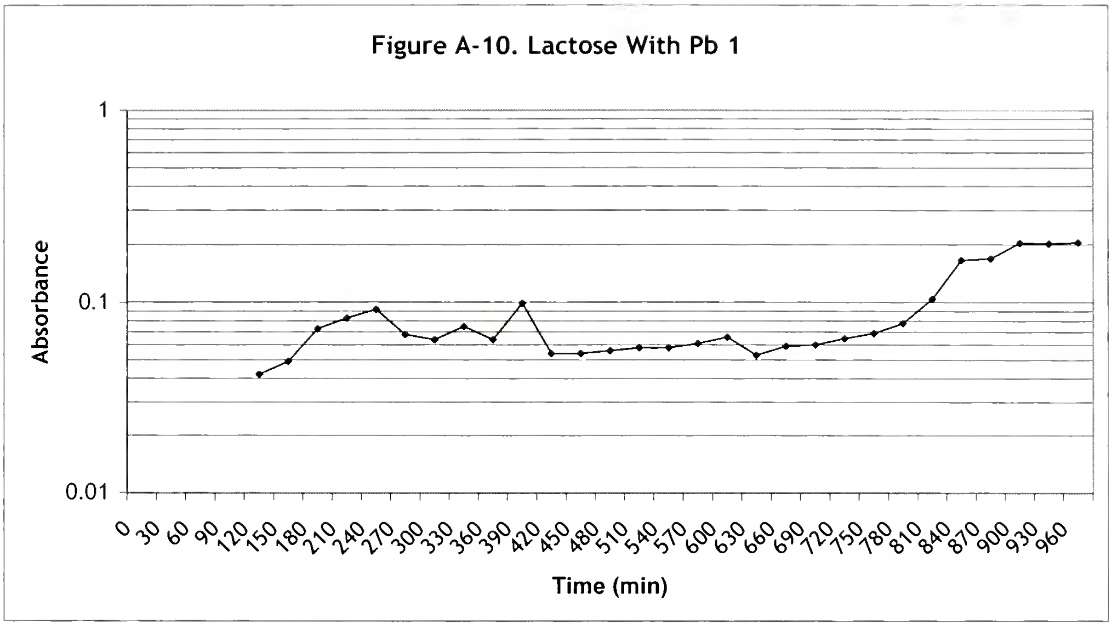


Absorbance increases from 0.182 to 0.366

Actual growth rate = (630 min -480 min) = 150 min

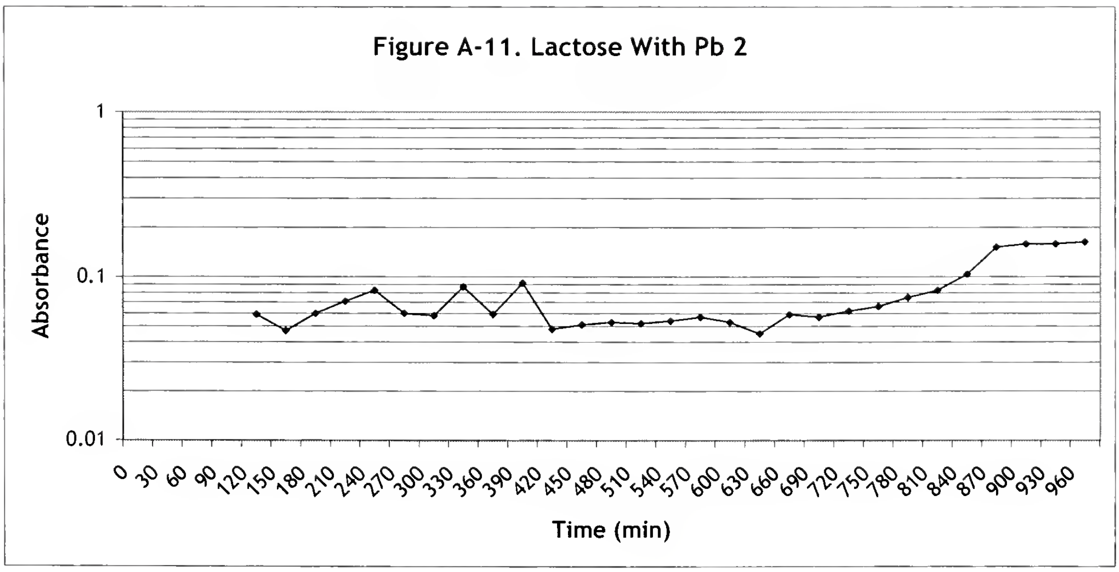
Data for *E.coli* from absorbance vs. time

Lactose only Lead at $1*10^{-3}$ M				
Time (min)	Run 1	Run 2	Run 3	Average
0	0	0	0	0
30	0	0	0	0
60	0	0	0	0
90	0	0	0	0
120	0.042	0.059	0.062	0.054
150	0.049	0.047	0.041	0.046
180	0.073	0.060	0.054	0.062
210	0.083	0.071	0.076	0.077
240	0.092	0.083	0.088	0.088
270	0.068	0.060	0.072	0.067
300	0.064	0.058	0.055	0.059
330	0.075	0.087	0.070	0.077
360	0.064	0.059	0.065	0.063
390	0.099	0.092	0.095	0.095
420	0.054	0.048	0.040	0.047
450	0.054	0.051	0.054	0.053
480	0.056	0.053	0.057	0.055
510	0.058	0.052	0.058	0.056
540	0.058	0.054	0.055	0.056
570	0.061	0.057	0.060	0.059
600	0.066	0.053	0.060	0.060
630	0.053	0.045	0.051	0.050
660	0.059	0.059	0.066	0.061
690	0.060	0.057	0.065	0.061
720	0.065	0.062	0.076	0.068
750	0.069	0.066	0.087	0.074
780	0.078	0.075	0.097	0.083
810	0.104	0.083	0.121	0.103
840	0.166	0.104	0.171	0.147
870	0.169	0.152	0.188	0.170
900	0.204	0.159	0.198	0.187
930	0.202	0.159	0.203	0.188
960	0.205	0.163	0.205	0.191



Absorbance increases from 0.104 to 0.204

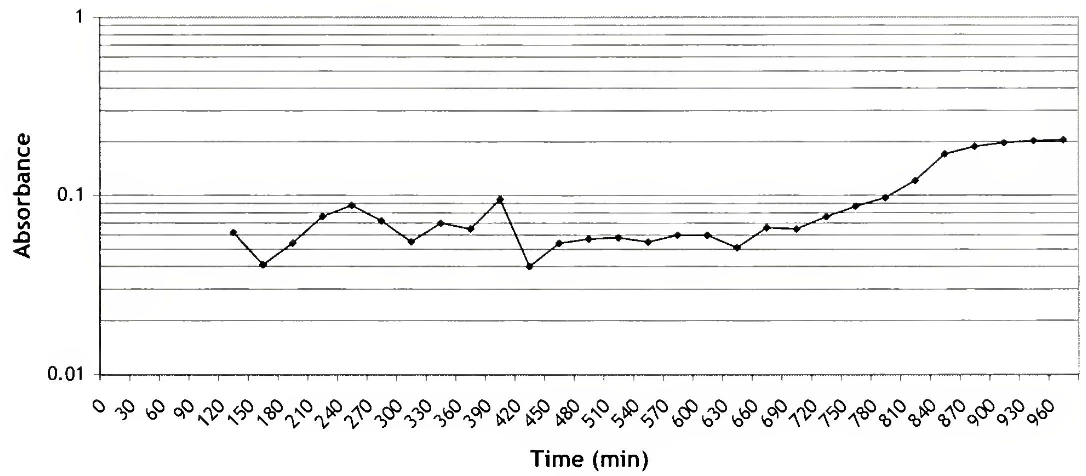
Actual growth rate = (900 min -810 min) = 90 min



Absorbance increases from 0.075 to 0.152

Actual growth rate = (870 min -780 min) = 90 min

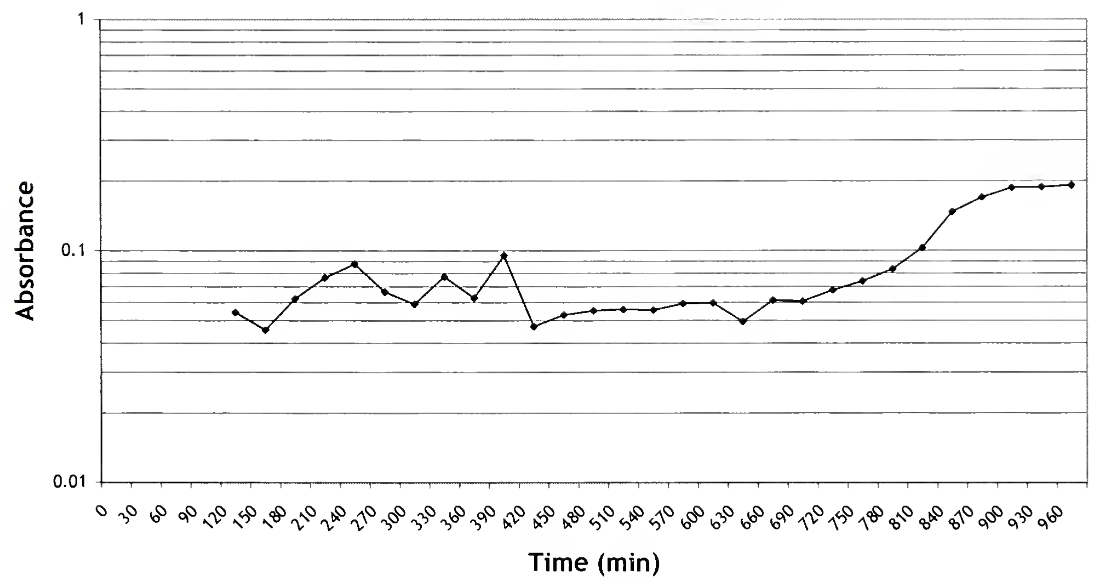
Figure A-12. Lactose With Pb 3



Absorbance increases from 0.087 to 0.171

Actual growth rate = (840 min -750 min) = 90 min

Figure A-13. Average Lactose With Pb



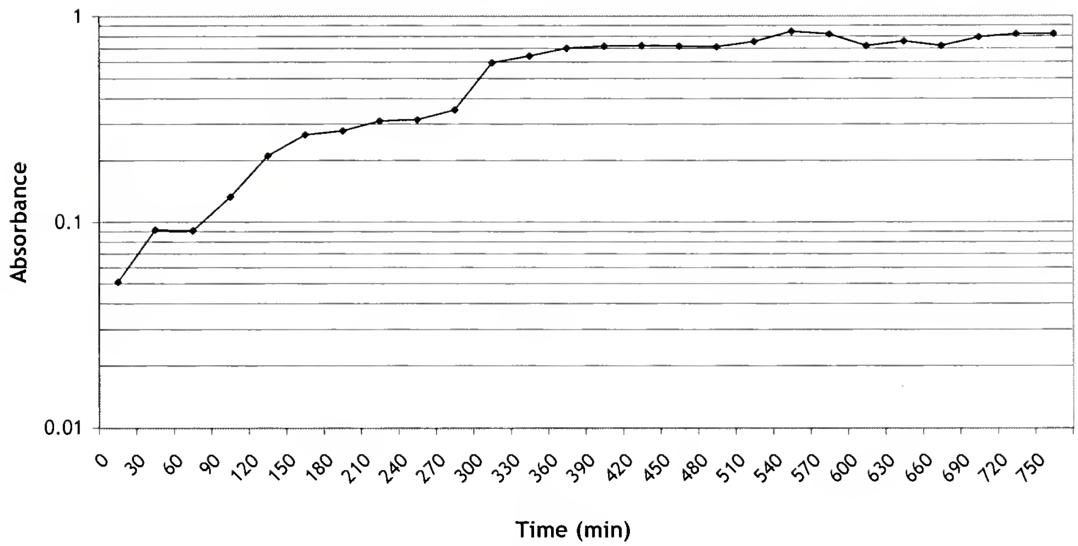
Absorbance increases from 0.074 to 0.147

Actual growth rate = (840 min -750 min) = 90 min

Data for *E.coli* from absorbance vs. time in M9 media

Glucose control				
Time(min)	Run1	Run2	Run3	Average
0	0	0	0	0
0	0.051	0.049	0.051	0.050
30	0.092	0.083	0.089	0.088
60	0.091	0.085	0.09	0.088
90	0.133	0.117	0.129	0.125
120	0.211	0.216	0.213	0.214
150	0.267	0.234	0.259	0.251
180	0.278	0.278	0.272	0.278
210	0.311	0.290	0.308	0.301
240	0.316	0.556	0.411	0.436
270	0.352	0.544	0.482	0.448
300	0.596	0.646	0.546	0.621
330	0.644	0.698	0.612	0.671
360	0.702	0.726	0.698	0.714
390	0.718	0.760	0.712	0.739
420	0.722	0.786	0.736	0.754
450	0.716	0.830	0.758	0.773
480	0.712	0.845	0.763	0.779
510	0.754	0.830	0.782	0.792
540	0.845	0.870	0.843	0.858
570	0.821	0.845	0.841	0.833
600	0.720	0.840	0.836	0.780
630	0.758	0.845	0.841	0.802
660	0.720	0.845	0.838	0.783
690	0.792	0.865	0.835	0.829
720	0.820	0.840	0.838	0.830
750	0.820	0.845	0.837	0.833

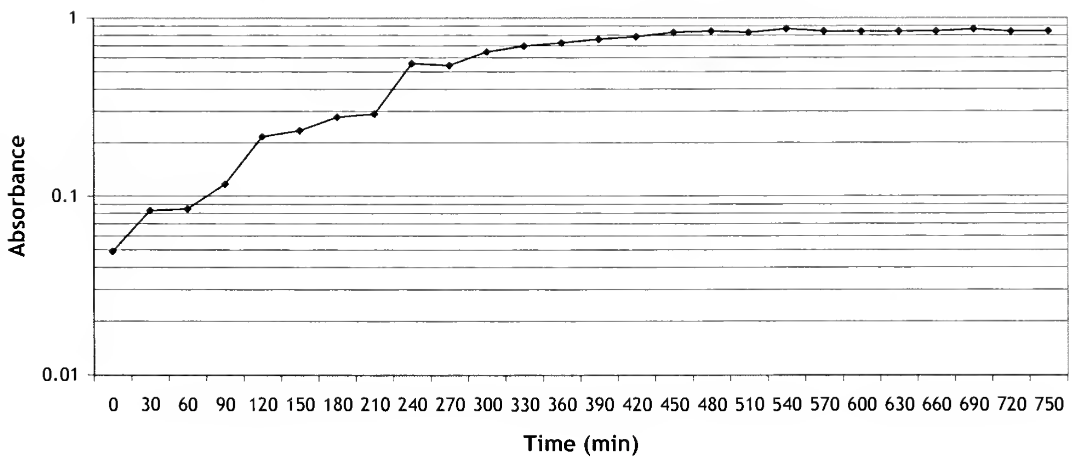
Figure A-14. Glucose Control (first set of data)



Absorbance increases from 0.133 to 0.267

Actual growth rate = (150 min -90 min) = 60 min

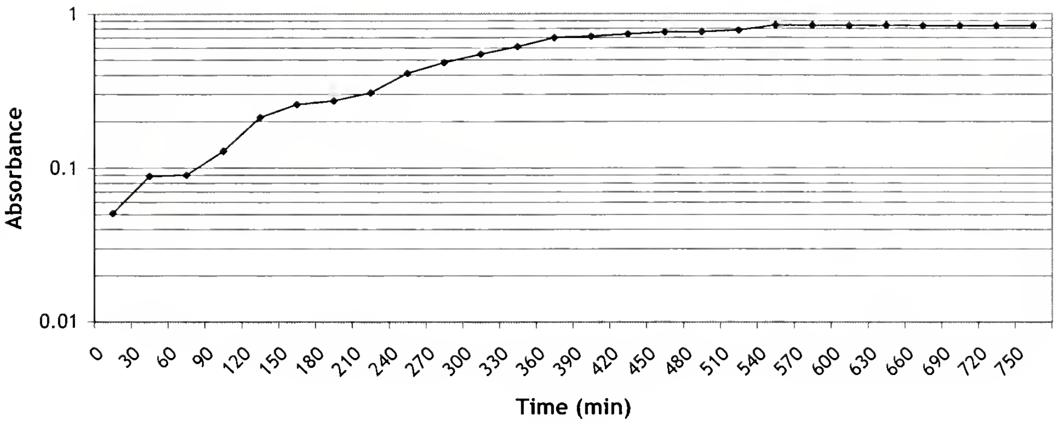
Figure A-15. Glucose Control (second set of data)



Absorbance increases from 0.278 to 0.556

Actual growth rate = (240 min -180 min) = 60 min

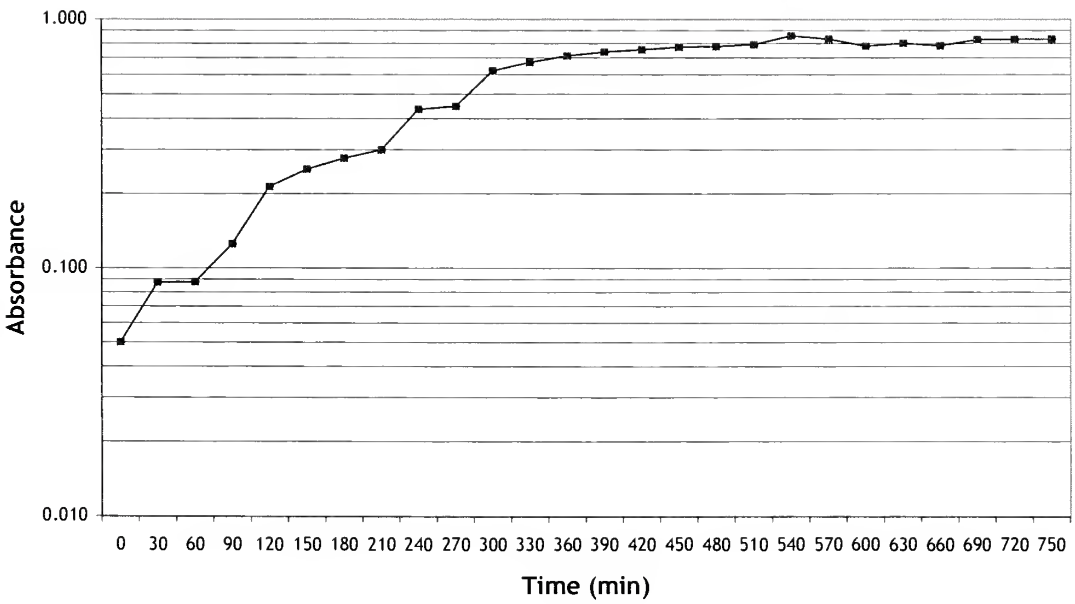
Figure A-16. Glucose Control (third set of data)



Absorbance increases from 0.129 to 0.259

Actual growth rate = **(150 min -90 min) = 60 min**

Figure A-17. Average Glucose Control



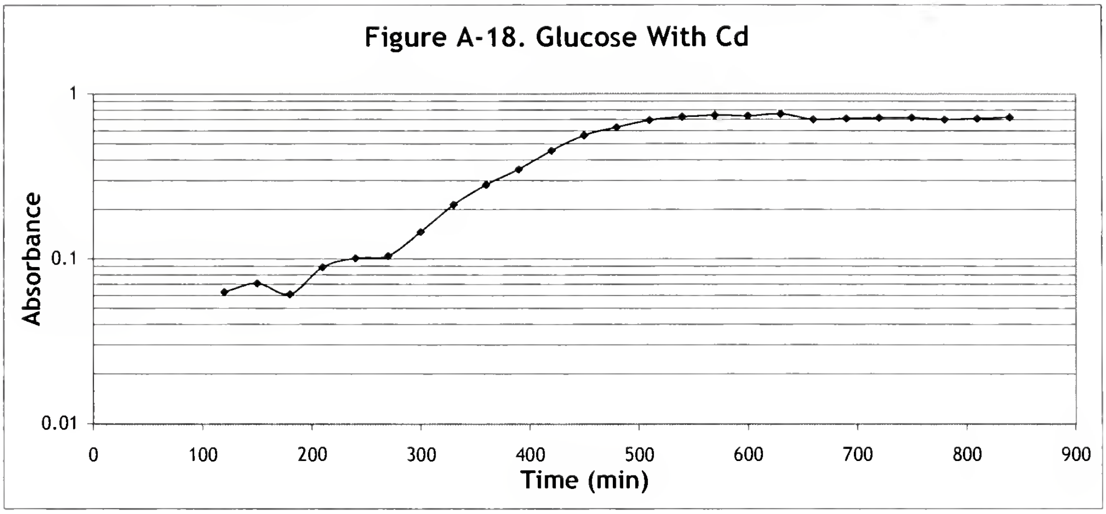
Absorbance increases from 0.125 to .0.251

Actual growth rate = **(150 min -90 min) = 60 min**

Data for *E.coli* from absorbance vs. time in M9 media

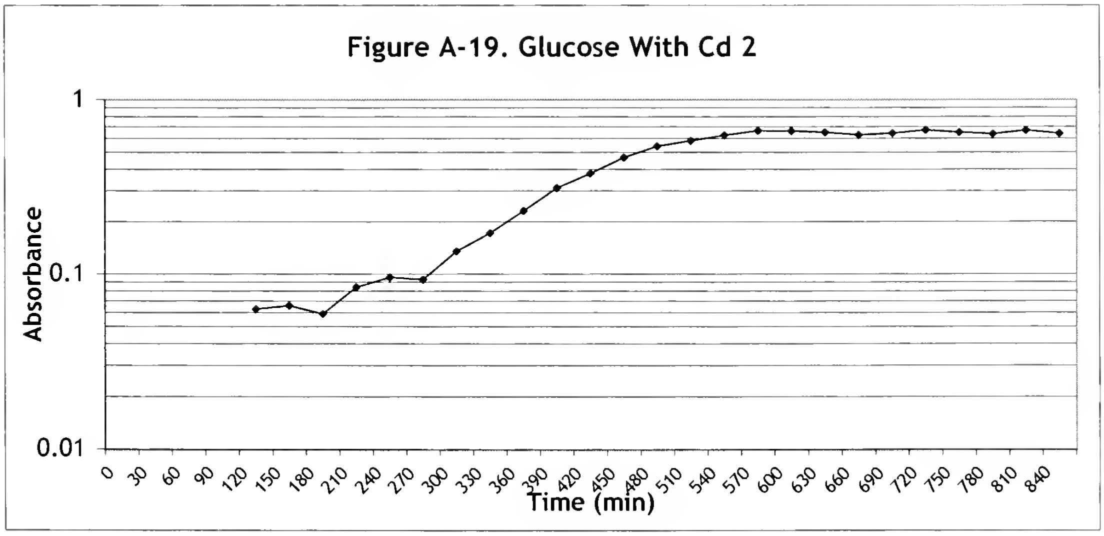
Glucose and Cd at $1*10^{-3}M$

Time (min)	Run 1	Run 2	Run 3	Run 4	Average
0	0	0	0	0	0
30	0	0	0	0	0
60	0	0	0	0	0
90	0	0	0	0	0
120	0.063	0.063	0.036	0.075	0.059
150	0.071	0.066	0.079	0.079	0.074
180	0.061	0.059	0.063	0.095	0.070
210	0.089	0.084	0.075	0.083	0.083
240	0.101	0.096	0.086	0.094	0.094
270	0.104	0.093	0.082	0.094	0.093
300	0.146	0.135	0.089	0.120	0.123
330	0.214	0.172	0.136	0.159	0.170
360	0.283	0.231	0.169	0.221	0.226
390	0.351	0.312	0.275	0.291	0.307
420	0.456	0.378	0.280	0.340	0.364
450	0.562	0.468	0.365	0.400	0.449
480	0.628	0.542	0.424	0.474	0.517
510	0.696	0.584	0.466	0.504	0.563
540	0.728	0.628	0.549	0.546	0.613
570	0.742	0.666	0.572	0.580	0.640
600	0.736	0.662	0.600	0.575	0.643
630	0.756	0.650	0.606	0.572	0.646
660	0.702	0.630	0.584	0.562	0.620
690	0.712	0.644	0.576	0.556	0.622
720	0.718	0.672	0.564	0.520	0.619
750	0.720	0.652	0.560	0.560	0.623
780	0.702	0.638	0.554	0.565	0.615
810	0.712	0.668	0.550	0.543	0.618
840	0.722	0.640	0.546	0.546	0.614



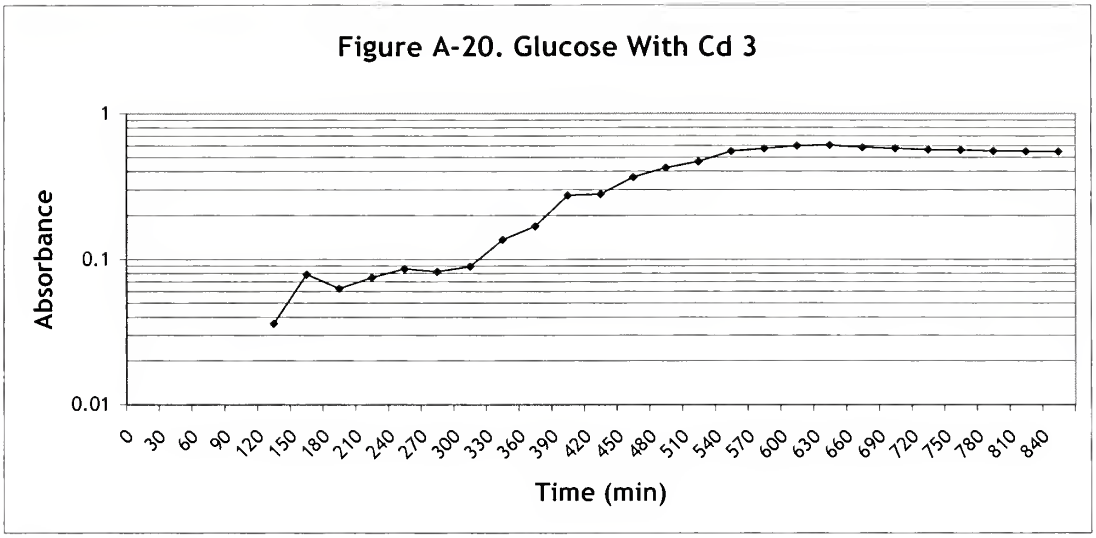
Absorbance increases from 0.365 to 0.728

Actual growth rate = **(540 min -390 min) = 150 min**



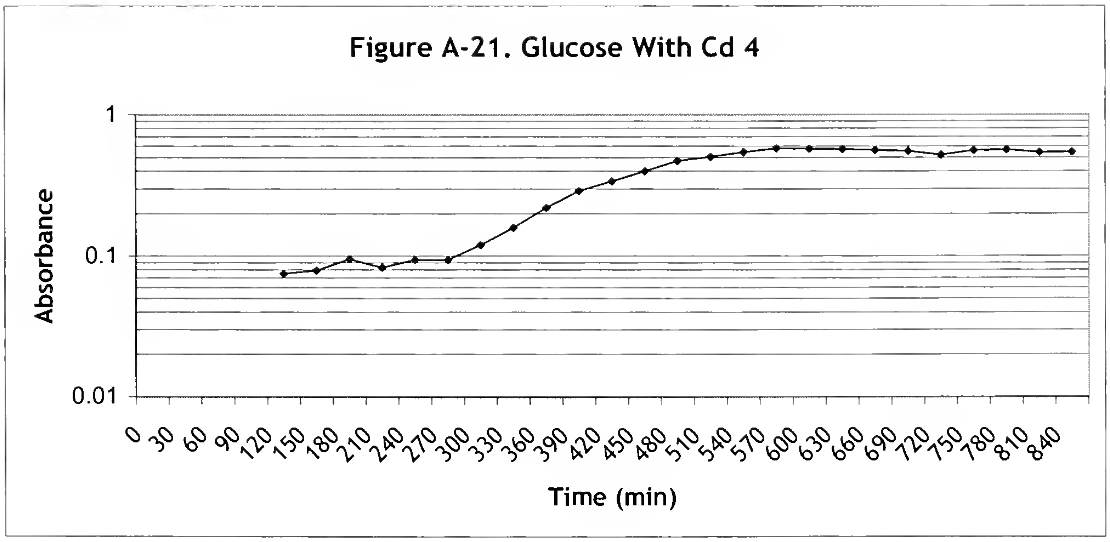
Absorbance increases from 0.312 to 0.628

Actual growth rate = **(540 min -390 min) = 150 min**



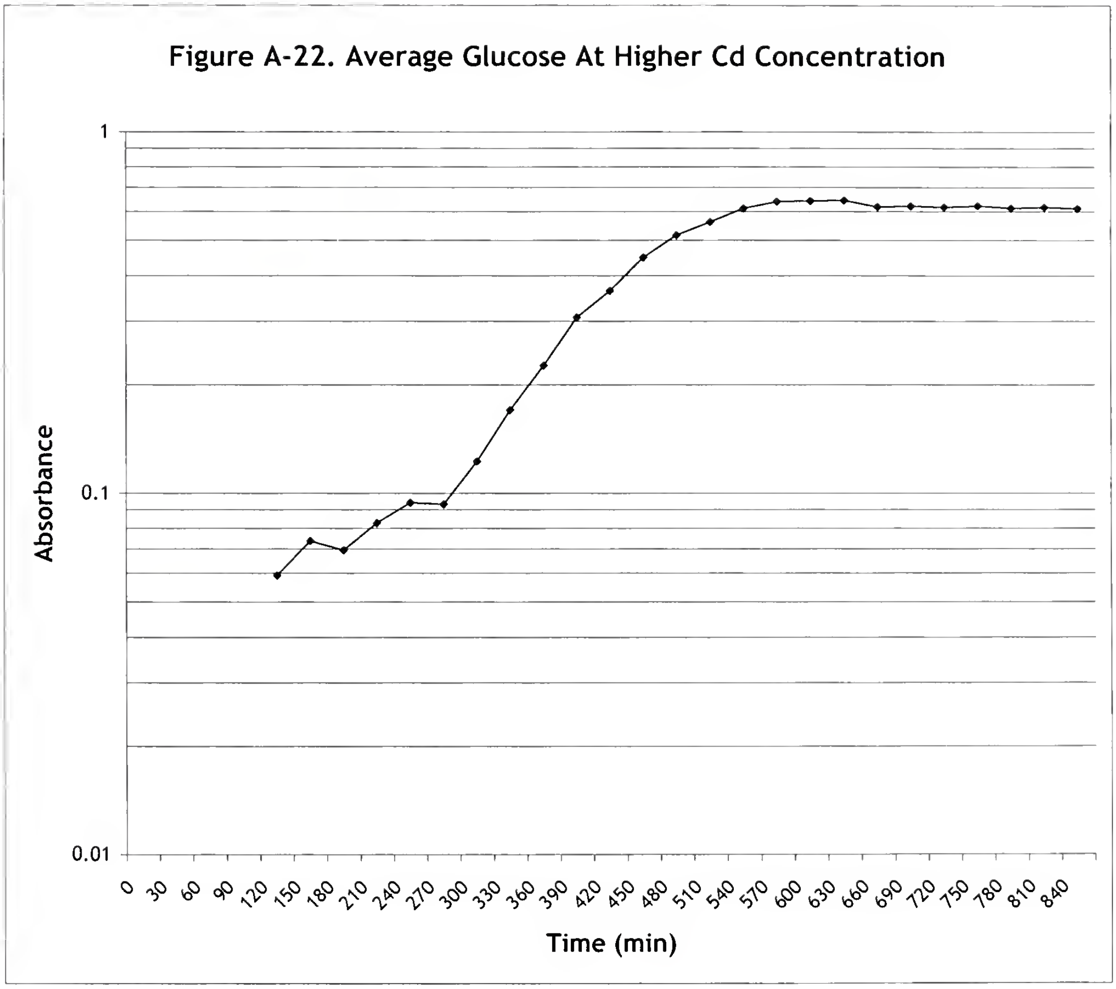
Absorbance increases from 0.275 to 0.549

Actual growth rate = (540 min -390 min) = 150 min



Absorbance increases from 0.291 to 0.580

Actual growth rate = (540 min -390 min) = 150 min



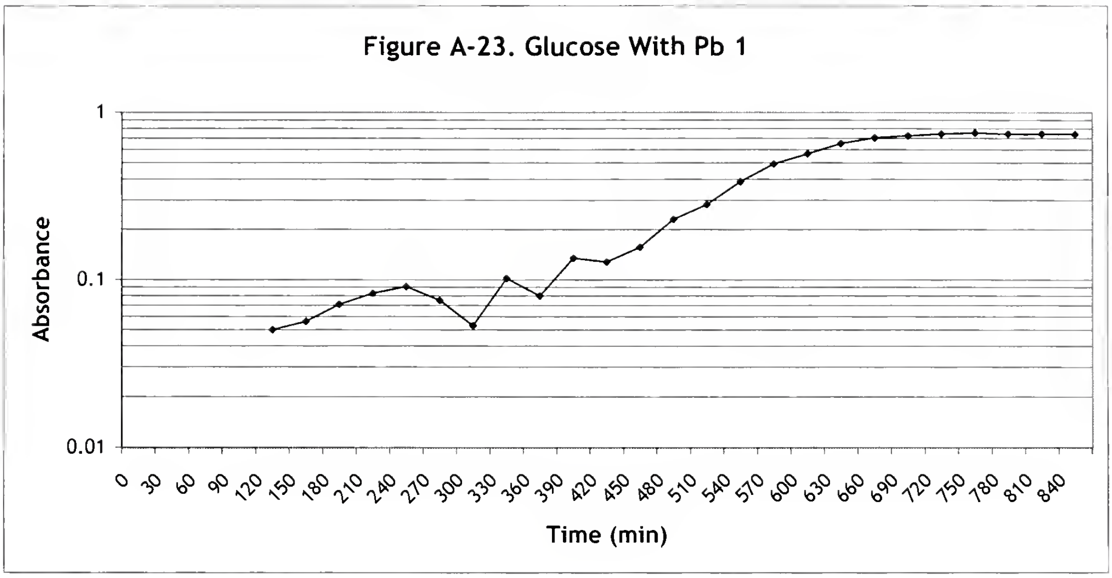
Absorbance increases from 0.307 to 0.613

Actual growth rate = (540 min -390 min) = 150 min

Data for *E.coli* from absorbance vs. time in M9 media

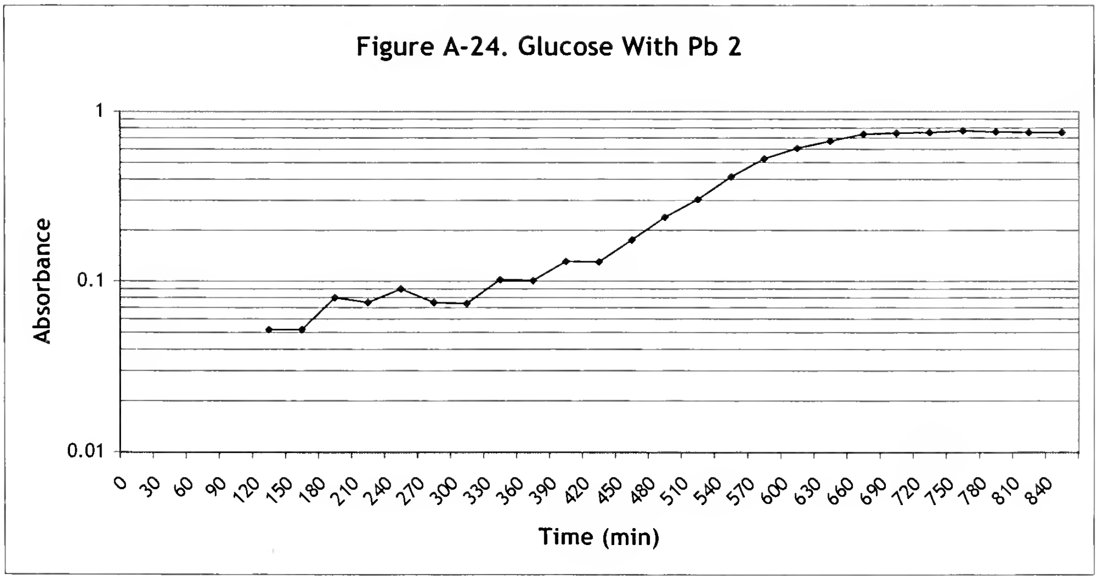
Glucose and Pb at $1*10^{-3}$ M

Time (min)	Run 1	Run 2	Run 3	Run 4	Average
0	0	0	0	0	0
30	0	0	0	0	0
60	0	0	0	0	0
90	0	0	0	0	0
120	0.050	0.052	0.048	0.069	0.055
150	0.056	0.052	0.050	0.067	0.056
180	0.071	0.080	0.073	0.087	0.078
210	0.083	0.075	0.092	0.093	0.086
240	0.091	0.090	0.099	0.097	0.094
270	0.075	0.075	0.079	0.075	0.076
300	0.053	0.074	0.074	0.076	0.069
330	0.102	0.102	0.091	0.092	0.097
360	0.080	0.101	0.087	0.066	0.084
390	0.135	0.131	0.130	0.143	0.135
420	0.128	0.130	0.109	0.126	0.123
450	0.157	0.175	0.121	0.163	0.154
480	0.231	0.239	0.184	0.215	0.217
510	0.283	0.303	0.272	0.290	0.287
540	0.388	0.414	0.325	0.404	0.383
570	0.494	0.528	0.440	0.516	0.495
600	0.568	0.608	0.538	0.584	0.575
630	0.652	0.670	0.648	0.682	0.663
660	0.708	0.738	0.694	0.726	0.717
690	0.728	0.748	0.722	0.744	0.736
720	0.746	0.754	0.742	0.754	0.749
750	0.756	0.770	0.750	0.756	0.758
780	0.748	0.760	0.758	0.754	0.755
810	0.748	0.754	0.754	0.754	0.753
840	0.744	0.752	0.746	0.744	0.747



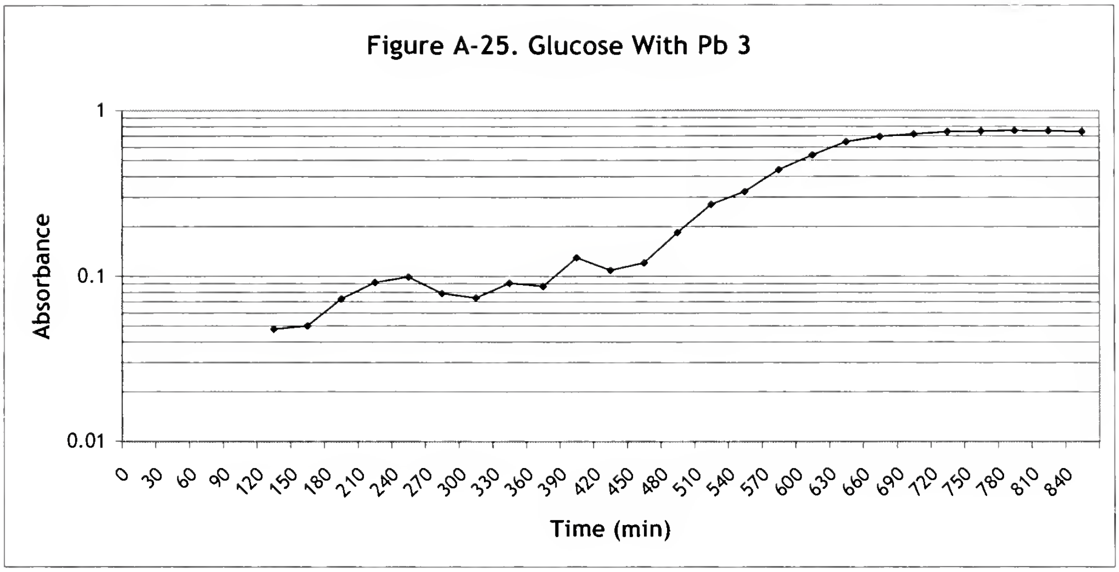
Absorbance increases from 0.283 to 0.568

Actual growth rate = (600 min -510 min) = 90 min



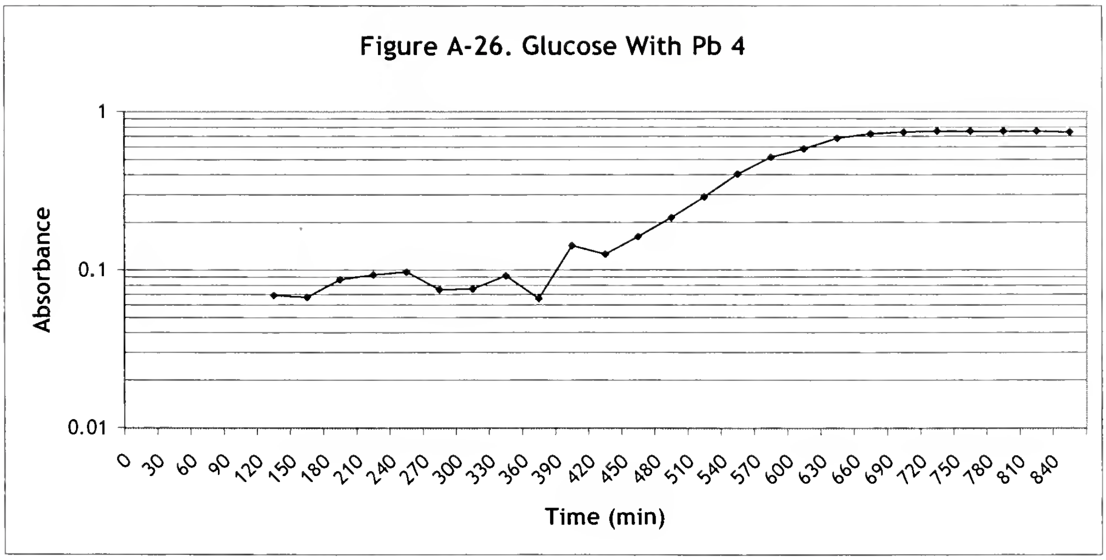
Absorbance increases from 0.303 to 0.608

Actual growth rate = (600 min -510 min) = 90 min



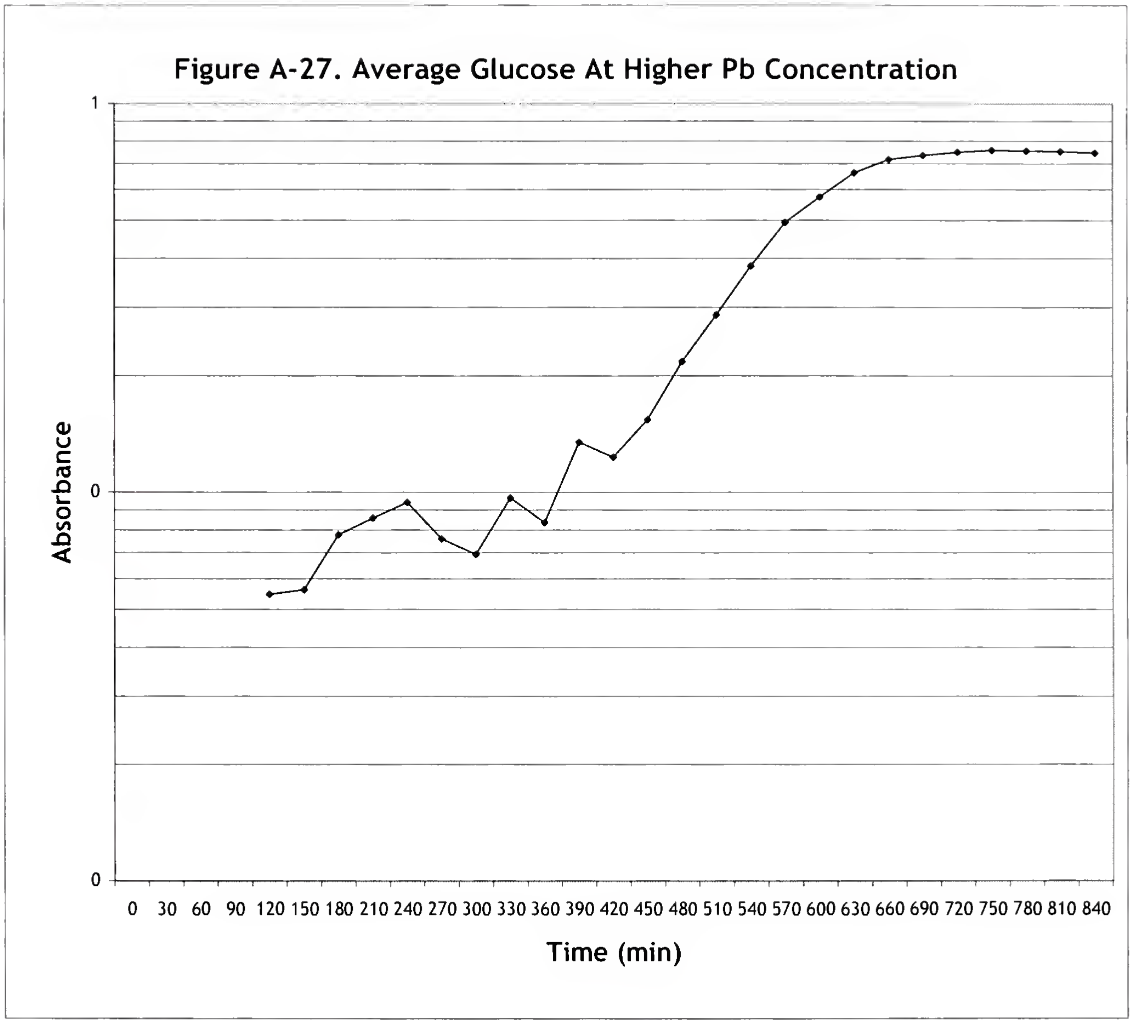
Absorbance increases from 0.325 to 0.648

Actual growth rate = (630 min -540 min) = 90 min



Absorbance increases from 0.290 to 0.584

Actual growth rate = (600 min -510 min) = 90 min



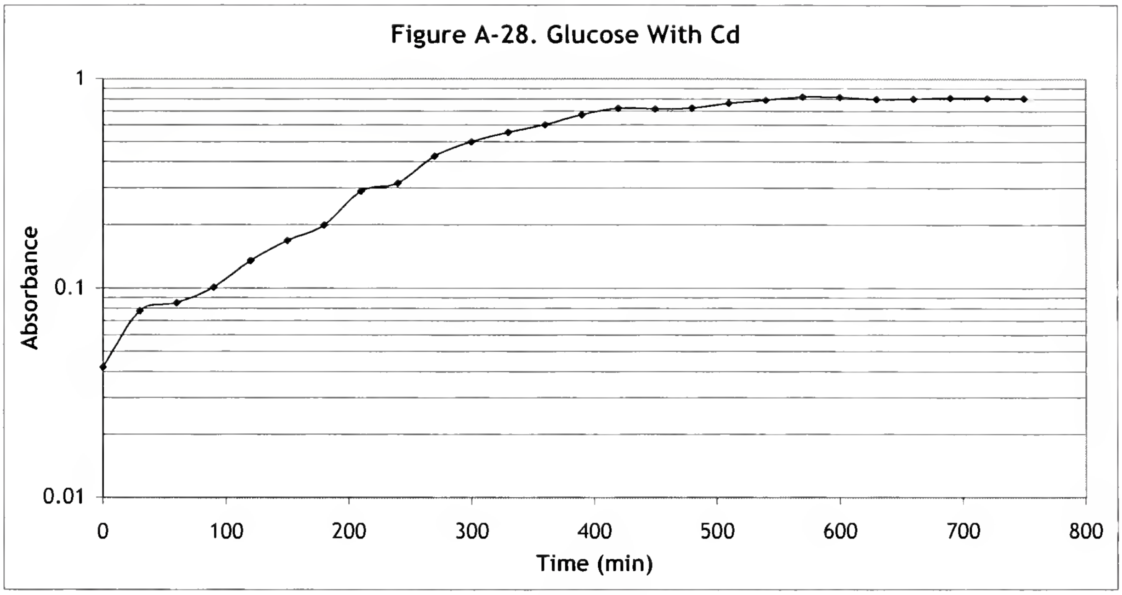
Absorbance increases from 0.287 to 0.575

Actual growth rate = (600 min -510 min) = 90 min

Data for *E.coli* from absorbance vs. time in M9 media

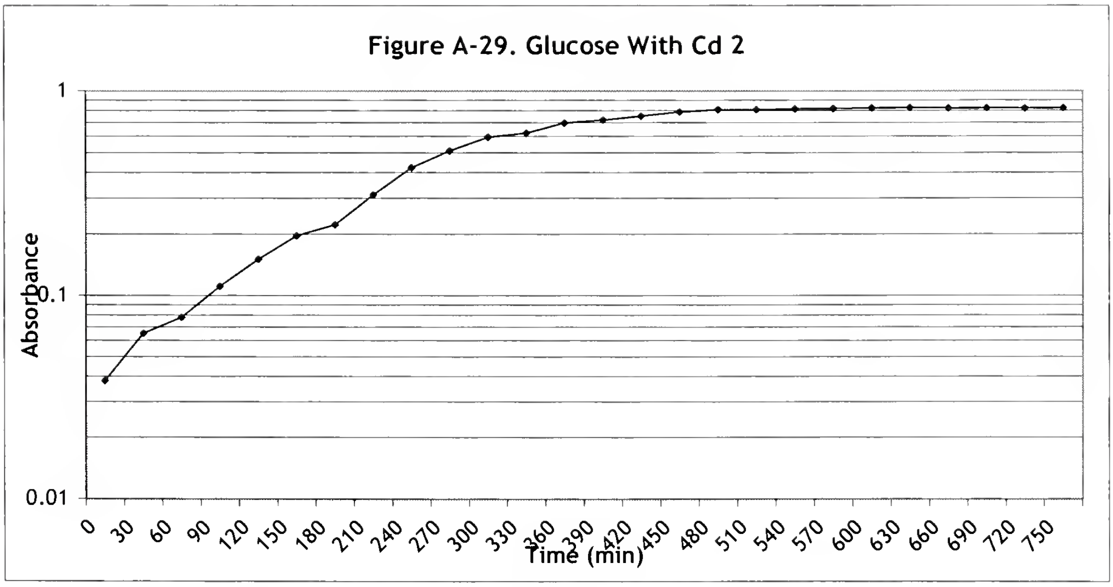
Glucose and Cd at $1*10^{-5}$M

Time(min)	Run1	Run2	Run3	Average
0	0	0	0	0
0	0.042	0.038	0.041	0.040
30	0.078	0.065	0.072	0.072
60	0.085	0.078	0.082	0.082
90	0.101	0.111	0.108	0.107
120	0.135	0.151	0.142	0.143
150	0.168	0.197	0.183	0.183
180	0.199	0.223	0.217	0.213
210	0.289	0.311	0.302	0.301
240	0.316	0.423	0.374	0.371
270	0.426	0.511	0.464	0.467
300	0.499	0.594	0.537	0.543
330	0.553	0.623	0.583	0.586
360	0.602	0.698	0.652	0.651
390	0.672	0.720	0.691	0.694
420	0.722	0.754	0.733	0.736
450	0.716	0.788	0.754	0.753
480	0.725	0.811	0.769	0.768
510	0.763	0.810	0.785	0.786
540	0.792	0.815	0.801	0.803
570	0.821	0.819	0.812	0.817
600	0.816	0.826	0.818	0.820
630	0.799	0.828	0.821	0.816
660	0.802	0.824	0.819	0.815
690	0.812	0.826	0.823	0.820
720	0.808	0.822	0.825	0.818
750	0.806	0.824	0.824	0.818



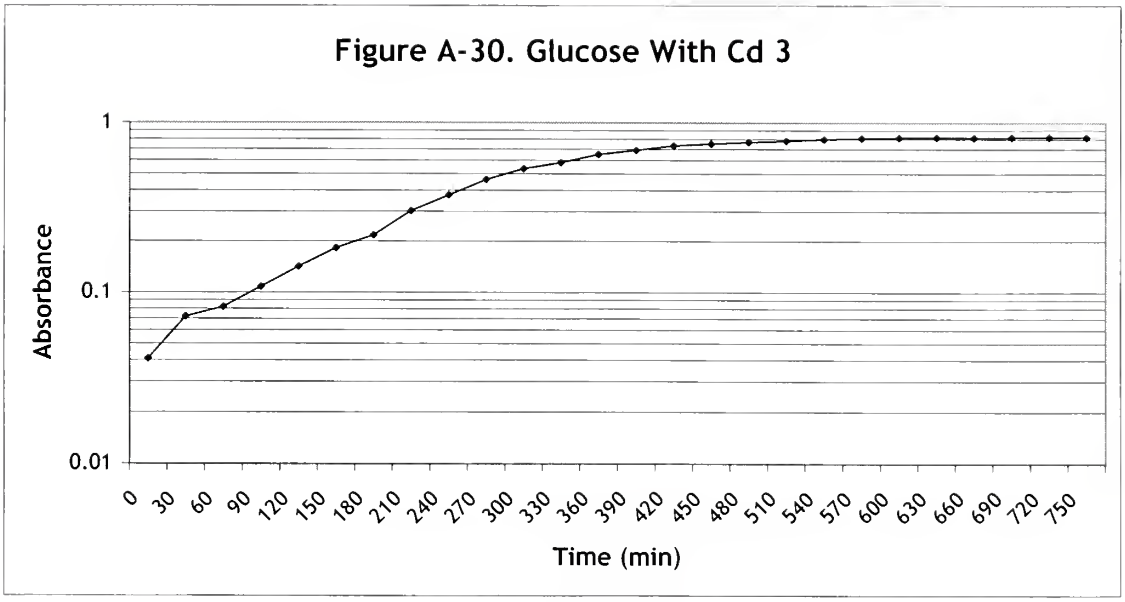
Absorbance increases from 0.101 to 0.199

Actual growth rate = **(180 min -90 min) = 90 min**



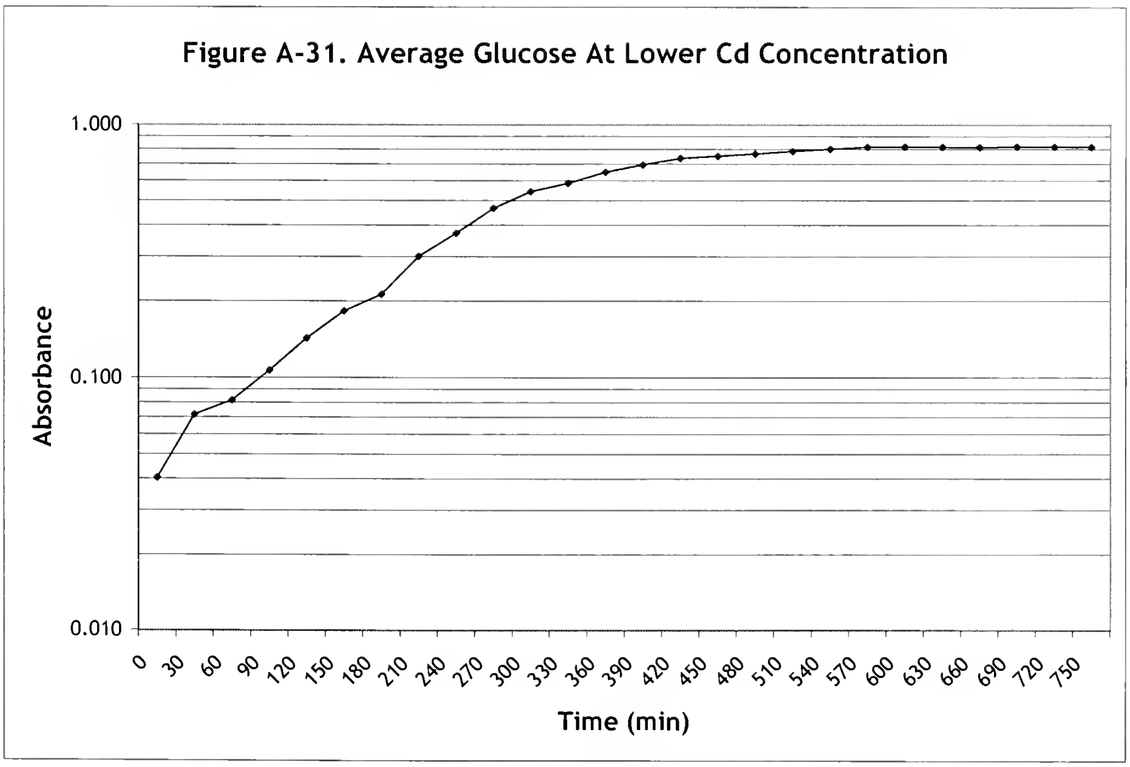
Absorbance increases from 0.111 to .0.223

Actual growth rate = **(180 min -90 min) = 90 min**



Absorbance increases from 0.108 to .0.217

Actual growth rate = (180 min -90 min) = 90 min



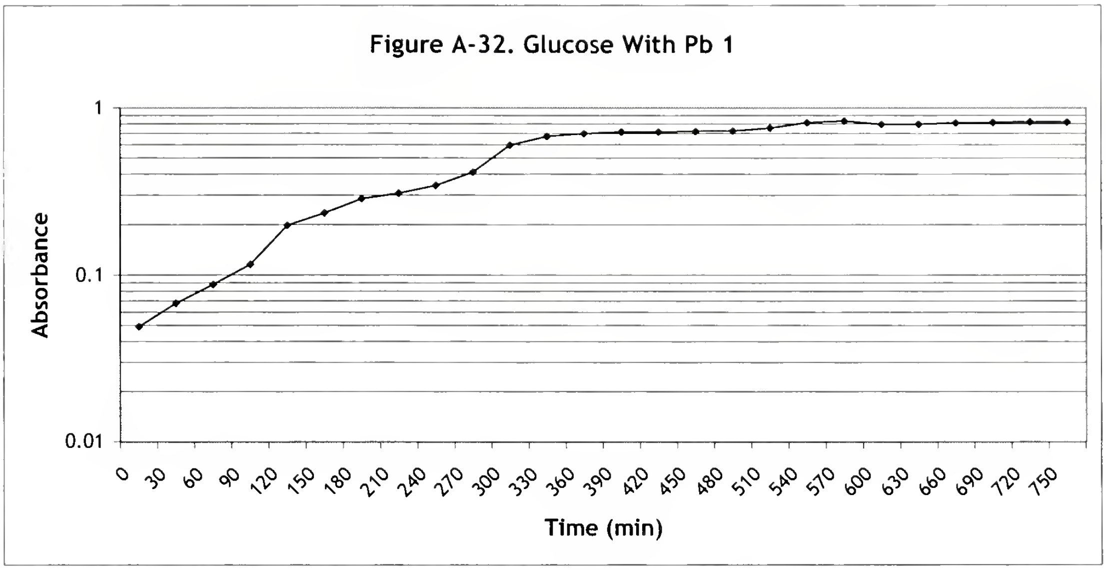
Absorbance increases from 0.107 to .0.213

Actual growth rate = (180 min -90 min) = 90 min

Data for *E.coli* from absorbance vs. time in M9 media

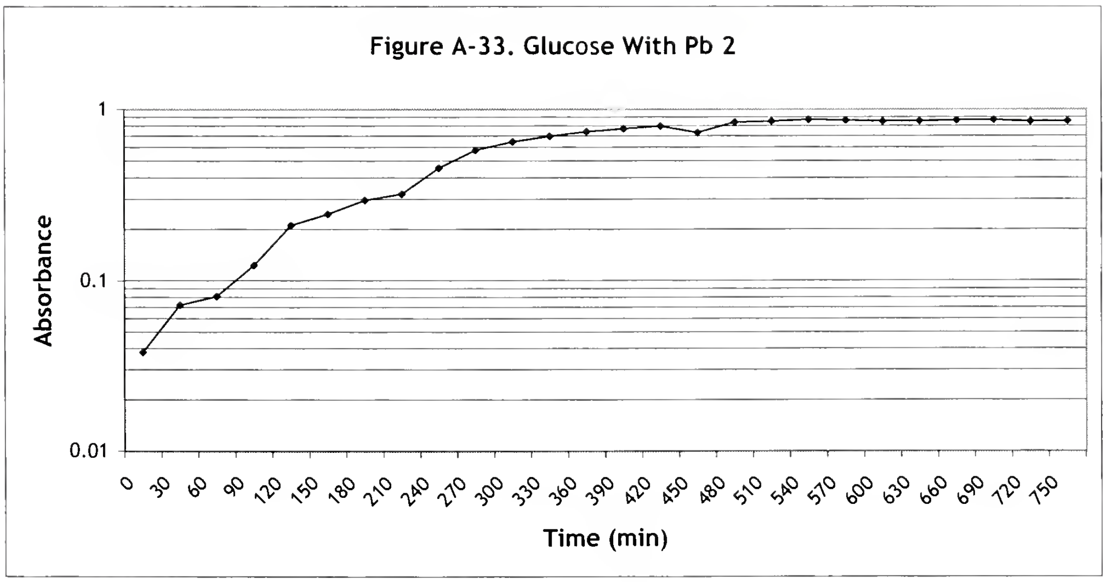
Glucose and Pb at $1*10^{-5}M$

Time(min)	Run1	Run2	Run3	Average
0	0	0	0	0
0	0.049	0.038	0.042	0.043
30	0.068	0.072	0.067	0.069
60	0.088	0.081	0.084	0.084
90	0.116	0.123	0.118	0.119
120	0.198	0.211	0.202	0.204
150	0.235	0.245	0.237	0.239
180	0.285	0.296	0.288	0.290
210	0.308	0.322	0.314	0.315
240	0.342	0.456	0.386	0.395
270	0.411	0.578	0.491	0.493
300	0.596	0.646	0.617	0.620
330	0.672	0.698	0.689	0.686
360	0.702	0.741	0.717	0.720
390	0.718	0.771	0.739	0.743
420	0.716	0.796	0.746	0.753
450	0.722	0.732	0.752	0.735
480	0.725	0.842	0.764	0.777
510	0.756	0.852	0.798	0.802
540	0.814	0.870	0.835	0.840
570	0.834	0.861	0.847	0.847
600	0.795	0.852	0.851	0.833
630	0.798	0.855	0.839	0.831
660	0.812	0.862	0.842	0.839
690	0.815	0.865	0.845	0.842
720	0.822	0.852	0.847	0.840
750	0.818	0.855	0.842	0.838



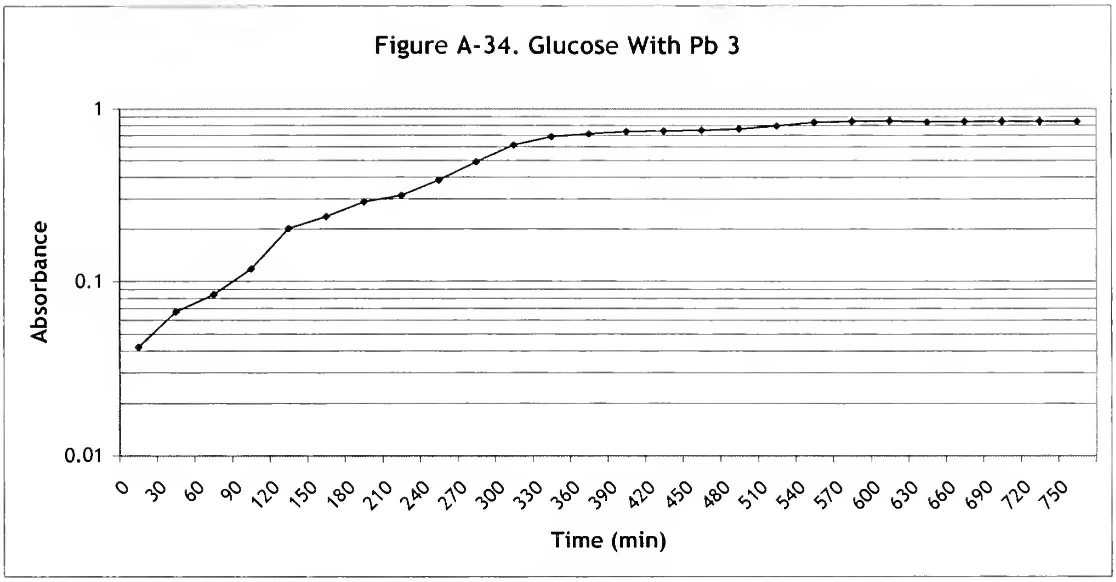
Absorbance increases from 0.116 to 0.235

Actual growth rate = **(150 min -90 min) = 60 min**



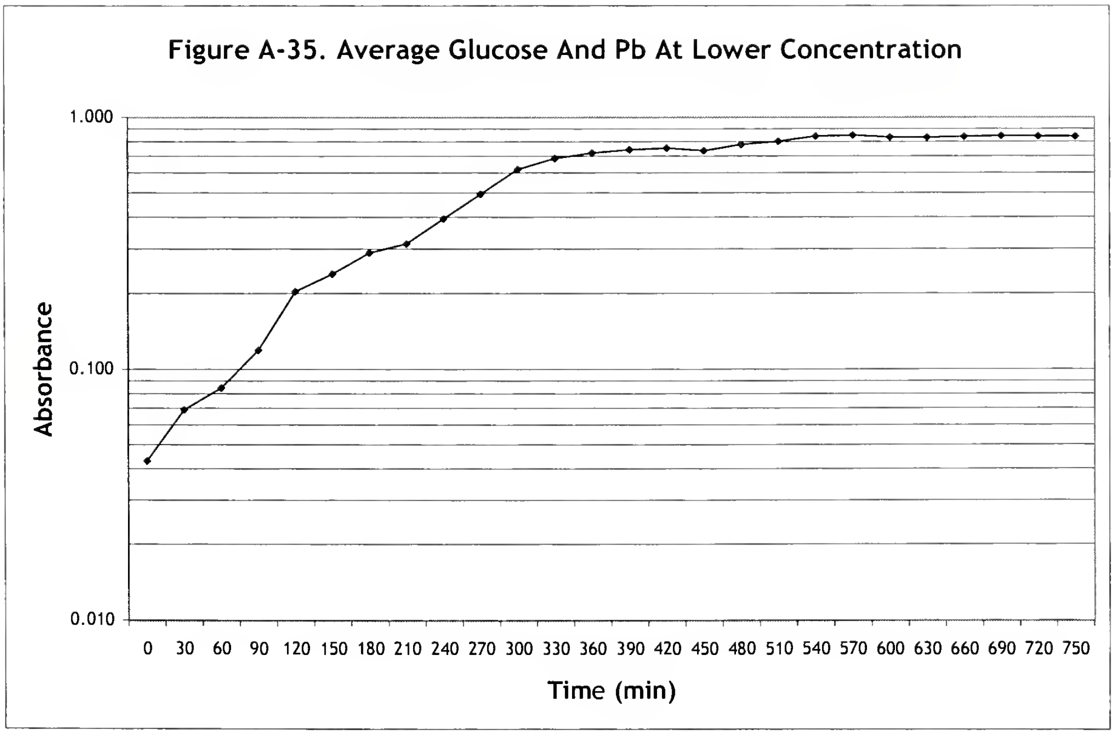
Absorbance increases from 0.123 to 0.245

Actual growth rate = **(150 min -90 min) = 60 min**



Absorbance increases from 0.118 to 0.237

Actual growth rate = (150 min -90 min) = 60 min



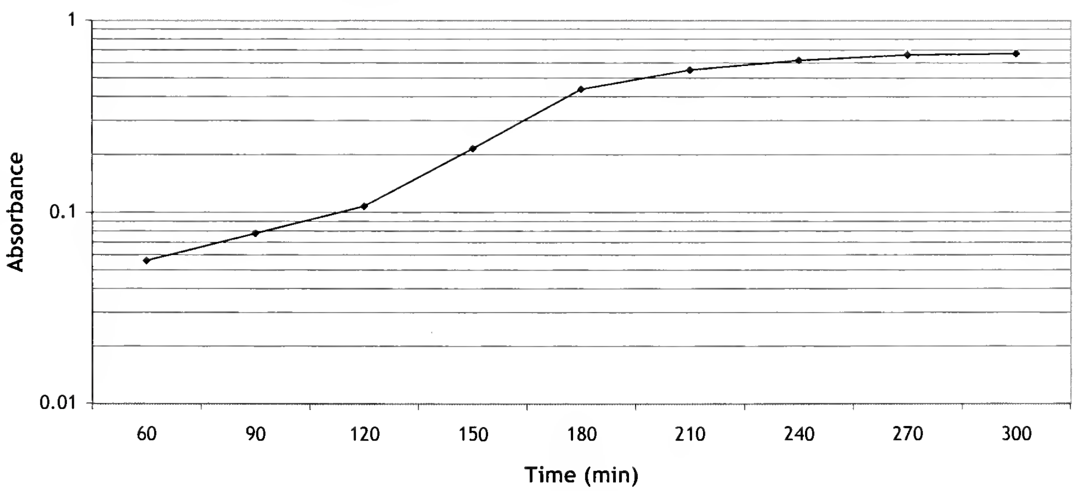
Absorbance increases from 0.119 to .0.239

Actual growth rate = (150 min -90 min) = 60 min

Data for *E.coli* from absorbance vs. time in LB Broth- Rich Media

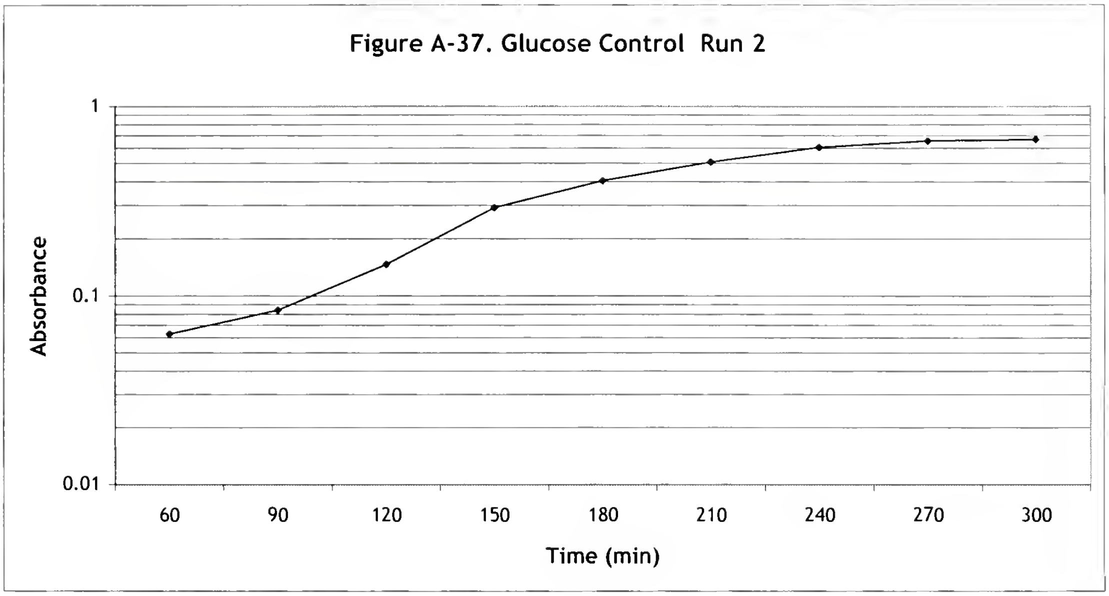
Glucose control					
Time(min)	Run1	Run2	Run3	Run4	Average
0					
30					
60	0.056	0.063	0.058	0.057	0.059
90	0.078	0.084	0.081	0.077	0.080
120	0.108	0.147	0.116	0.132	0.126
150	0.215	0.292	0.234	0.261	0.251
180	0.436	0.404	0.421	0.412	0.418
210	0.550	0.508	0.521	0.524	0.526
240	0.618	0.608	0.612	0.615	0.613
270	0.660	0.658	0.657	0.653	0.657
300	0.672	0.668	0.681	0.669	0.673

Figure A-36. Glucose Control Run 1



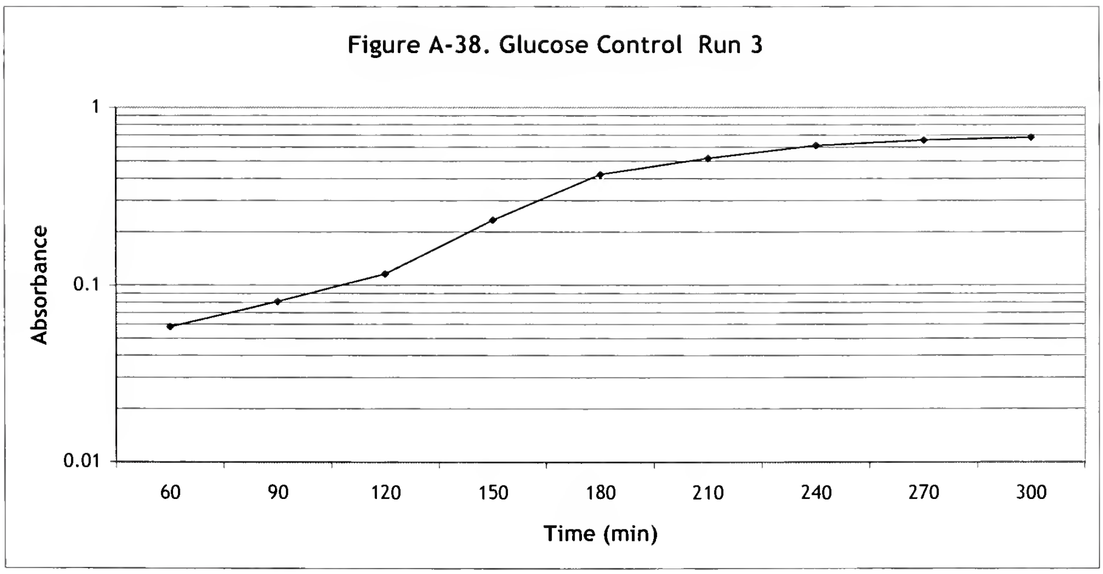
Absorbance increases from 0.108 to .0.215

Actual growth rate = (150 min -120 min) = 30 min



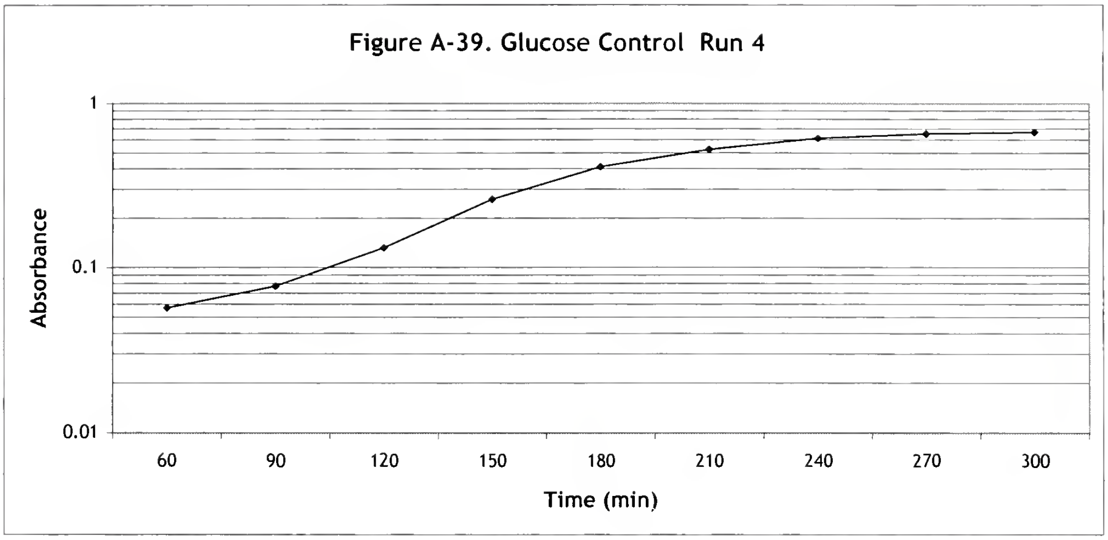
Absorbance increases from 0.147 to .0.292

Actual growth rate = (150 min -120 min) = 30 min



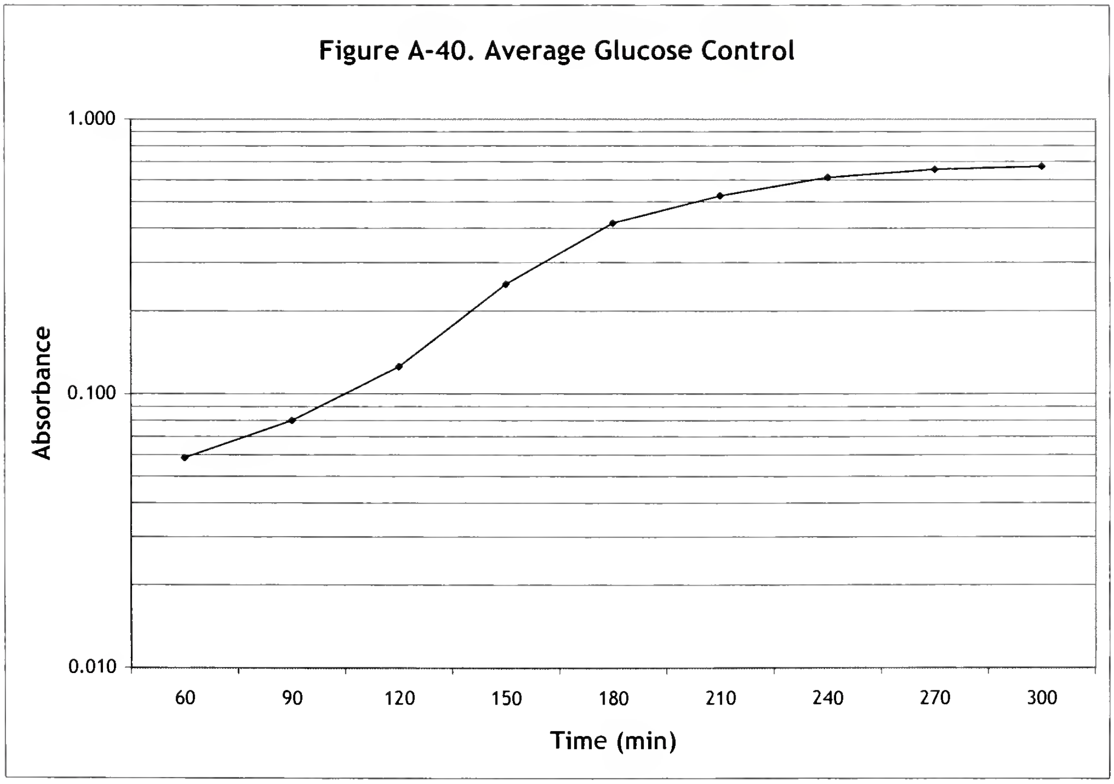
Absorbance increases from 0.116 to .0.234

Actual growth rate = (150 min -120 min) = 30 min



Absorbance increases from 0.132 to .0.261

Actual growth rate = **(150 min -120 min) = 30 min**



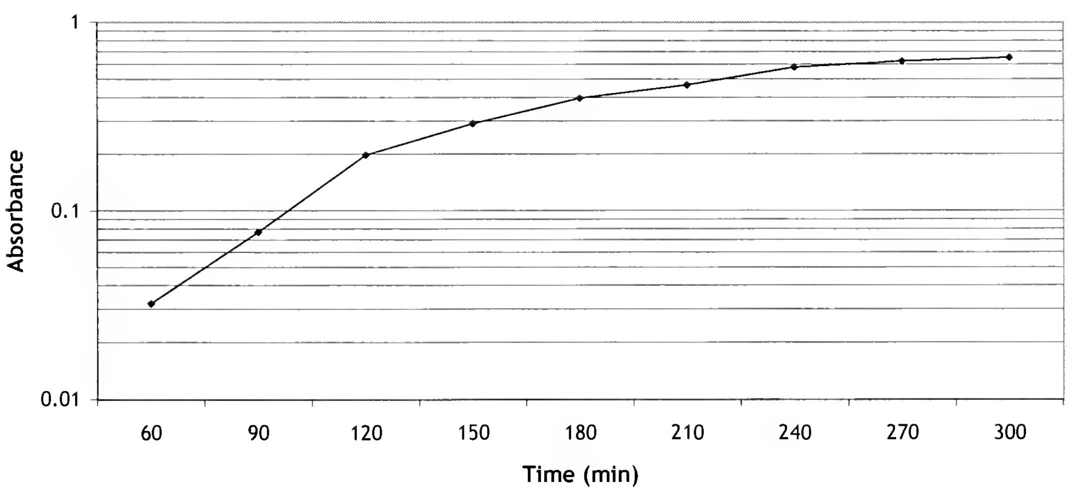
Absorbance increases from 0.126 to .0.251

Actual growth rate = **(150 min -120 min) = 30 min**

Data for *E.coli* from absorbance vs. time in LB Broth- Rich Media

Glucose With 1*10^{-5} M Cd					
Time(min)	Run1	Run2	Run3	Run4	Average
0					
30					
60	0.032	0.034	0.028	0.031	0.031
90	0.077	0.073	0.069	0.072	0.073
120	0.198	0.161	0.172	0.176	0.177
150	0.291	0.266	0.254	0.258	0.267
180	0.397	0.326	0.344	0.351	0.355
210	0.467	0.448	0.432	0.441	0.447
240	0.578	0.567	0.598	0.561	0.576
270	0.624	0.562	0.642	0.641	0.617
300	0.652	0.605	0.682	0.678	0.654

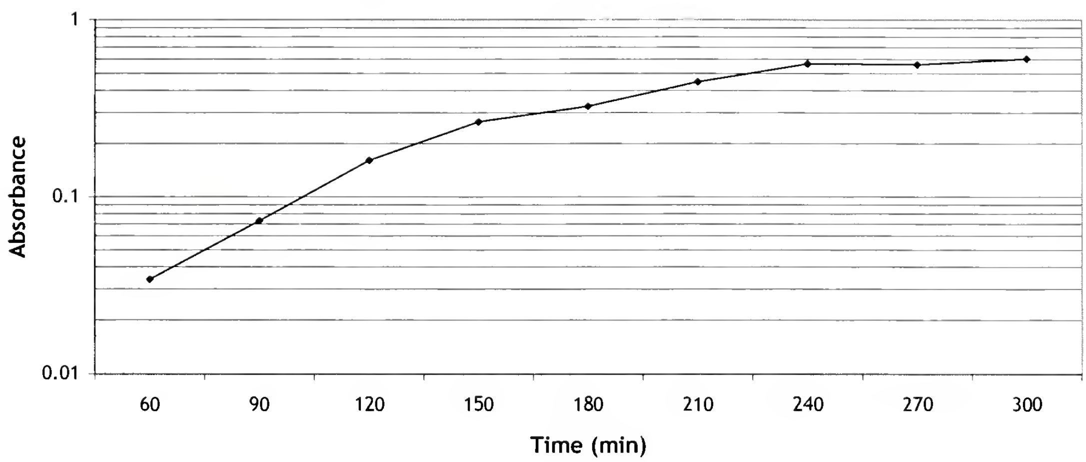
Figure A-41. Glucose With Cd Run 1



Absorbance increases from 0.198 to .0.397

Actual growth rate = (180 min - 120min) = 60 min

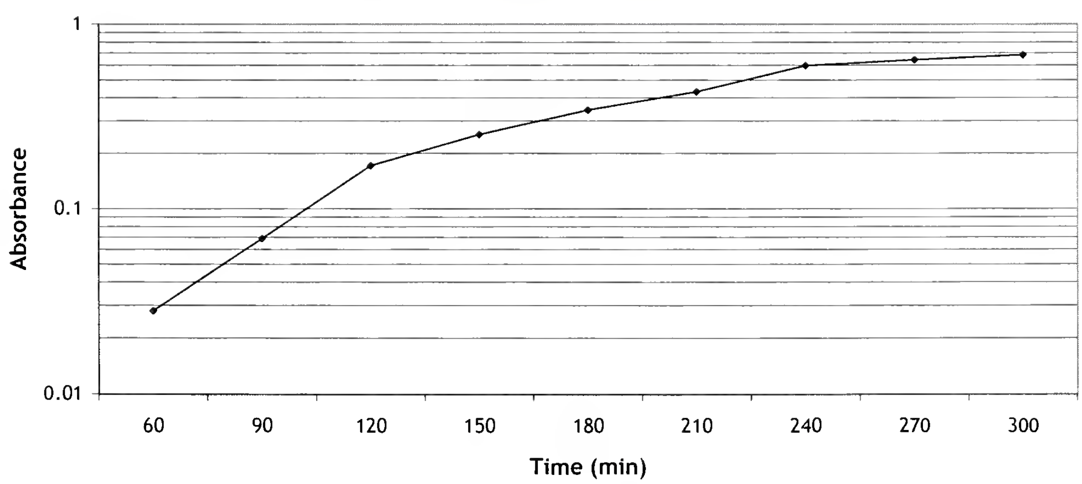
Figure A-42. Glucose With Cd Run 2



Absorbance increases from 0.161 to .0.326

Actual growth rate = (180 min - 120min) = 60 min

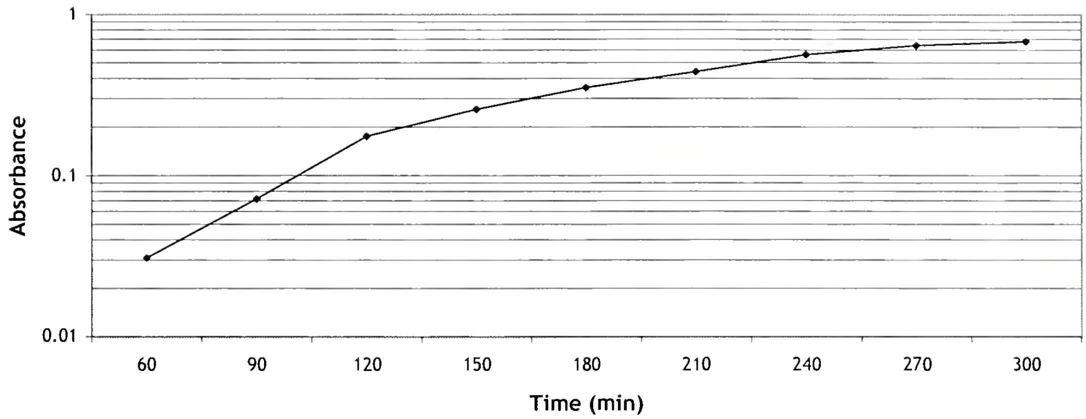
Figure A-43. Glucose With Cd Run 3



Absorbance increases from 0.172 to .0.344

Actual growth rate = (180 min - 120min) = 60 min

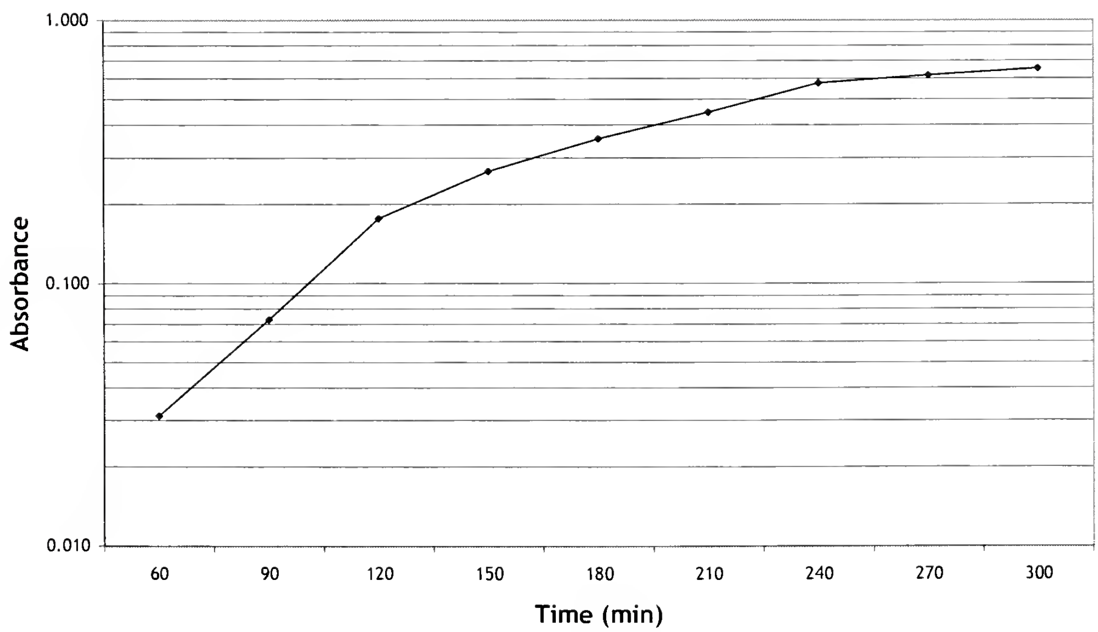
Figure A-44. Glucose With Cd Run 4



Absorbance increases from 0.176 to .0.351

Actual growth rate = (180 min - 120min) = 60 min

Figure A-45. Average Glucose With Cd



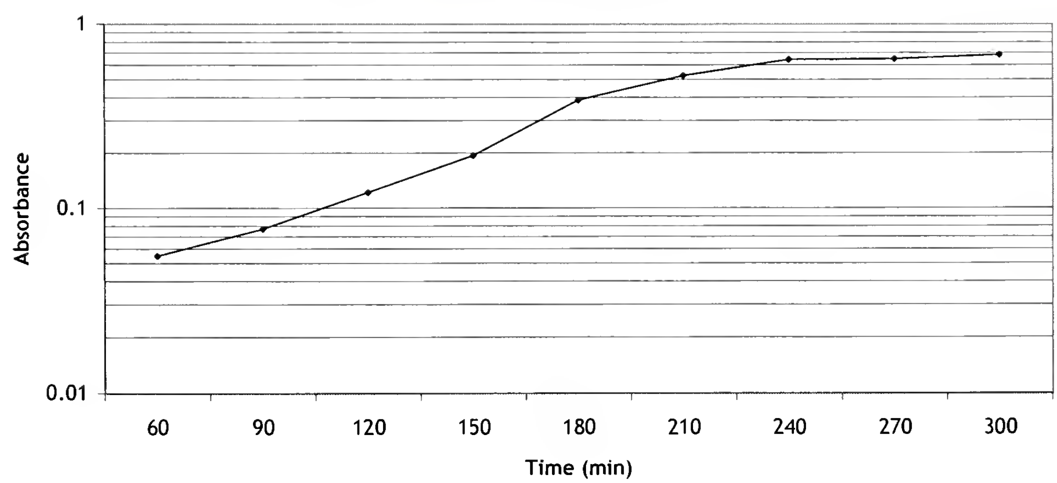
Absorbance increases from 0.177 to .0.355

Actual growth rate = (180 min - 120min) = 60 min

Data for *E.coli* from absorbance vs. time in LB Broth- Rich Media

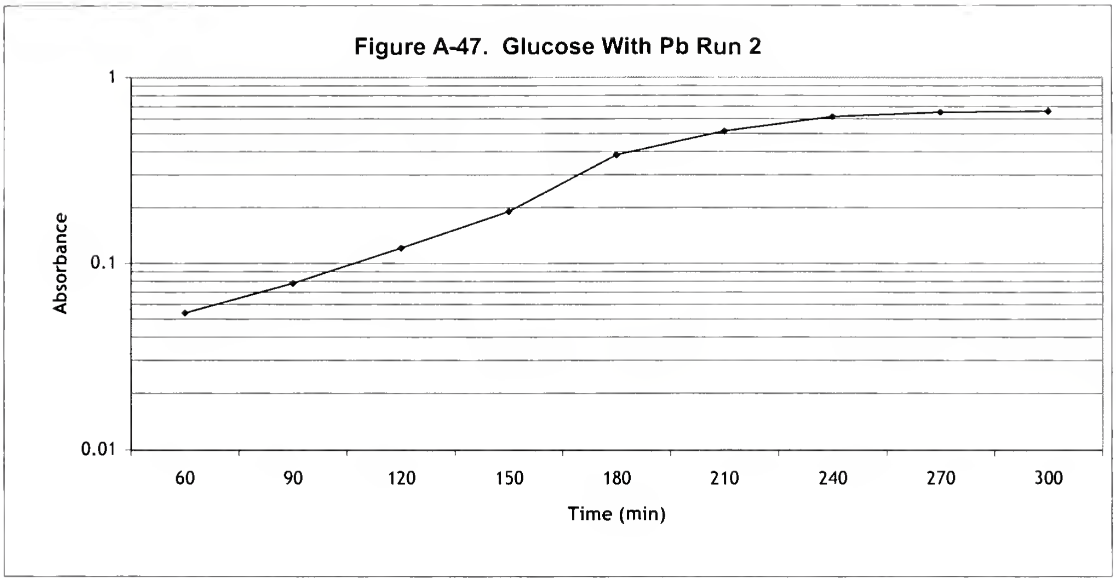
Glucose With $1*10^{-5}$ M Pb					
Time(min)	Run1	Run2	Run3	Run4	Average
0					
30					
60	0.055	0.054	0.028	0.048	0.046
90	0.077	0.078	0.052	0.071	0.070
120	0.122	0.121	0.101	0.108	0.113
150	0.193	0.191	0.185	0.181	0.188
180	0.388	0.384	0.369	0.366	0.377
210	0.526	0.516	0.412	0.504	0.490
240	0.642	0.618	0.484	0.611	0.589
270	0.648	0.652	0.578	0.648	0.632
300	0.682	0.661	0.663	0.674	0.670

Figure A-46. Glucose With Pb Run 1



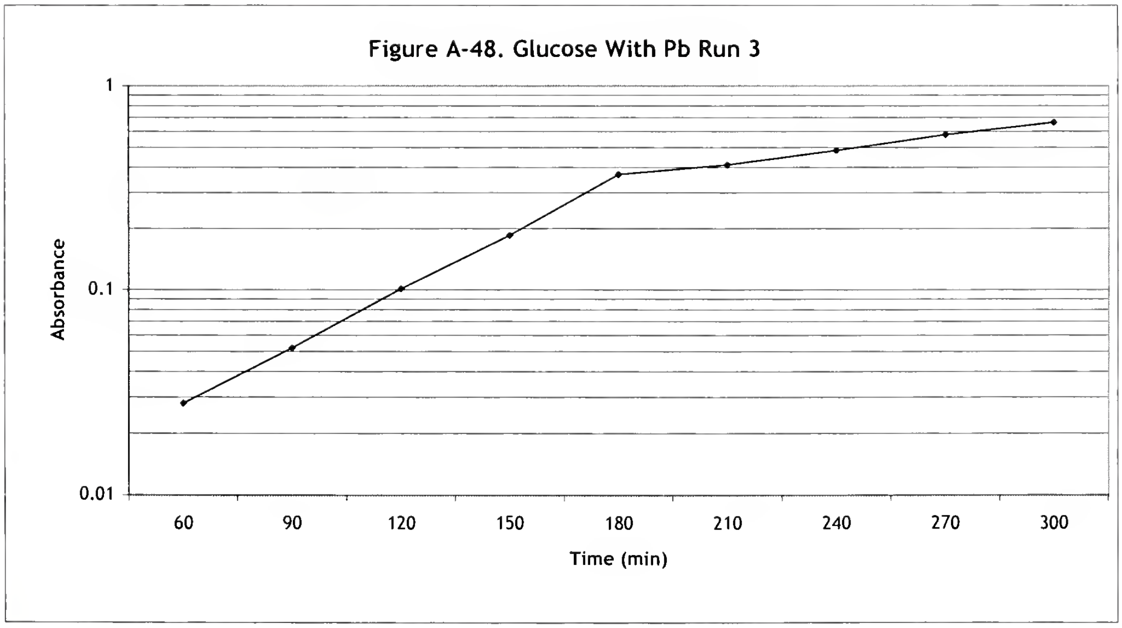
Absorbance increases from 0.193 to .0.388

Actual growth rate = (180 min -150 min) = 30 min



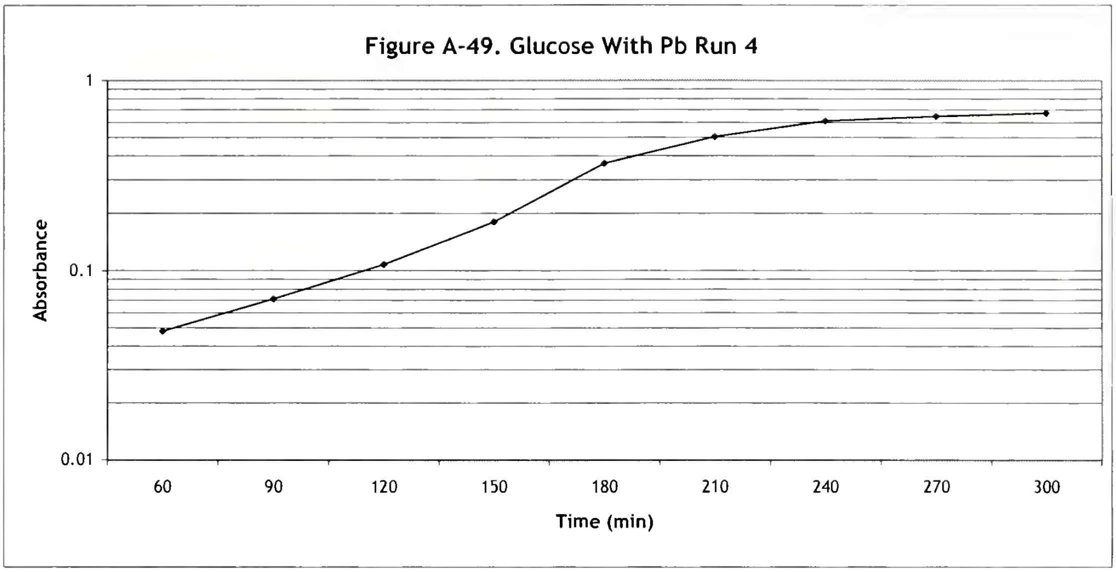
Absorbance increases from 0.191 to .0.384

Actual growth rate = (180 min -150 min) = 30 min



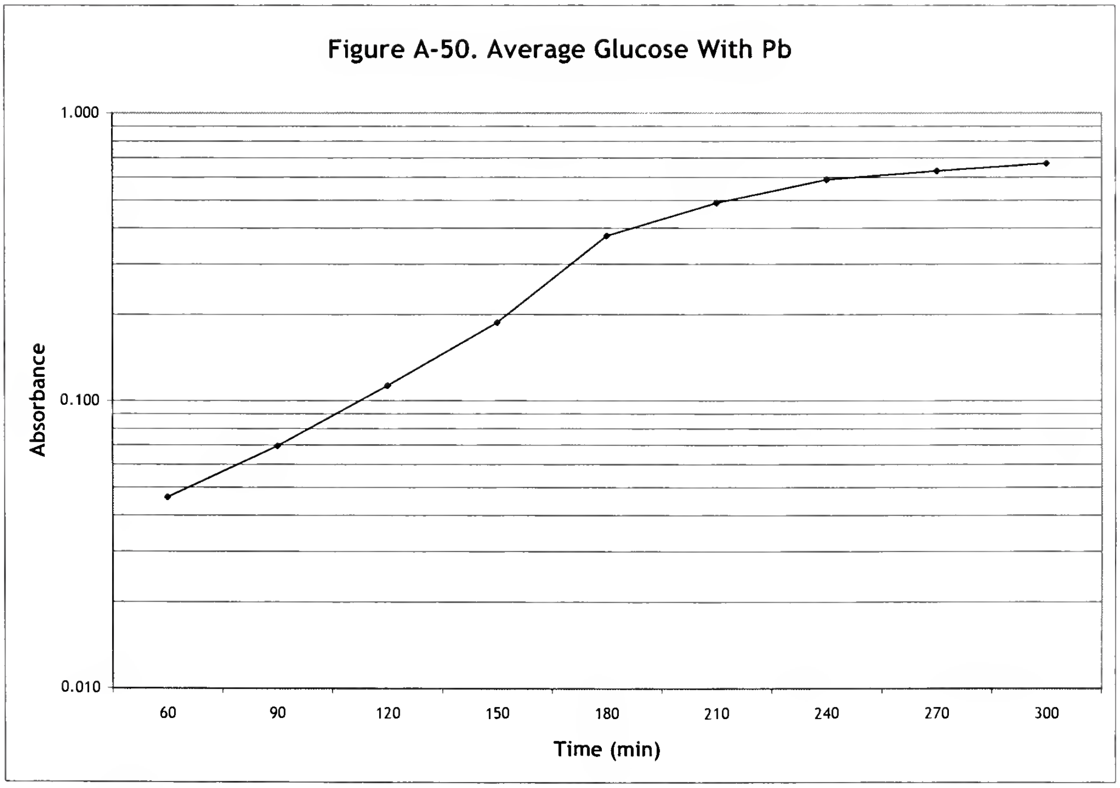
Absorbance increases from 0.185 to .0.369

Actual growth rate = (180 min -150 min) = 30 min



Absorbance increases from 0.181 to .0.366

Actual growth rate = (150 min -120 min) = 30 min



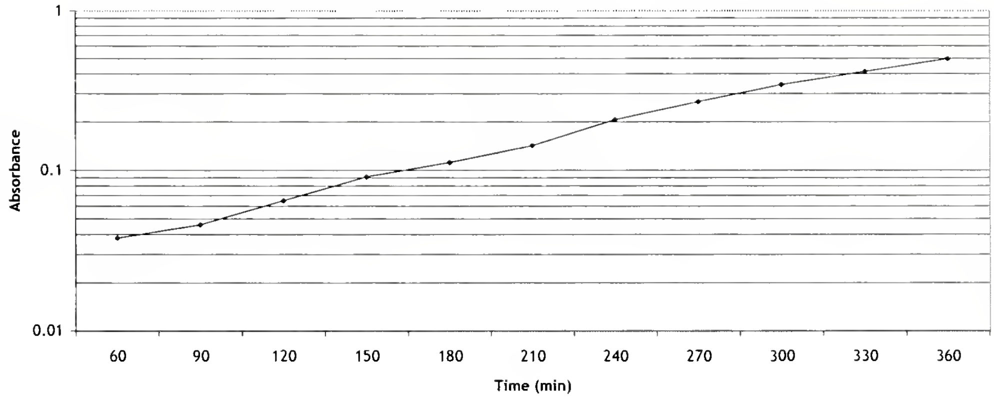
Absorbance increases from 0.188 to .0.377

Actual growth rate = (150 min -120 min) = 30 min

Data for *E.coli* from absorbance vs. time in LB Broth- Rich Media

Glucose With $1*10^{-3}$ M Cd				
Time(min)	Run1	Run2	Run3	Average
0				
30				
60	0.038	0.028	0.042	0.036
90	0.046	0.051	0.063	0.053
120	0.065	0.058	0.068	0.064
150	0.091	0.078	0.075	0.081
180	0.112	0.108	0.098	0.106
210	0.142	0.138	0.114	0.131
240	0.206	0.199	0.204	0.203
270	0.268	0.245	0.242	0.252
300	0.342	0.323	0.298	0.321
330	0.414	0.399	0.409	0.407
360	0.498	0.422	0.455	0.458

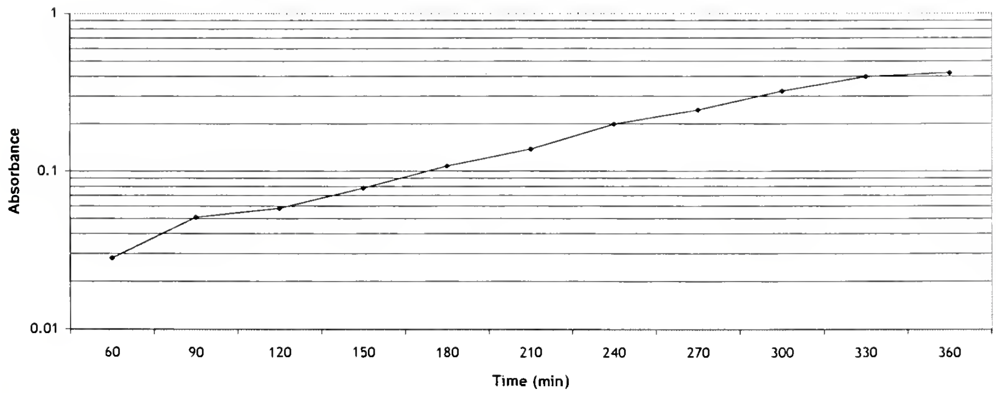
Figure A-51. Glucose With Higher Cd Concentration Run 1



Absorbance increases from 0.206 to .0.414

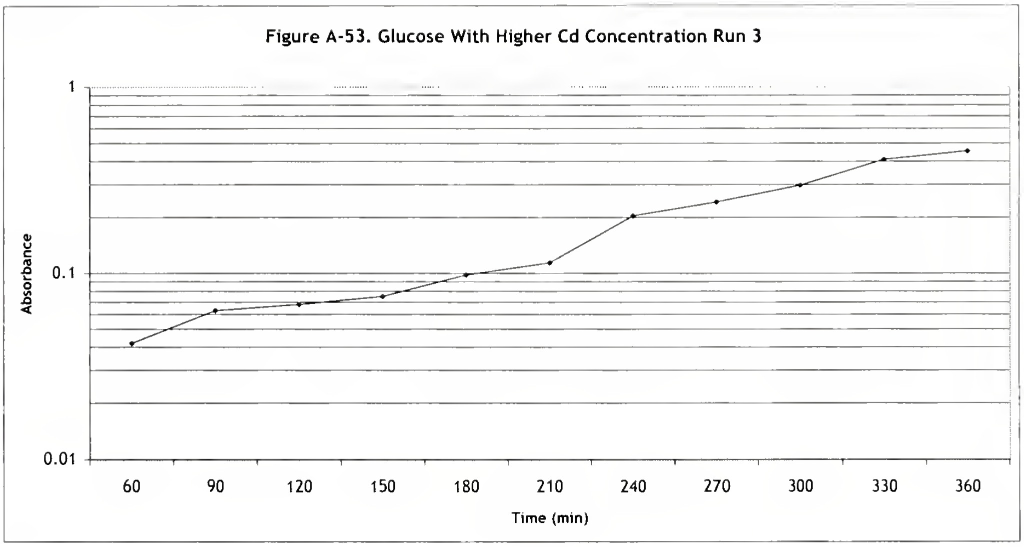
Actual growth rate = **(330 min - 240min) = 90 min**

Figure A-52. Glucose With Higher Cd Concentration Run 2



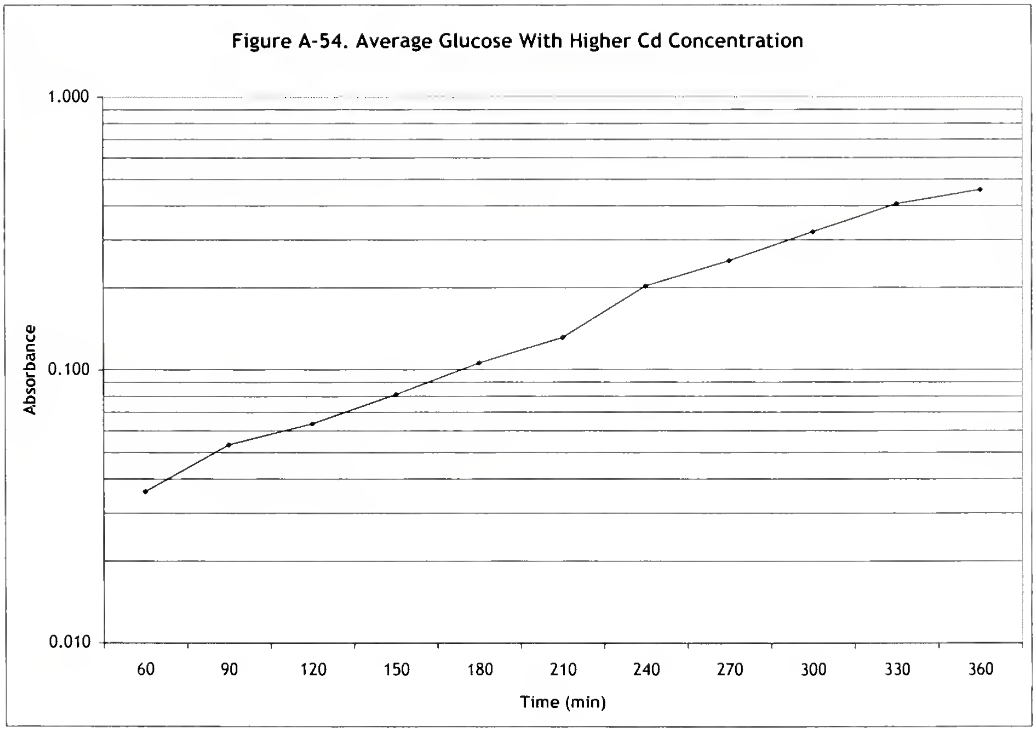
Absorbance increases from 0.199 to .0.399

Actual growth rate = **(330 min - 240min) = 90 min**



Absorbance increases from 0.204 to .0.409

Actual growth rate = (330 min - 240min) = 90 min



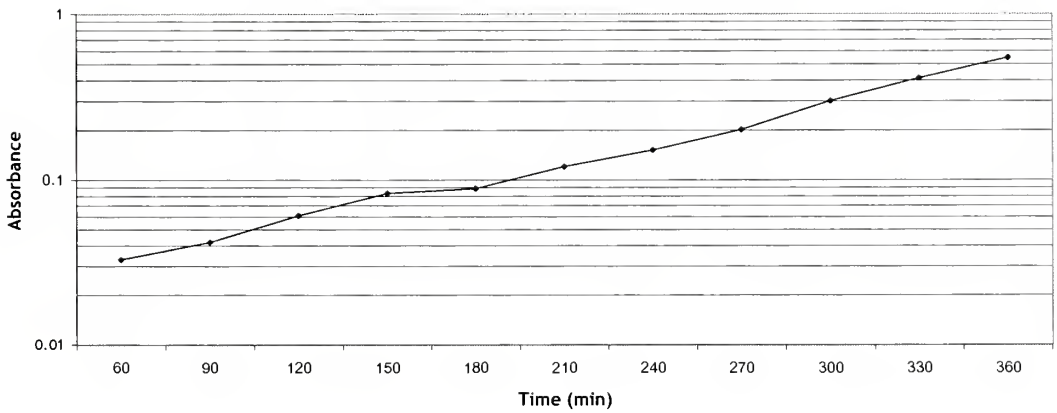
Absorbance increases from 0.203 to .0.407

Actual growth rate = (330 min - 240min) = 90 min

Data for *E.coli* from absorbance vs. time in LB Broth- Rich Media

Glucose With $1*10^{-3}$ M Pb				
Time(min)	Run1	Run2	Run3	Average
0				
30				
60	0.033	0.028	0.039	0.033
90	0.042	0.034	0.054	0.043
120	0.061	0.039	0.071	0.057
150	0.083	0.052	0.092	0.076
180	0.089	0.071	0.096	0.085
210	0.121	0.101	0.108	0.110
240	0.152	0.138	0.144	0.145
270	0.202	0.196	0.175	0.191
300	0.301	0.279	0.217	0.266
330	0.412	0.375	0.354	0.380
360	0.548	0.414	0.428	0.463

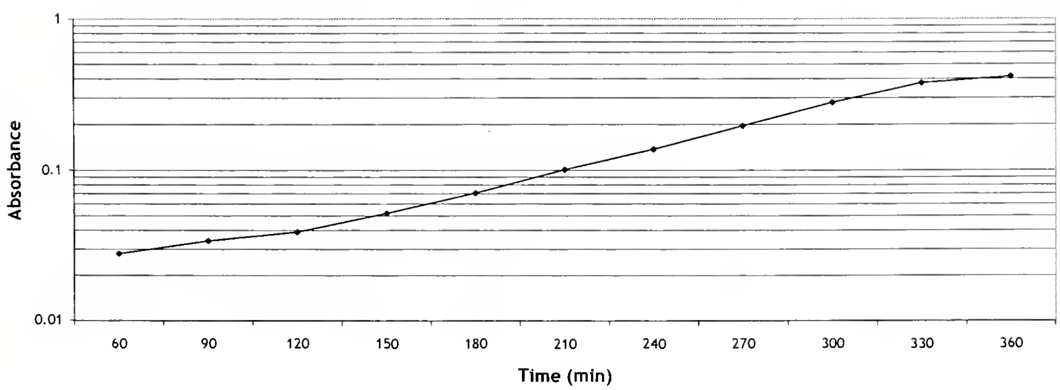
Figure A-55. Glucose With Higher Pb Concentration Run 1



Absorbance increases from 0.152 to .0.301

Actual growth rate = (300 min - 240min) = 60 min

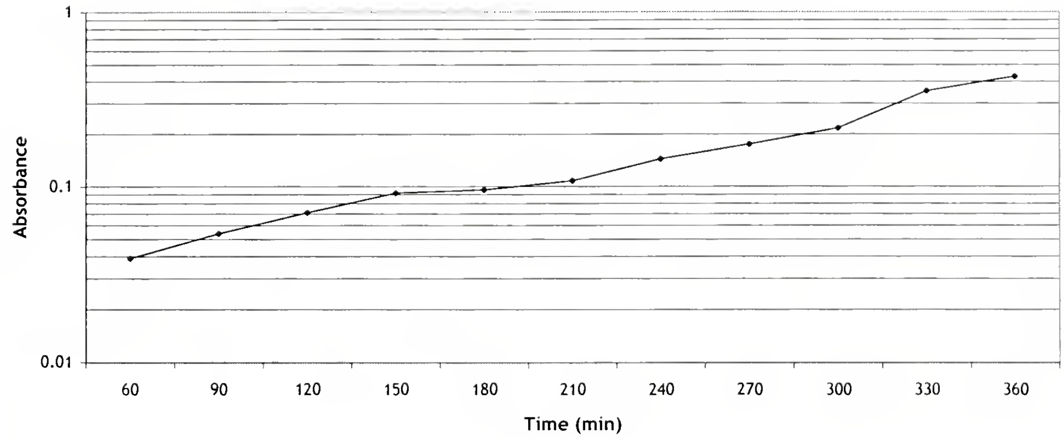
Figure A-56. Glucose With Higher Pb Concentration Run 2



Absorbance increases from 0.138 to .0.279

Actual growth rate = (300 min - 240min) = 60 min

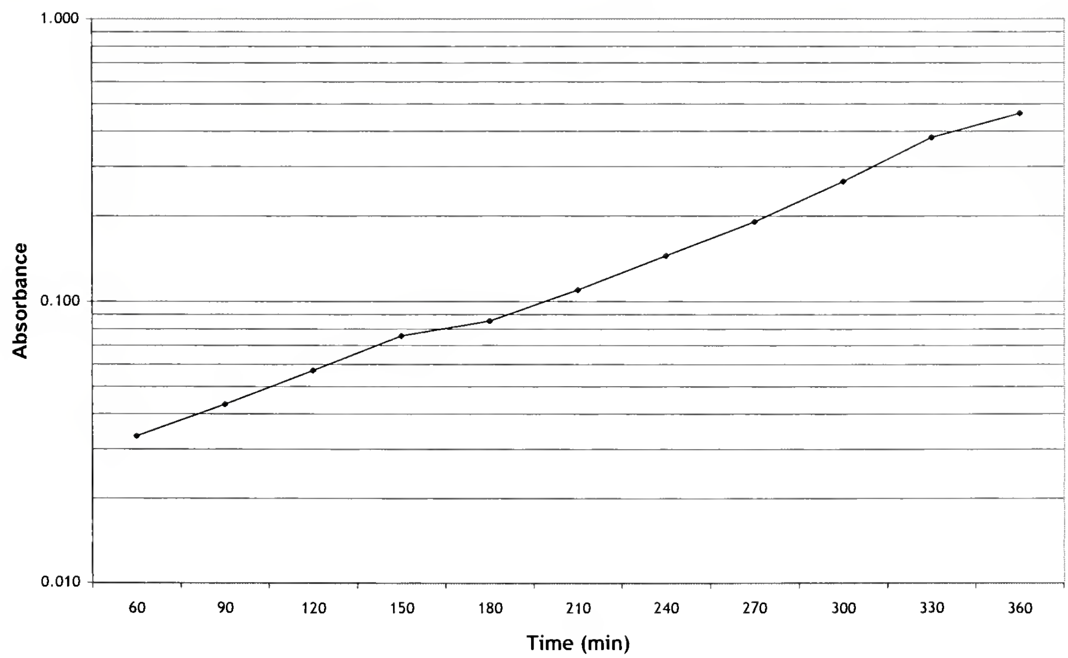
Figure A-57. Glucose With Higher Pb Concentration Run 3



Absorbance increases from 0.108 to .0.217

Actual growth rate = **(300 min - 210min) = 90 min**

Figure A-58. Average Glucose With Higher Pb Concentration



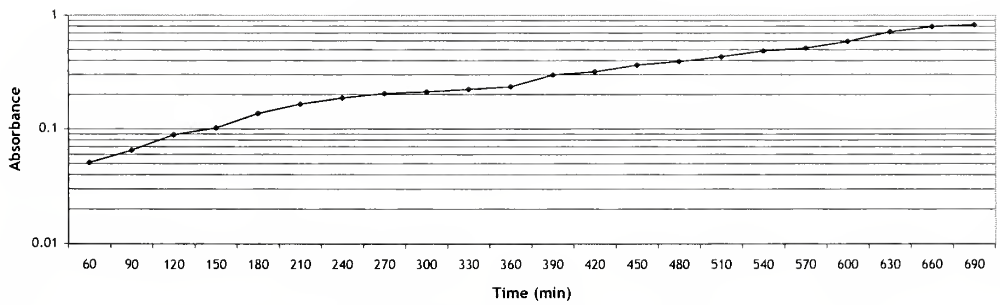
Absorbance increases from 0.191 to .0.380

Actual growth rate = **(330 min - 270min) = 60 min**

Data for *E.coli* from absorbance vs. time in M9 media
with no casamino acids (poor media)

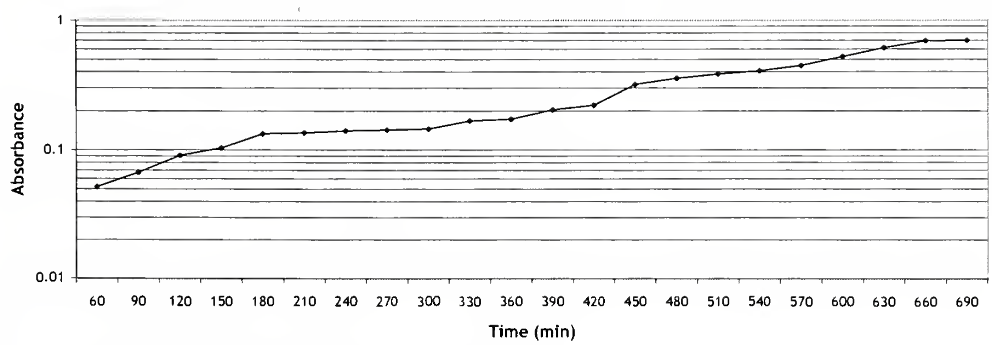
Glucose control				
Time(min)	Run1	Run2	Run3	Average
0				
30				
60	0.051	0.052	0.058	0.052
90	0.065	0.067	0.071	0.066
120	0.089	0.091	0.099	0.090
150	0.102	0.104	0.106	0.103
180	0.137	0.134	0.141	0.136
210	0.165	0.136	0.158	0.151
240	0.187	0.141	0.167	0.164
270	0.204	0.143	0.172	0.174
300	0.211	0.146	0.181	0.179
330	0.222	0.168	0.193	0.195
360	0.234	0.173	0.205	0.204
390	0.298	0.205	0.227	0.243
420	0.318	0.222	0.265	0.268
450	0.365	0.321	0.321	0.336
480	0.391	0.358	0.384	0.378
510	0.432	0.386	0.41	0.409
540	0.485	0.407	0.456	0.446
570	0.514	0.446	0.492	0.480
600	0.592	0.526	0.555	0.559
630	0.718	0.613	0.687	0.666
660	0.794	0.698	0.753	0.746
690	0.826	0.702	0.812	0.764

Figure A-59. Glucose Control Run 1

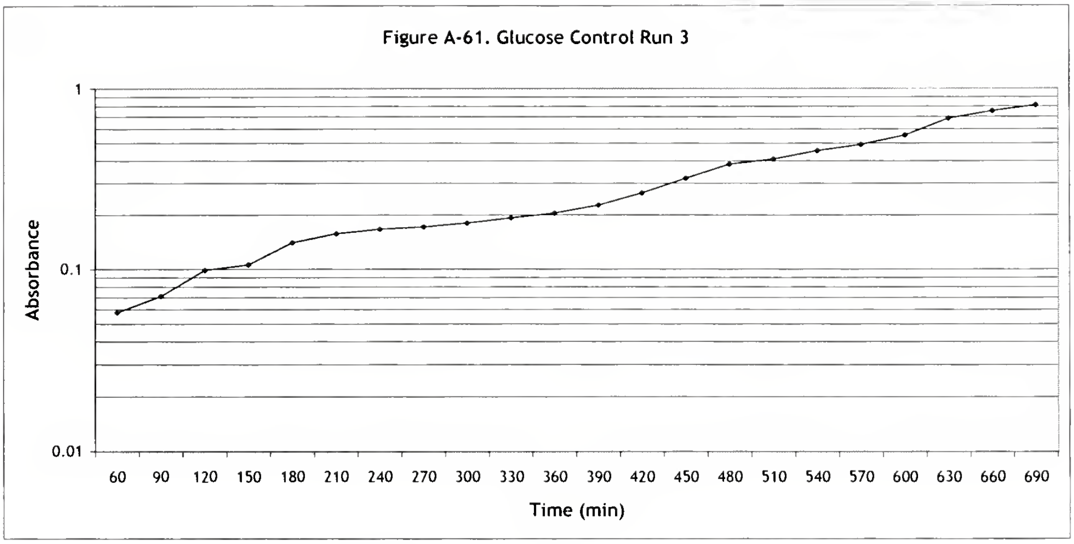


Absorbance increases from 0.102 to .0.204
Actual growth rate = **(270 min -150 min) = 120 min**

Figure A-60. Glucose Control Run 2

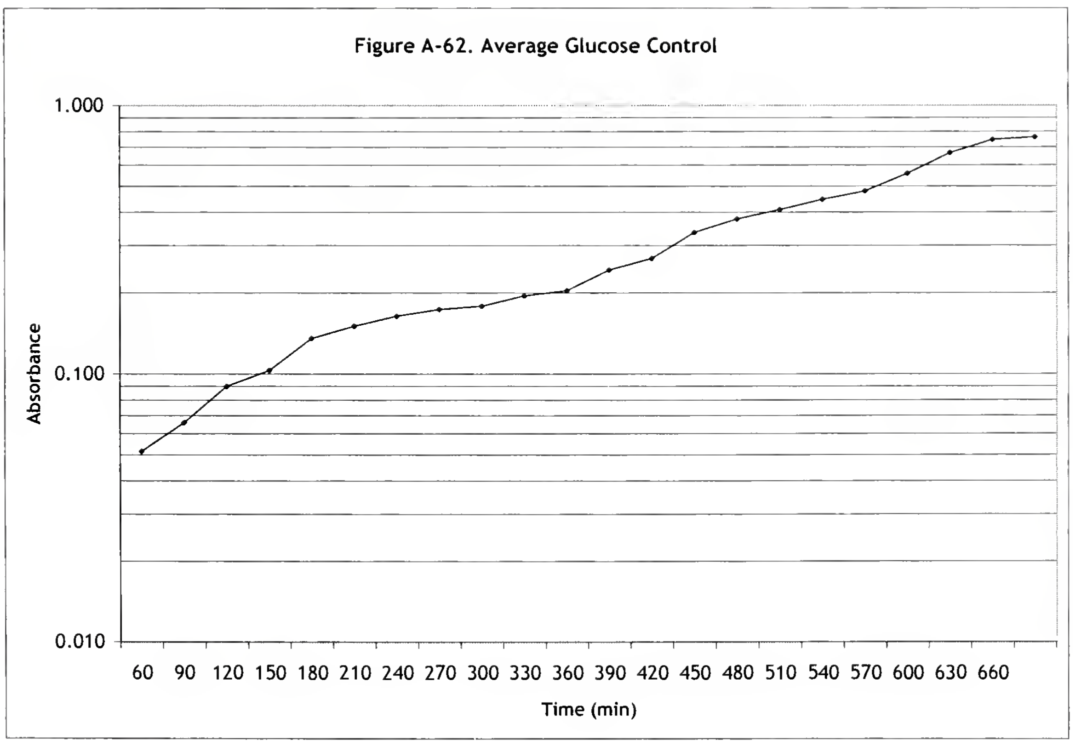


Absorbance increases from 0.205 to .0.407
Actual growth rate = **(540 min -390 min) = 150 min**



Absorbance increases from 0.205 to .0.41

Actual growth rate = (510 min -360 min) = 150 min



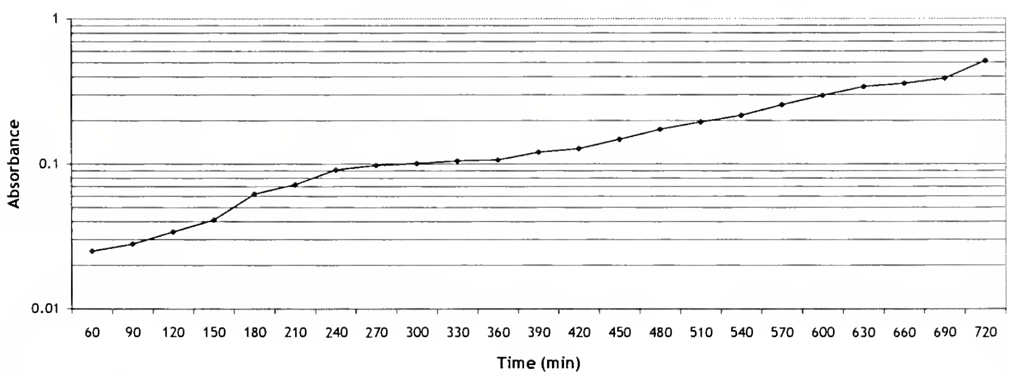
Absorbance increases from 0.103 to .0.204

Actual growth rate = (510 min -360 min) = 150 min

Data for *E.coli* from absorbance vs. time in M9 media
with no casamino acids (poor media)

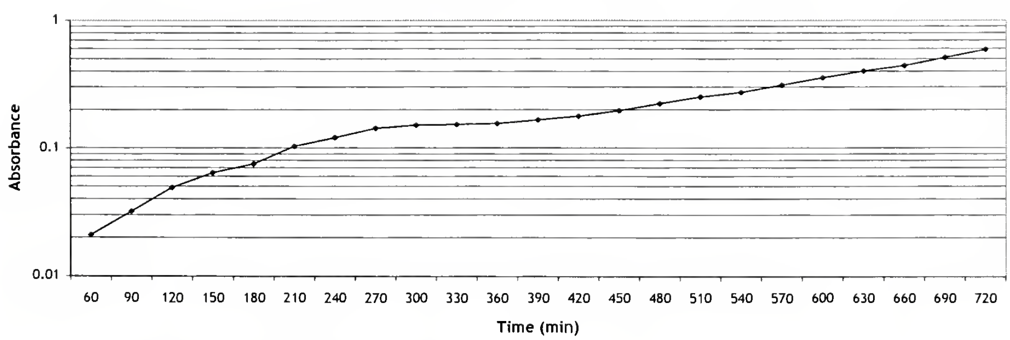
Glucose With $1*10^{-5}$ M Cd				
Time(min)	Run1	Run2	Run3	Average
0				
30				
60	0.025	0.021	0.032	0.023
90	0.028	0.032	0.038	0.030
120	0.034	0.049	0.042	0.042
150	0.041	0.064	0.059	0.053
180	0.062	0.075	0.079	0.069
210	0.072	0.103	0.095	0.088
240	0.091	0.121	0.108	0.106
270	0.098	0.143	0.126	0.121
300	0.101	0.151	0.142	0.126
330	0.105	0.153	0.148	0.129
360	0.107	0.156	0.153	0.132
390	0.121	0.166	0.165	0.144
420	0.128	0.178	0.174	0.153
450	0.148	0.196	0.187	0.172
480	0.174	0.223	0.201	0.199
510	0.195	0.251	0.224	0.223
540	0.217	0.273	0.251	0.247
570	0.256	0.312	0.292	0.287
600	0.298	0.356	0.328	0.327
630	0.342	0.402	0.364	0.369
660	0.359	0.443	0.4	0.401
690	0.391	0.511	0.498	0.467
720	0.514	0.59	0.569	0.558

Figure A-63. Glucose With Cd Run 1

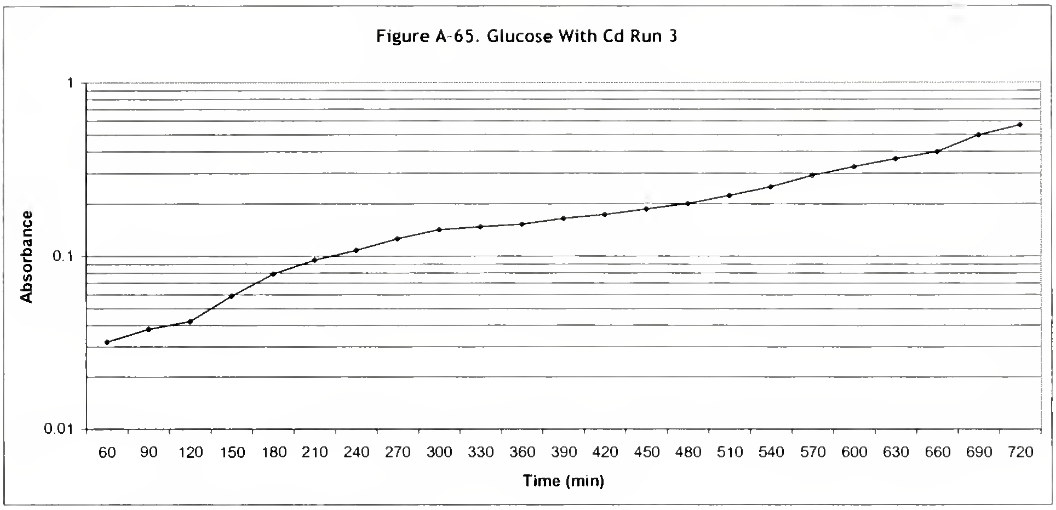


Absorbance increases from 0.195 to .0.387
Actual growth rate = (690 min - 510min) = 180 min

Figure A-64. Glucose With Cd Run 2

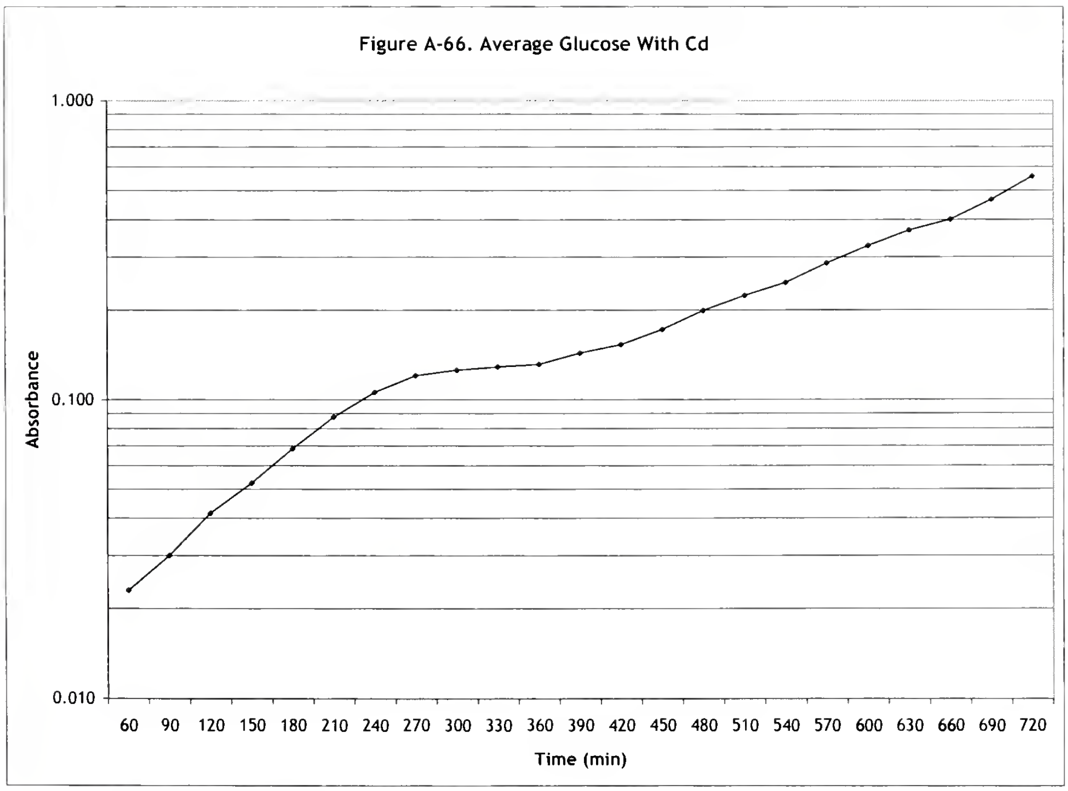


Absorbance increases from 0.223 to .0.443
Actual growth rate = (660 min - 480min) = 180 min



Absorbance increases from 0.201 to .0.4

Actual growth rate = **(660 min - 480min) = 180 min**



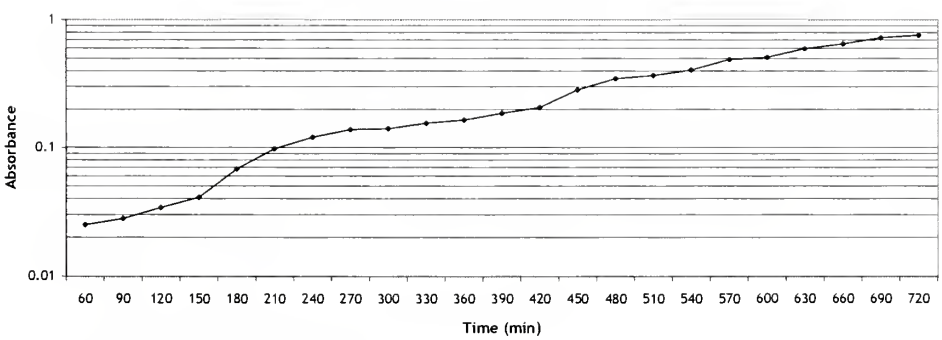
Absorbance increases from 0.199 to .0.401

Actual growth rate = **(660 min - 480min) = 180 min**

Data for *E.coli* from absorbance vs. time in M9 media
with no casamino acids (poor media)

Glucose With $1*10^{-5}$ M Pb				
Time(min)	Run1	Run2	Run3	Average
60	0.025	0.021	0.032	0.026
90	0.028	0.029	0.033	0.030
120	0.034	0.043	0.036	0.038
150	0.041	0.054	0.048	0.048
180	0.068	0.073	0.071	0.071
210	0.098	0.071	0.099	0.089
240	0.121	0.079	0.102	0.101
270	0.139	0.078	0.114	0.110
300	0.141	0.085	0.121	0.116
330	0.156	0.089	0.128	0.124
360	0.165	0.099	0.131	0.132
390	0.186	0.102	0.148	0.145
420	0.207	0.126	0.163	0.165
450	0.283	0.145	0.174	0.201
480	0.347	0.163	0.196	0.235
510	0.369	0.198	0.227	0.265
540	0.406	0.206	0.294	0.302
570	0.491	0.312	0.362	0.388
600	0.512	0.423	0.454	0.463
630	0.597	0.484	0.512	0.531
660	0.653	0.511	0.582	0.582
690	0.726	0.533	0.631	0.630
720	0.762	0.576	0.694	0.677

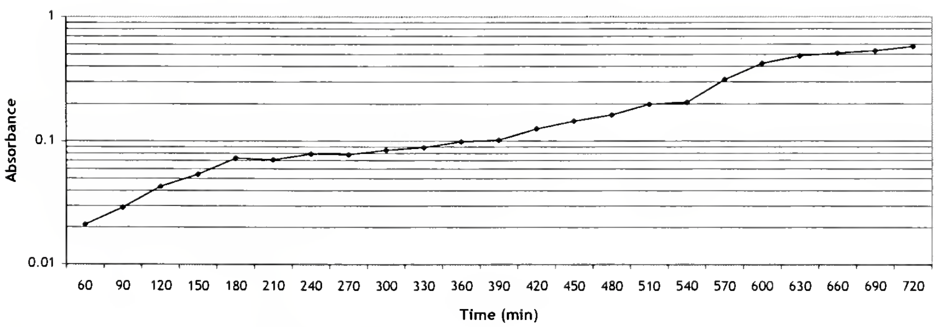
Figure A-67. Glucose With Pb Run 1



Absorbance increases from 0.141 to .0.283

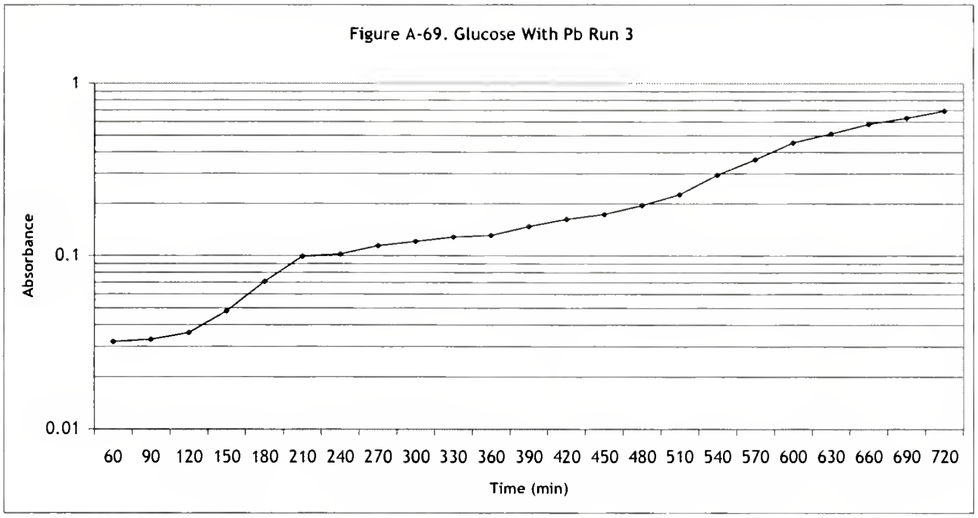
Actual growth rate = (450 min - 300min) = 150 min

Figure A-68. Glucose With Pb Run 2



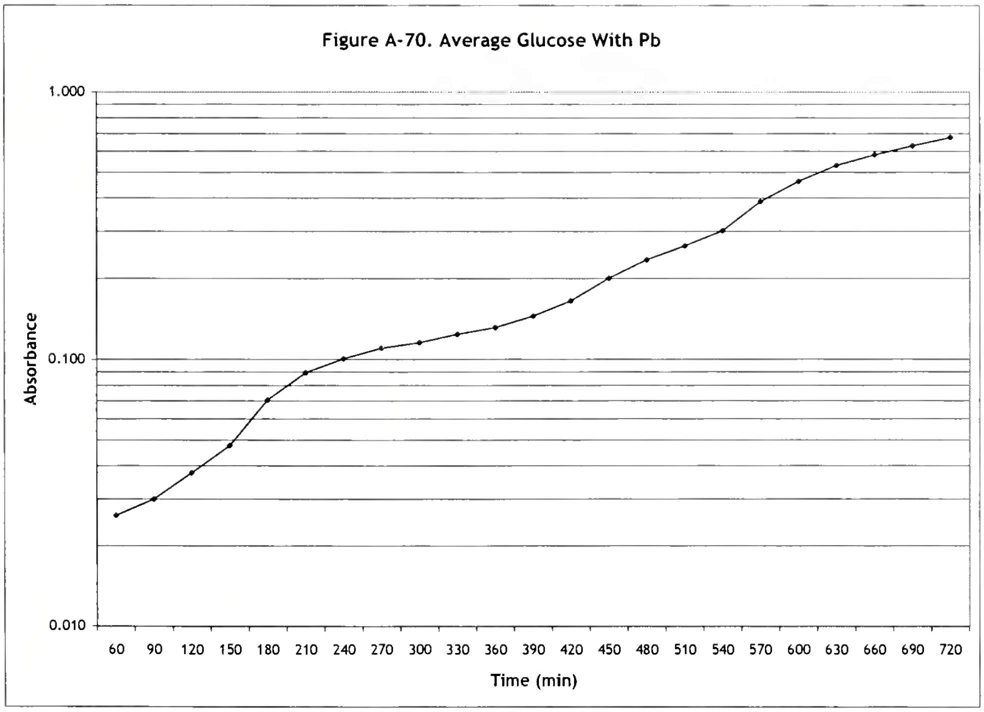
Absorbance increases from 0.102 to .0.206

Actual growth rate = (540 min - 390min) = 150 min



Absorbance increases from 0.148 to .0.294

Actual growth rate = (540 min - 390min) = 150 min



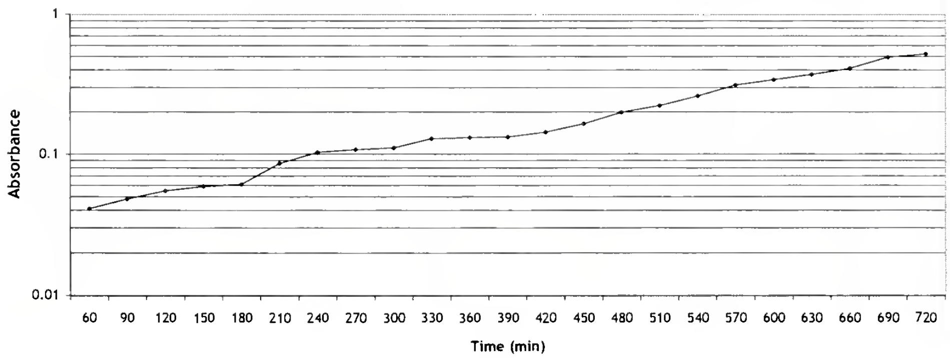
Absorbance increases from 0.132 to .0.265

Actual growth rate = (510 min - 360min) = 150 min

Data for *E.coli* from absorbance vs. time in M9 media
with no casamino acids (poor media)

Glucose With $1*10^{-3}$ M Cd				
Time(min)	Run1	Run2	Run3	Average
60	0.041	0.053	0.051	0.048
90	0.048	0.068	0.056	0.057
120	0.055	0.073	0.069	0.066
150	0.059	0.112	0.075	0.082
180	0.061	0.131	0.098	0.097
210	0.086	0.128	0.102	0.105
240	0.103	0.135	0.108	0.115
270	0.108	0.131	0.114	0.118
300	0.111	0.135	0.121	0.122
330	0.129	0.142	0.128	0.133
360	0.131	0.149	0.136	0.139
390	0.133	0.151	0.149	0.144
420	0.144	0.155	0.153	0.151
450	0.165	0.168	0.162	0.165
480	0.199	0.175	0.171	0.182
510	0.223	0.184	0.213	0.207
540	0.261	0.193	0.243	0.232
570	0.312	0.248	0.258	0.273
600	0.342	0.301	0.306	0.316
630	0.373	0.342	0.351	0.355
660	0.412	0.381	0.396	0.396
690	0.495	0.438	0.449	0.461
720	0.522	0.489	0.482	0.498

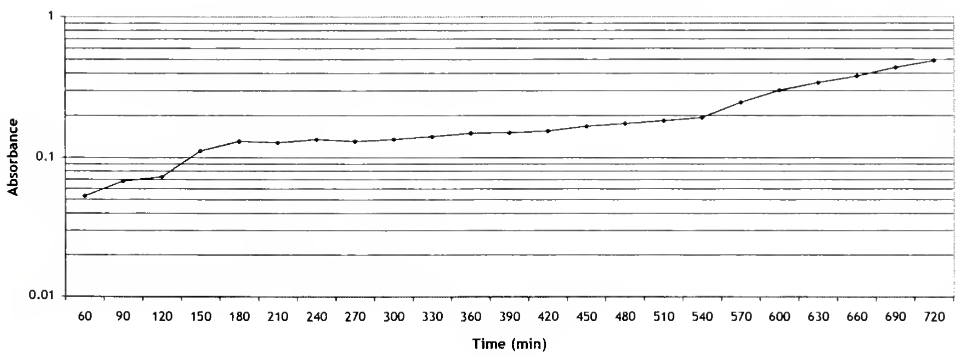
Figure A-71. Glucose With Higher Cd Concentration Run 1



Absorbance increases from 0.129 to .0.261

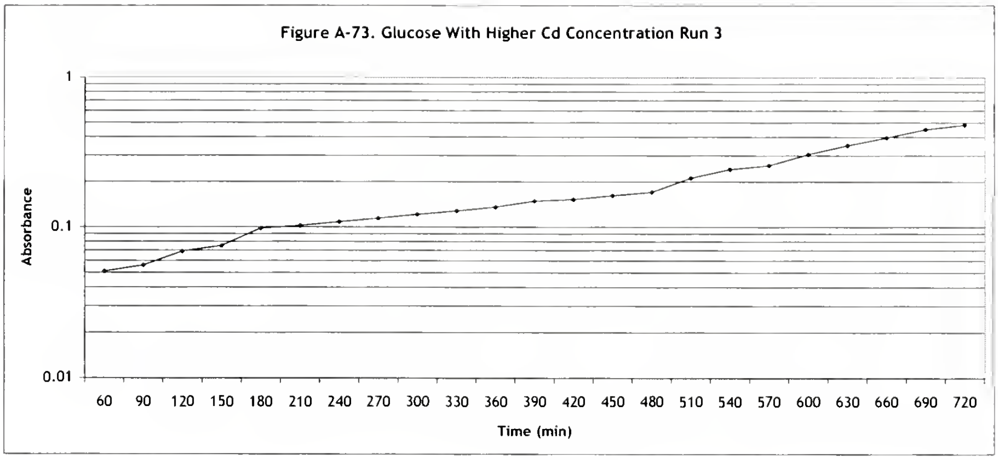
Actual growth rate = (540 min - 330min) = 210 min

Figure A-72. Glucose With Higher Cd Concentration Run 2



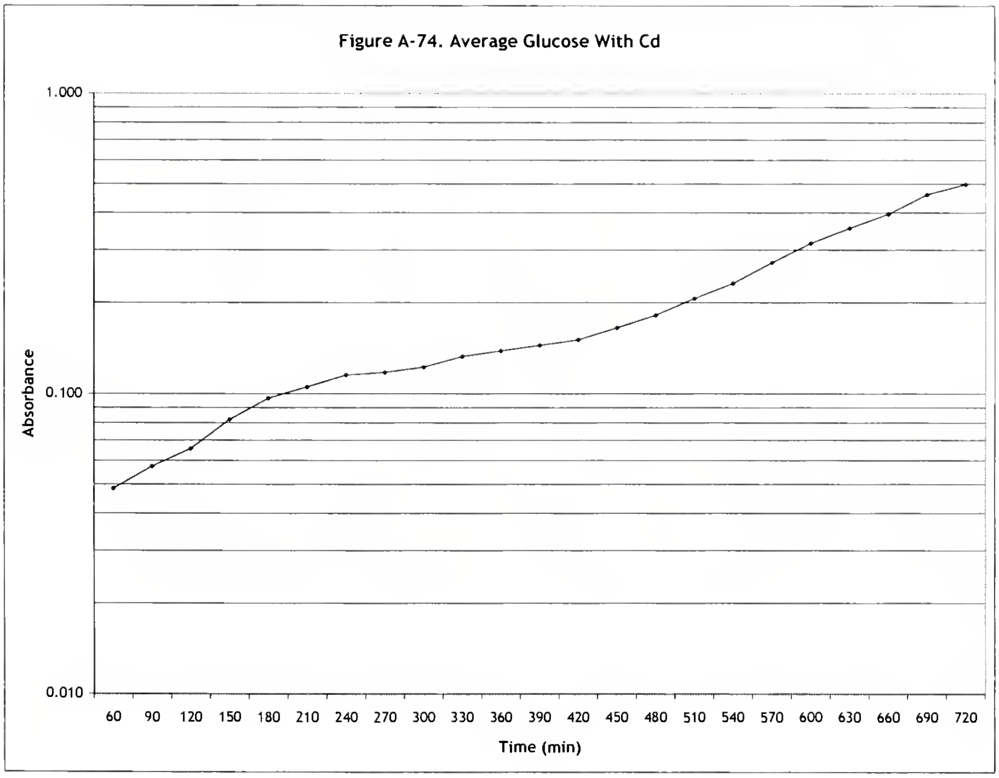
Absorbance increases from 0.151 to .0.301

Actual growth rate = (600 min - 390min) = 210 min



Absorbance increases from 0.128 to 0.258

Actual growth rate = (570 min - 330min) = 240 min



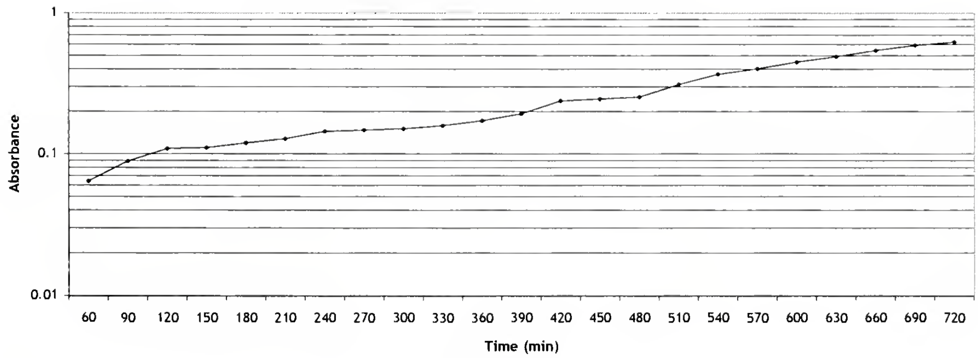
Absorbance increases from 0.139 to .0.280

Actual growth rate = (570 min - 360min) = 210 min

Data for *E.coli* from absorbance vs. time in M9 media
with no casamino acids (poor media)

Glucose With $1*10^{-3}$ M Pb				
Time(min)	Run1	Run2	Run3	Average
60	0.064	0.021	0.041	0.042
90	0.089	0.035	0.048	0.057
120	0.109	0.043	0.055	0.069
150	0.111	0.071	0.086	0.089
180	0.120	0.094	0.103	0.106
210	0.128	0.131	0.111	0.123
240	0.145	0.156	0.121	0.141
270	0.148	0.163	0.142	0.151
300	0.151	0.174	0.158	0.161
330	0.159	0.182	0.163	0.168
360	0.172	0.186	0.178	0.179
390	0.193	0.192	0.188	0.191
420	0.238	0.201	0.211	0.217
450	0.246	0.227	0.233	0.235
480	0.254	0.241	0.248	0.248
510	0.312	0.258	0.273	0.281
540	0.368	0.261	0.341	0.323
570	0.403	0.314	0.359	0.359
600	0.449	0.378	0.377	0.401
630	0.493	0.422	0.412	0.442
660	0.544	0.458	0.495	0.499
690	0.592	0.491	0.522	0.535
720	0.625	0.516	0.543	0.561

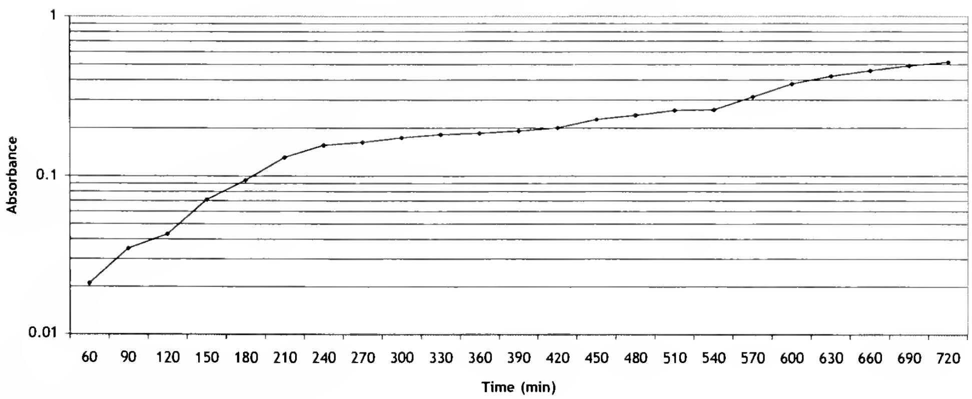
Figure A-75.Glucose With Higher Pb Concentration Run 1



Absorbance increases from 0.179 to .0.359

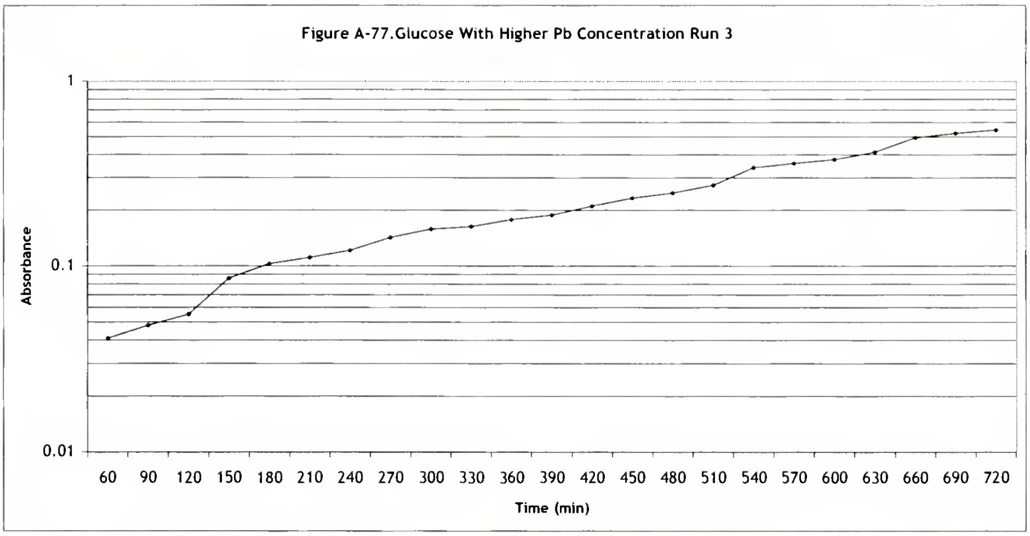
Actual growth rate = (570 min - 360min) = 210 min

Figure A-76.Glucose With Higher Pb Concentration Run 2



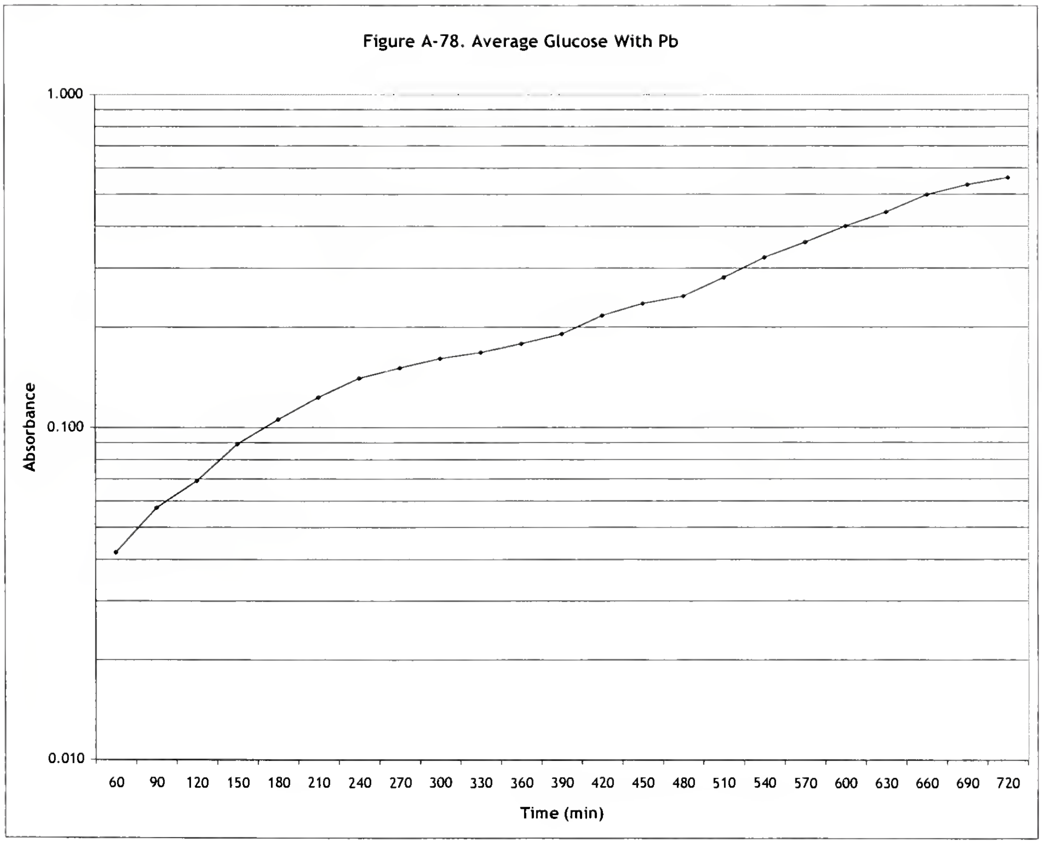
Absorbance increases from 0.179 to .0.359

Actual growth rate = (570 min - 360min) = 210 min



Absorbance increases from 0.188 to .0.377

Actual growth rate = (600 min - 390min) = 210 min



Absorbance increases from 0.179 to .0.359

Actual growth rate = (570 min - 360min) = 210 min

E.coli growth data

Table A-1. *E.coli* cell growth over period of time

	Dilution		
	1:25	1:50	
		(twice for all media)	
	25	50	Average
lactose	6	4	183
lactose and Cd	4	4	167
lactose and Pb	5	4	175
glucose	5	5	208
glucose and Cd	4	3	133
glucose and Pb	8	4	200

growth time: 6 hours

	Dilution		
	1:25	1:250	
		(twice for all media)	
	25	250	Average
lactose	15	1	292
lactose and Cd	7	1	225
lactose and Pb	8	1	233
glucose	12	1	267
glucose and Cd	4	1	200
glucose and Pb	11	1	258

growth time: 7 hours

	Oilution		
	1:25	1:250	
(twice la, la.+Cd, la.+Pb, glu+Pb)		(twice glu)	
	25	250	Average
lactose	36	3	850
lactose and Cd	12	1	283
lactose and Pb	17	2	450
glucose	31	2	592
glucose and Cd	5	1	208
glucose and Pb	13	1	300

growth tim 8 hours

	Oilution		
	1:25	1:250	
	(twice la.+Cd,glu,glu+Pb)	(twice for la.,la.+Pb,glu+Cd)	
	25	250	Average
lactose	49	5	1242
lactose and Cd	18	2	467
lactose and Pb	19	2	492
glucose	35	3	833
glucose and Cd	6	1	217
glucose and Pb	16	2	433

growth time: 9 hours

	Oilution		
	1:25	1 250	
	(twice la.,la+Cd,glu)	(twice la.+Pb,glu+Cd,glu+Pb)	
	25	250	Average
lactose	56	5	1350
lactose and Cd	37	3	867
lactose and Pb	21	2	508
glucose	41	4	1017
glucose and Cd	7	1	225
glucose and Pb	17	2	475

growth time: 10 hours

	Dilution				
	1:50	1: 500	1:2,500	1 :25,000	
	twice(glu,glu+Cd,glu+Pb)	(twice la+Cd,la.+Pb)			
	50	500	2,500	25,000	Average
lactose	84	7	1	0	3400
lactose and Cd	17	2	0	0	950
lactose and Pb	22	2	0	0	1033
glucose	26	2	0	0	1200
glucose and Cd	9	1	0	0	467
glucose and Pb	13	1	0	0	600

growth time: 11 hours

	Dilution				
	1:50	1: 500	1:2,500	1 :25,000	
	(twice la+Cd,glu+Cd,glu+Pb)	(twice la.+Pb,glu)			
	50	500	2,500	25,000	Average
lactose	104	11	2	0	5233
lactose and Cd	21	2	0	0	1033
lactose and Pb	26	5	0	0	2100
glucose	27	2, 3	0	0	1283
glucose and Cd	11	1	0	0	533
glucose and Pb	17	1	0	0	733

growth time: 12 hours

	Dilution				
ice for la.+Cd,glu,glu +Cd,glu+Pb)		1: 500	1:2,500	1 :25,000	
	50	500	2,500	25,000	Average
lactose	171	19	4	0	9350
lactose and Cd	24	2	0	0	1133
lactose and Pb	44	6	1	0	2567
glucose	31	3	0	0	1567
glucose and Cd	17	1	0	0	733
glucose and Pb	18	1	0	0	767

growth time: 12.5 hours

	Dilution	1:500			
	1:50 twice (glu+Pb)	(twice la+Cd,glu,glu+Cd)	1:2,500	1 :25,000	
	50	500	2,500	25,000	Average
lactose	321	38	8	0	18350
lactose and Cd	25	2	1	0	1583
lactose and Pb	64	7	1	0	3067
glucose	33	4	0	0	2050
glucose and Cd	17	2	0	0	950
glucose and Pb	20	2	0	0	1000

growth time: 13 hours

	Dilution	1:500			
	1:50 twice (glu+Pb)	(twice la+Cd,glu,glu+Cd)	1:2,500	1 :25,000	
	50	500	2,500	25,000	Average
lactose	341	39	9	0	19683
lactose and Cd	25	3	1	0	1750
lactose and Pb	69	8	2	0	4150
glucose	41	6	0	0	2517
glucose and Cd	21	3	0	0	1350
glucose and Pb	21	2	0	0	1033

growth time: 14 hours

	Dilution				
	1:50	1: 500	1:2,500	1 :25,000	
	twice(glu+Pb)	twice(glu+Cd)			
	50	500	2,500	25,000	Average
lactose	100+	218	44	4	106333
lactose and Cd	48	5	1	0	2467
lactose and Pb	242	23	5	0	12033
glucose	66	6	2	0	3767
glucose and Cd	36	4	0	0	1933
glucose and Pb	50	6	0	0	2667

growth time: 15 hours

	Oilution					
	1:50	1: 500	1:2,500	1 :25,000	1:125,000	
	50	500	2,500	25,000	125,000	Average
lactose	100+	100+	124	13	3	336667
lactose and Cd	82	8	2	0	0	4367
lactose and Pb	100+	48	8	1	0	23000
glucose	183	18	4	0	0	9383
glucose and Cd	68	8	2	0	0	4133
glucose and Pb	78	9	2	0	0	4467

growth time: 16 hours

	Oilution						
	1:50	1:500	1:2,500	1:25,000	1:125,000		
	50	500	2,500	25,000	125,000		Average
		twice(glu,glu+Cd)					
lactose	100+	100+	224	22	5		578333
lactose and Cd	165	16	4	0	0		8750
lactose and Pb	100+	294	64	6	0		152333
glucose	100+	46	9	0	0		22667
glucose and Cd	96	10	0	0	0		4933
glucose and Pb	113	14	2	0	0		5883

growth time: 17 hours

	Oilution					
	1:50	1:500	1:2,500	1:25,000	1:125,000	
	50	500	2,500	25,000	125,000	Average
		twice(glu+Cd)				
lactose	100+	100+	232	25	5	610000
lactose and Cd	218	22	6	0	0	12300
lactose and Pb	100+	100+	165	16	3	395833
glucose	100+	132	27	3	0	69500
glucose and Cd	115	10	0	0	0	5250
glucose and Pb	143	16	3	0	0	7550

growth time: 18 hours

	Dilution				
	1:50	1:500	1:2,500	1:25,000	
	50	500	2,500	25,000	Average
		twice(glu+Cd)	twice(la.+Pb)	twice(la.)	
lactose	100+	100+	256	27	663333
lactose and Cd	264	26	6	0	13733
lactose and Pb	100+	100+	175	16	425000
glucose	100+	258	53	5	128833
glucose and Cd	114	10	2	0	5400
glucose and Pb	149	17	3	0	7817

growth time: 19 hours

	Oilution			
	1:2,500	1:25,000	1:125,000	
	3 times(la+Cd,glu+Cd,glu +Pb)			
	2,500	25,000	125,000	Average
lactose	282	28	6	718333
lactose and Cd	7	0	0	17500
lactose and Pb	172	16	3	401667
glucose	51	4	1	117500
glucose and Cd	2-twice, 3	0	0	5833
glucose and Pb	3	0	0	7500

growth time: 19.5 hours

	Dilution			
	1:2,500	1:25,000	1:125,000	
3 times(glu +Cd, glu +Pb)		twice(la.,la+Cd)		
	2,500	25,000	125,000	Average
lactose	100+	29	6	733333
lactose and Cd	8	1	0	23333
lactose and Pb	181	18	4	467500
glucose	52	5	1	126667
glucose and Cd	2, 3-twice	0	0	6667
glucose and Pb	4, 3-twice	0	0	8333

growth time: 20 hours

	Dilution			
	1:2,500	1:25,000	1:125,000	
3 times(glu +Cd, glu +Pb)		twice(la.,la+Cd)		
	2,500	25,000	125,000	Average
lactose	100+	31	6	766667
lactose and Cd	9	1	0	24167
lactose and Pb	192	18	4	476666
glucose	98	9	2	240000
glucose and Cd	3	0	0	7500
glucose and Pb	4	0	0	10000

growth time: 21 hours

	Dilution			
	1:2,500	1:25,000	1:125,000	
3 times(glu +Cd, glu +Pb)		twice(la+Cd)		
	2,500	25,000	125,000	Average
lactose	330	31	7	825000
lactose and Cd	11	1	0	25833
lactose and Pb	199	19	4	490833
glucose	105	10	2	254167
glucose and Cd	3	0	0	7500
glucose and Pb	4	0	0	10000

growth time: 22 hours

	Dilution			
	1:2,500	1:25,000	1:125,000	
	3 times (glu +Pb)	twice(la+Cd)	twice(la.)	
	2,500	25,000	125,000	Average
lactose	100+	37	8	975000
lactose and Cd	15	1	0	29167
lactose and Pb	198	20	4	498333
glucose	119	12	2	282500
glucose and Cd	3-twice,	0	0	8333
glucose and Pb	5	0	0	12500

growth time: 23 hours

	Dilution			
	1:2,500	1:25,000	1:125,000	
	3 times(glu +Pb)	twice(la.,la+Cd)		
	2,500	25,000	125,000	Average
lactose	100+	41	8	1016667
lactose and Cd	14	2	0	45000
lactose and Pb	201	23	4	525833
glucose	123	13	3	335833
glucose and Cd	4, 5-twice	0	0	11667
glucose and Pb	6	0	0	15000

growth time: 24 hours

	Dilution			
	1:2,500	1:25,000	1:125,000	
	3 times(glu +Pb)	twice(la.,la+Cd)		
	2,500	25,000	125,000	Average
lactose	100+	48	9	1175000
lactose and Cd	18	2	0	48333
lactose and Pb	214	23	5	578333
glucose	141	14	3	359167
glucose and Cd	4, 6-twi	0	0	13333
glucose and Pb	7	0	0	17500

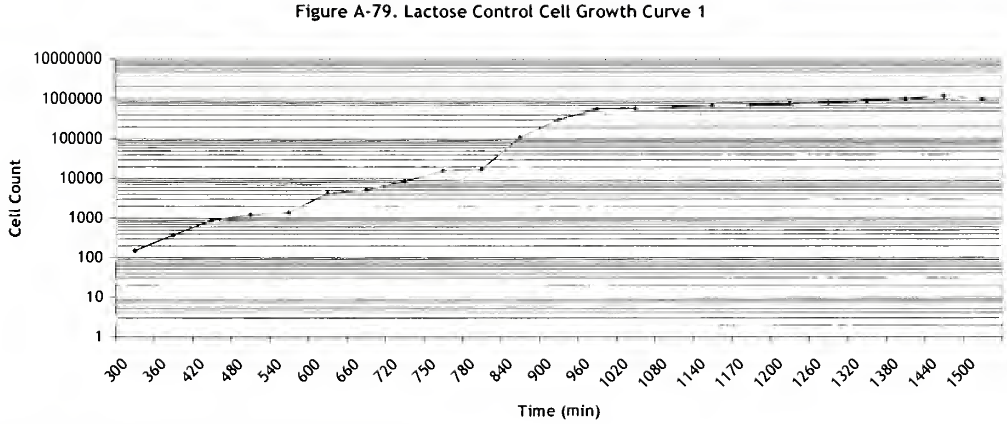
growth time: 25 hours

	Dilution			
	1:2,500	1:25,000	1:125,000	
twice(la+Cd), 3 times(glu +Cd, glu +Pb)				
	2,500	25,000	125,000	Average
lactose	100+	39	8	991667
lactose and Cd	15	1	0	33333
lactose and Pb	192	18	4	476667
glucose	118	12	2	281667
glucose and Cd	5	0	0	12500
glucose and Pb	5	0	0	12500

LACTOSE ONLY CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Average
300	150	200	200	183
360	375	250	250	292
420	900	900	750	850
480	1225	1250	1250	1242
540	1400	1400	1250	1350
600	4200	3500	2500	3400
660	5200	5500	5000	5233
720	8550	9500	10000	9350
750	16050	19000	20000	18350
780	17050	19500	22500	19683
840	109000	110000	100000	106333
900	310000	325000	375000	336667
960	560000	550000	625000	578333
1020	580000	625000	625000	610000
1080	640000	675000	675000	663333
1140	705000	700000	750000	718333
1170	725000	725000	750000	733333
1200	775000	775000	750000	766667
1260	825000	775000	875000	825000
1320	925000	1000000	1000000	975000
1380	1025000	1025000	1000000	1016667
1440	1200000	1200000	1125000	1175000
1500	975000	1000000	1000000	991667

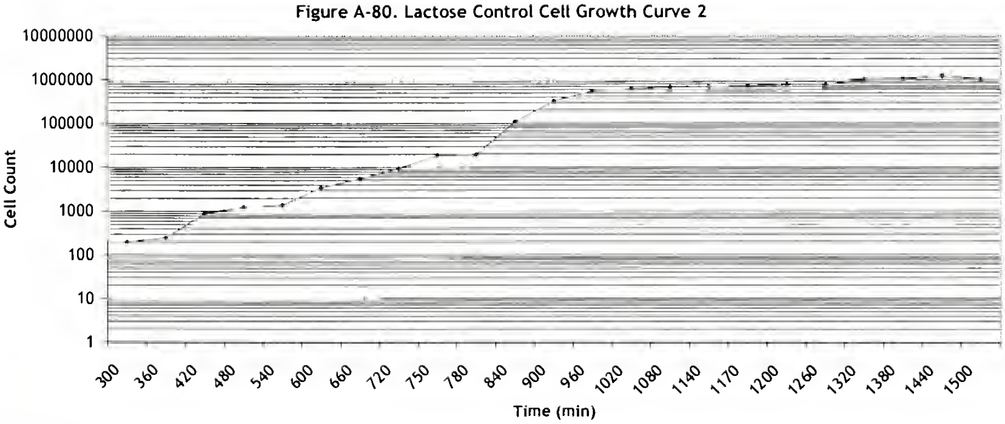
Figure A-79. Lactose Control Cell Growth Curve 1



Cell Count increases from 8550 to 17050

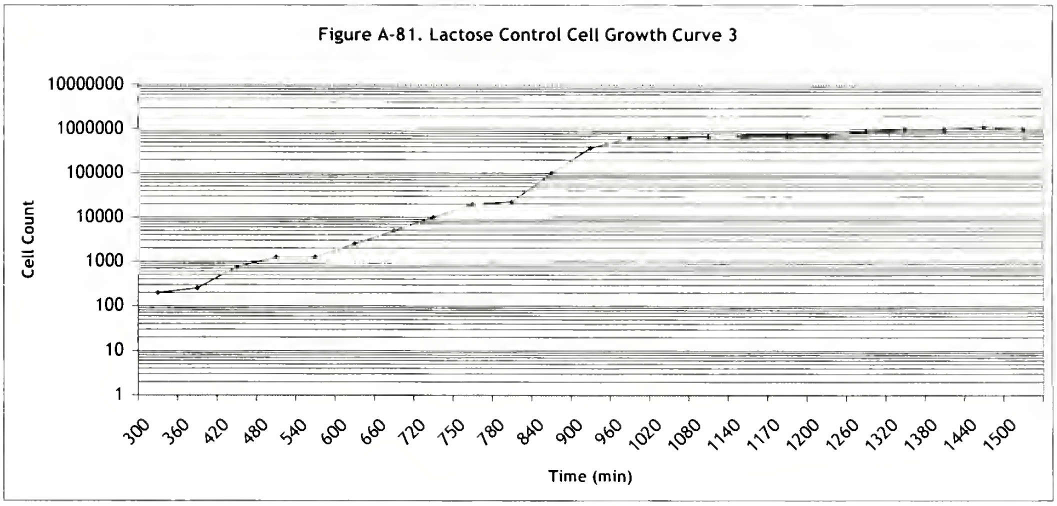
Actual growth rate = (780 min -720min) = 60 min

Figure A-80. Lactose Control Cell Growth Curve 2



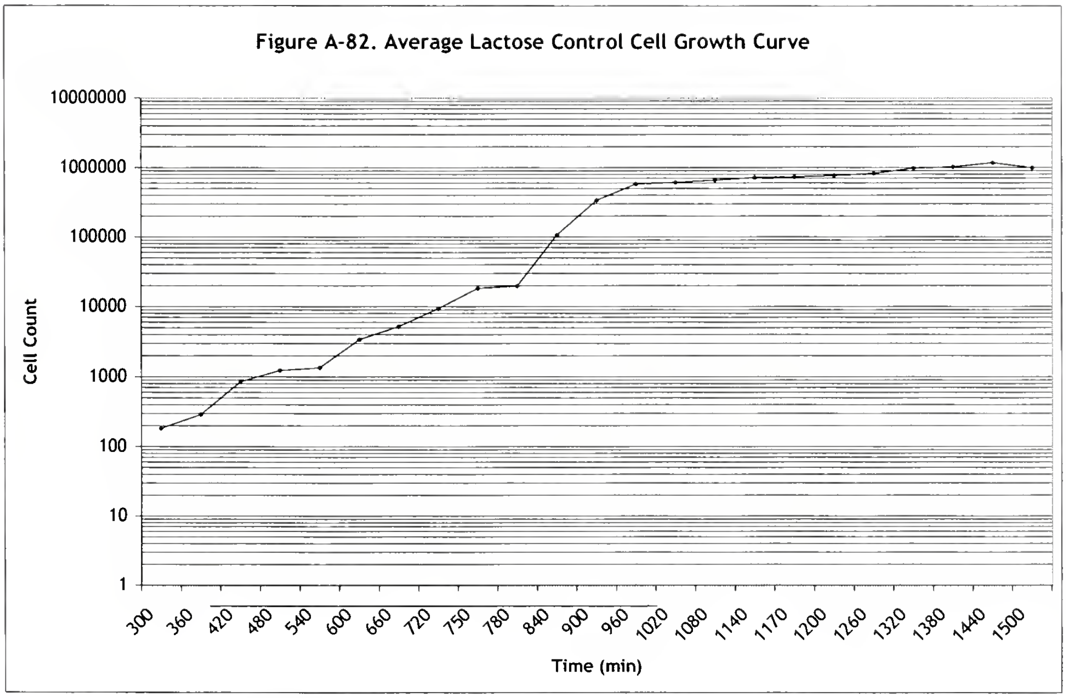
Cell Count increases from 9500 to 19000

Actual growth rate = (750 min -720min) = 30 min



Cell Count increases from 10000 to 20000

Actual growth rate = (750 min -720min) = 30 min



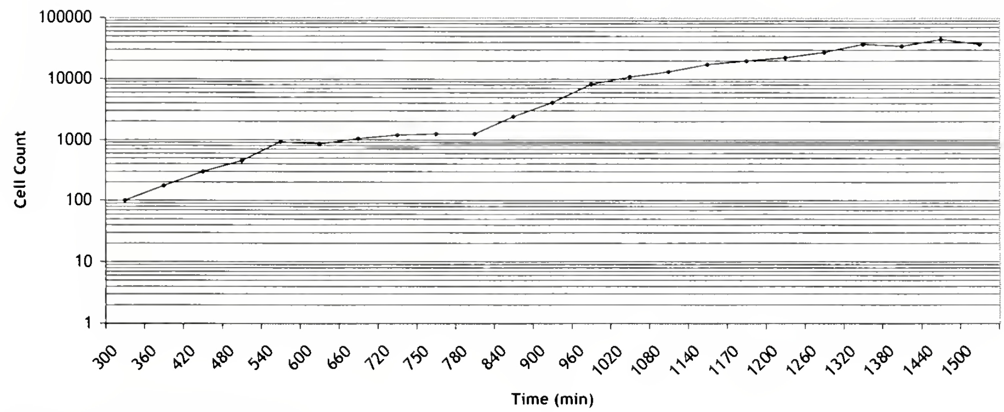
Cell Count increases from 9350 to 18350

Actual growth rate = (750 min -720min) = 30 min

LACTOSE WITH Cd CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Average
300	100	200	200	167
360	175	250	250	225
420	300	300	250	283
480	450	450	500	467
540	925	925	750	867
600	850	1000	1000	950
660	1050	1050	1000	1033
720	1200	1200	1000	1133
750	1250	1000	2500	1583
780	1250	1500	2500	1750
840	2400	2500	2500	2467
900	4100	5000	4000	4367
960	8250	8000	10000	8750
1020	10900	11000	15000	12300
1080	13200	13000	15000	13733
1140	17500	17500	17500	17500
1170	20000	25000	25000	23333
1200	22500	25000	25000	24167
1260	27500	25000	25000	25833
1320	37500	25000	25000	29167
1380	35000	50000	50000	45000
1440	45000	50000	50000	48333
1500	37500	37500	25000	33333

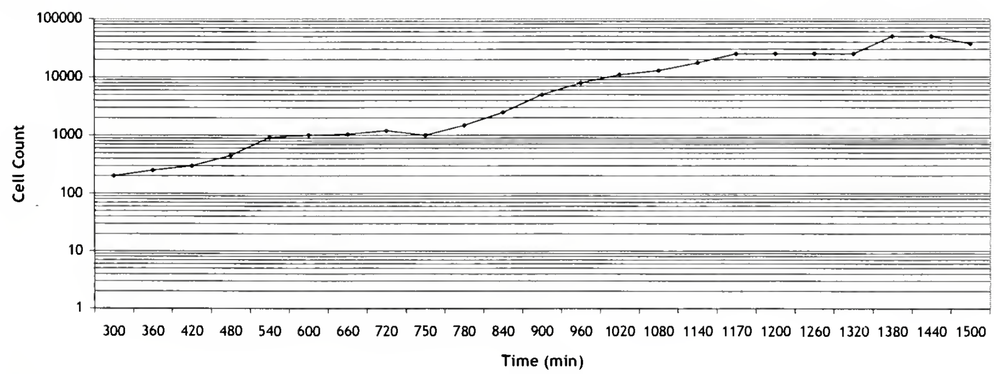
Figure A-83. Lactose With Cd Cell Growth Curve 1



Cell Count increases from 10900 to 22500

Actual growth rate = (1200 min -1020min) = 180 min

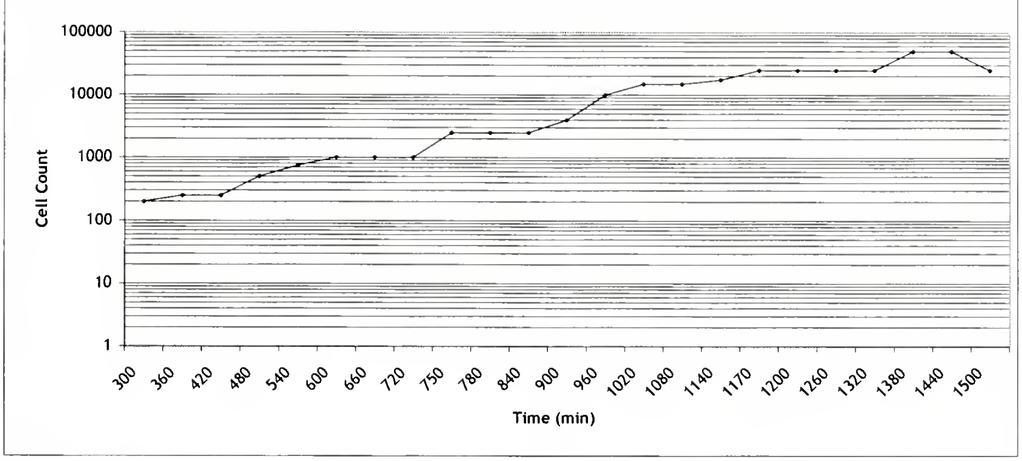
Figure A-84. Lactose With Cd Cell Growth Curve 2



Cell Count increases from 25000 to 50000

Actual growth rate = (1380 min -1170 min) = 210 min

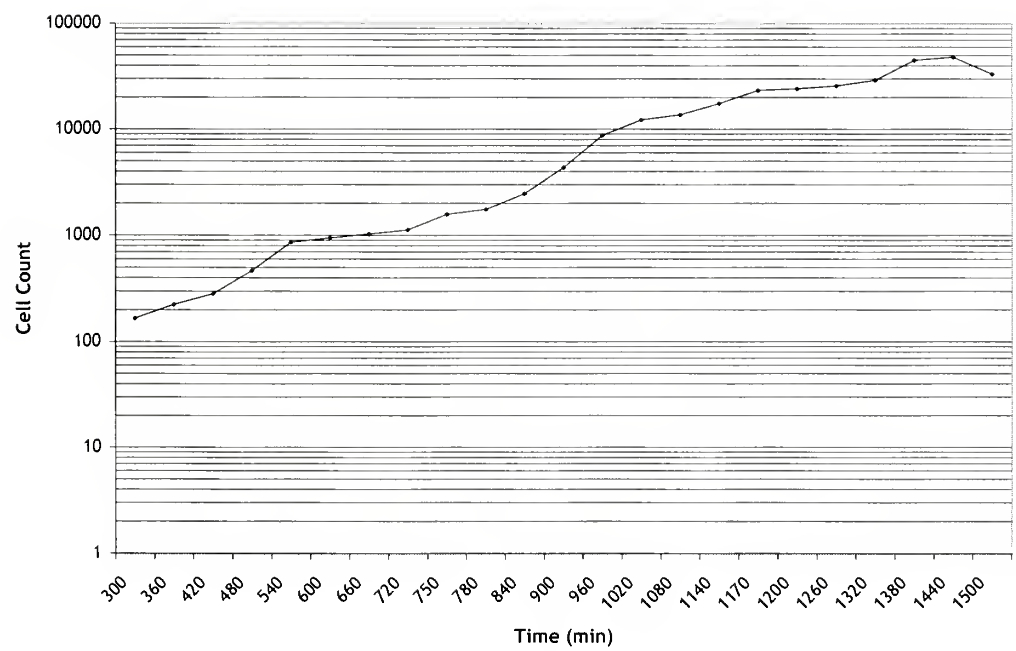
Figure A-85. Lactose With Cd Cell Growth Curve 3



Cell Count increases from 25000 to 50000

Actual growth rate = (1380 min -1170 min) = 210 min

Figure A-86. Average Lactose With Cd Cell Growth Curve



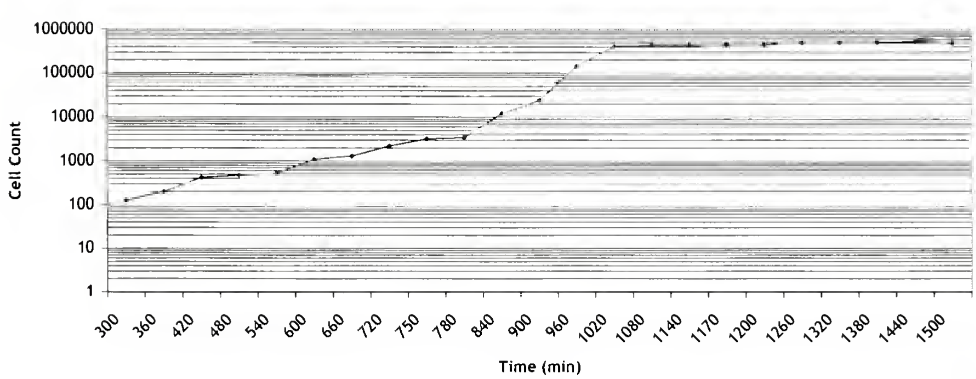
Cell Count increases from 8750 to 17500

Actual growth rate = (1140 min -960min) = 180 min

LACTOSE WITH Pb CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Average
300	125	200	200	175
360	200	250	250	233
420	425	425	500	450
480	475	500	500	492
540	525	500	500	508
600	1100	1000	1000	1033
660	1300	2500	2500	2100
720	2200	3000	2500	2567
750	3200	3500	2500	3067
780	3450	4000	5000	4150
840	12100	11500	12500	12033
900	24000	20000	25000	23000
960	147000	160000	150000	152333
1020	412500	400000	375000	395833
1080	437500	437500	400000	425000
1140	430000	400000	375000	401667
1170	452500	450000	500000	467500
1200	452500	450000	500000	467500
1260	497500	475000	500000	490833
1320	495000	500000	500000	498333
1380	502500	575000	500000	525833
1440	535000	575000	625000	578333
1500	480000	450000	500000	476667

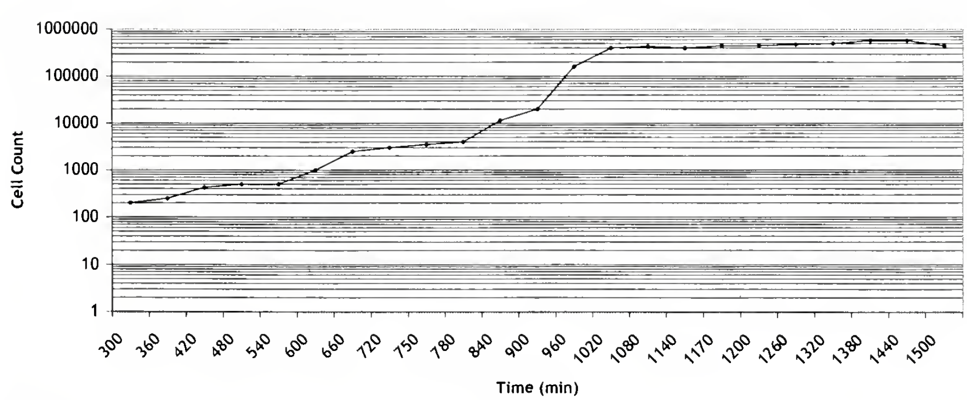
Figure A-87. Lactose With Pb Cell Growth Curve 1



Cell Count increases from 1100 to 2200

Actual growth rate = (720 min -600min) = 120 min

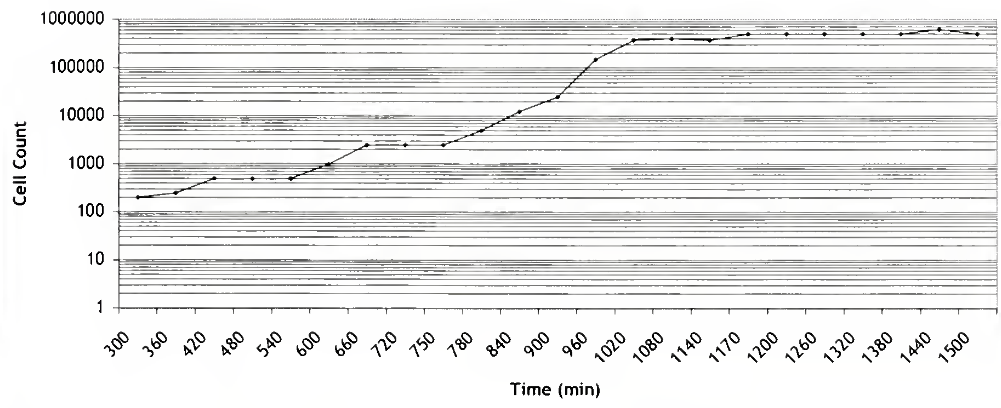
Figure A-88. Lactose With Pb Cell Growth Curve 2



Cell Count increases from 500 to 1000

Actual growth rate = (600 min -480min) = 120 min

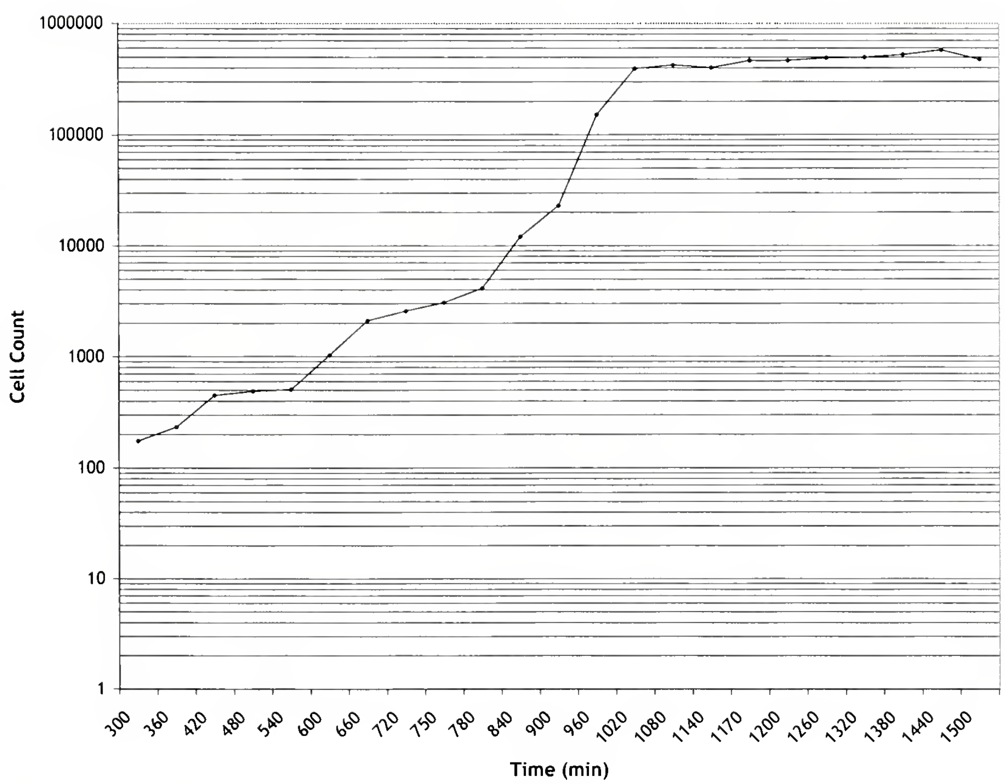
Figure A-89. Lactose With Pb Cell Growth Curve 3



Cell Count increases from 2500 to 5000

Actual growth rate = (780 min -660 min) = 120 min

Figure A-90. Average Lactose With Pb Cell Growth Curve



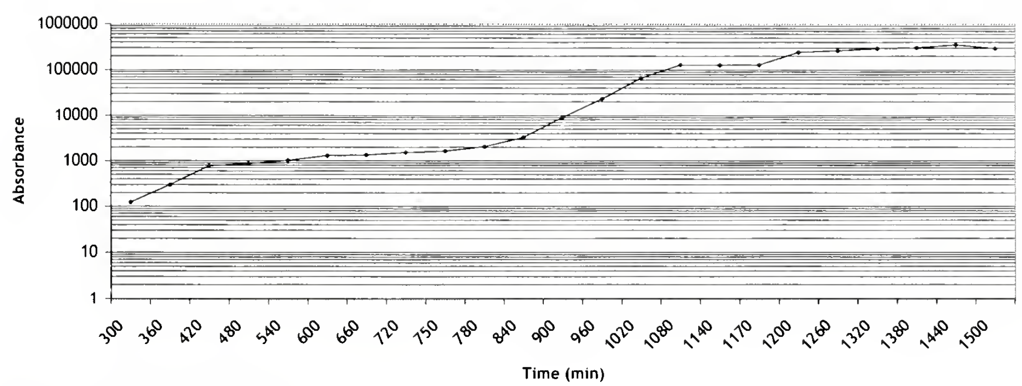
Cell Count increases from 2100 to 4150

Actual growth rate = (780 min -660min) = 120 min

GLUCOSE ONLY CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Average
300	125	250	250	208
360	300	250	250	267
420	775	500	500	592
480	875	750	875	833
540	1025	1000	1025	1017
600	1300	1000	1300	1200
660	1350	1000	1500	1283
720	1550	1500	1650	1567
750	1650	2000	2500	2050
780	2050	3000	2500	2517
840	3300	3000	5000	3767
900	9150	9000	10000	9383
960	23000	22500	22500	22667
1020	66000	67500	75000	69500
1080	129000	132500	125000	128833
1140	127500	100000	125000	117500
1170	130000	125000	125000	126667
1200	245000	225000	250000	240000
1260	262500	250000	250000	254167
1320	297500	300000	250000	282500
1380	307500	325000	375000	335833
1440	352500	350000	375000	359167
1500	295000	300000	250000	281667

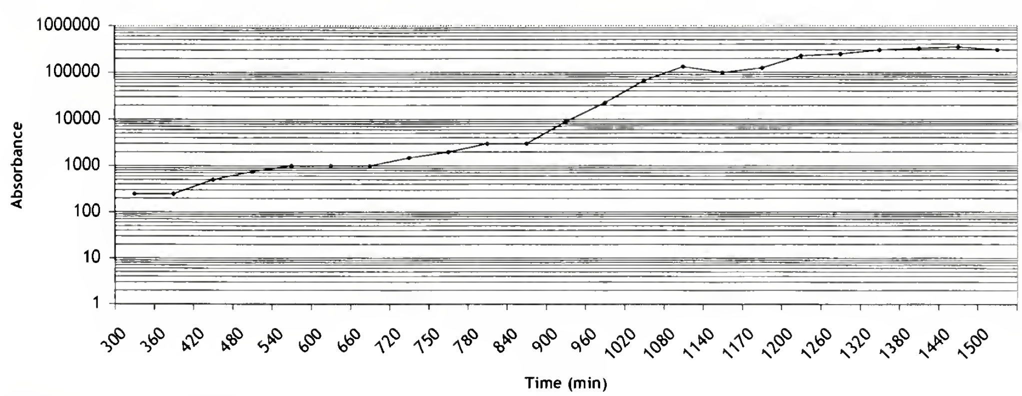
Figure A-91. Glucose Control Cell Growth Curve 1



Cell Count increases from 1650 to 3300

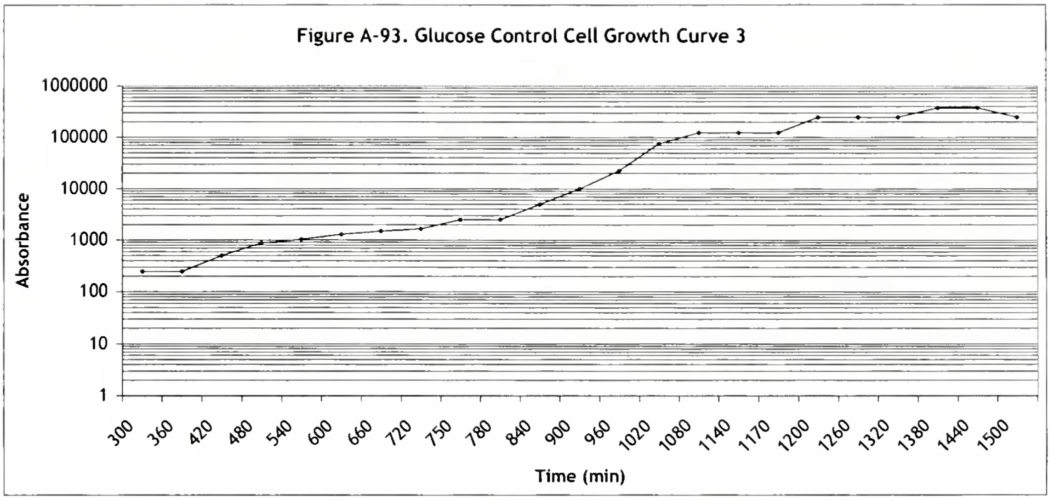
Actual growth rate = (840min -750min) = 90 min

Figure A-92. Glucose Control Cell Growth Curve 2



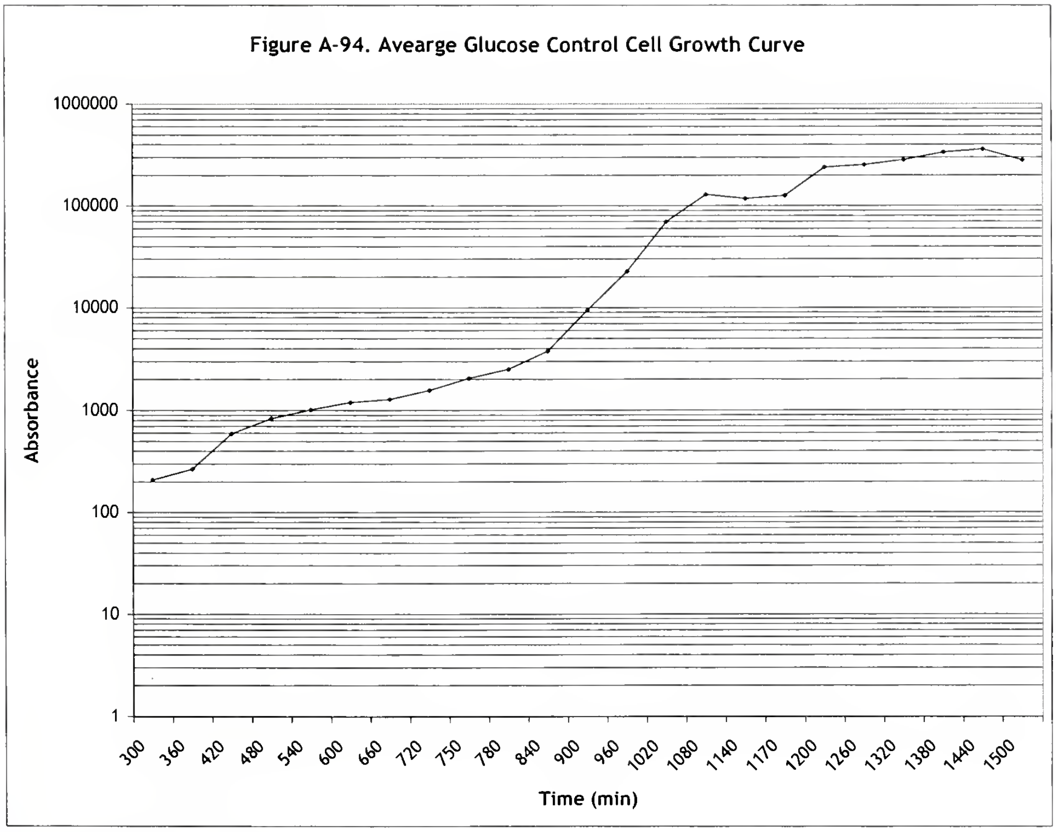
Cell Count increases from 1500 to 3000

Actual growth rate = (780min -720min) = 60 min



Cell Count increases from 5000 to 10000

Actual growth rate = (900min -840min) = 60 min



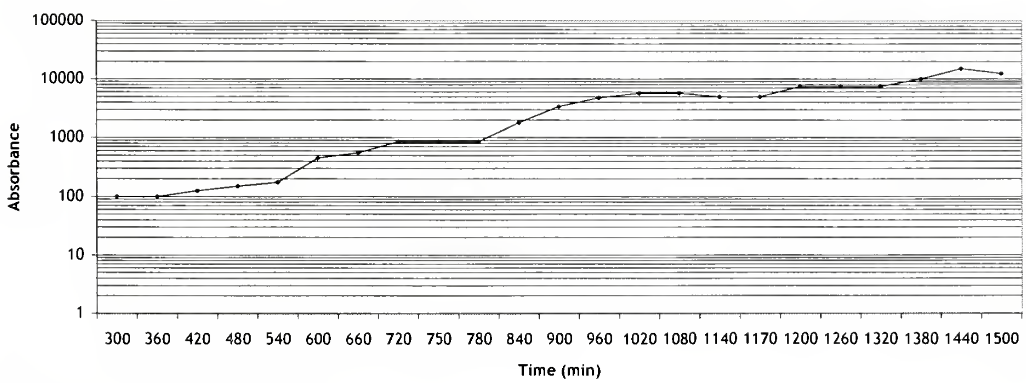
Cell Count increases from 126667 to 254167

Actual growth rate = (1260min -1170min) = 90 min

GLUCOSE WITH Cd CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Average
300	100	150	150	133
360	100	250	250	200
420	125	250	250	208
480	150	250	250	217
540	175	250	250	225
600	450	500	450	467
660	550	500	550	533
720	850	500	850	733
750	850	1000	1000	950
780	850	1000	1500	1117
840	1800	2000	2000	1933
900	3400	4000	5000	4133
960	4800	5000	5000	4933
1020	5750	5000	5000	5250
1080	5700	5000	5500	5400
1140	5000	5000	7500	5833
1170	5000	7500	7500	6667
1200	7500	7500	7500	7500
1260	7500	7500	7500	7500
1320	7500	7500	10000	8333
1380	10000	12500	12500	11667
1440	15000	10000	15000	13333
1500	12500	12500	12500	12500

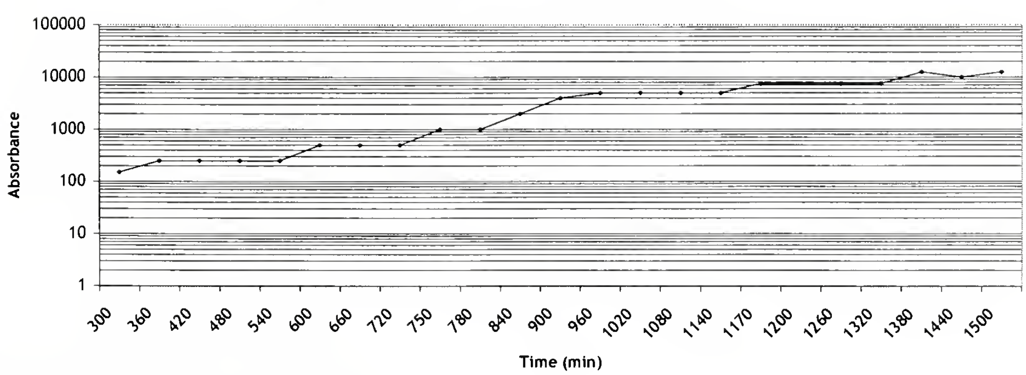
Figure A-95. Glucose With Cd Cell Growth Curve 1



Cell Count increases from 5000 to 10000

Actual growth rate = (1380min -1170min) = 210 min

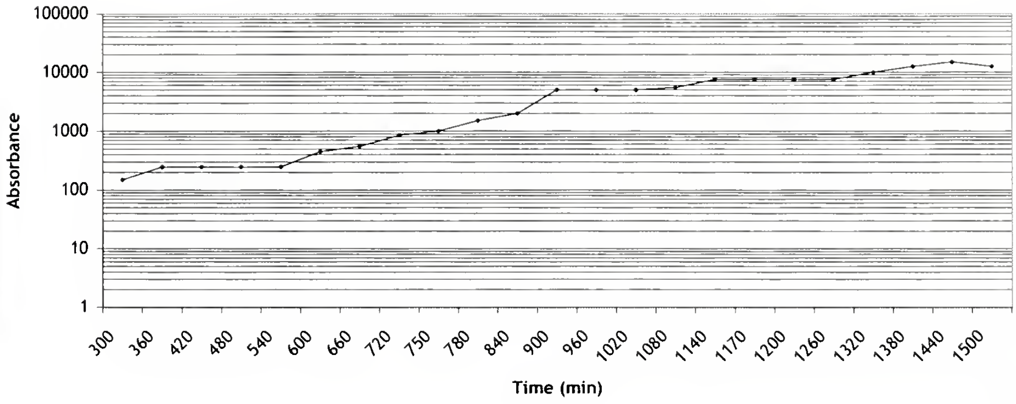
Figure A-96. Glucose With Cd Cell Growth Curve 2



Cell Count increases from 5000 to 10000

Actual growth rate = (1440min -1140min) = 300 min

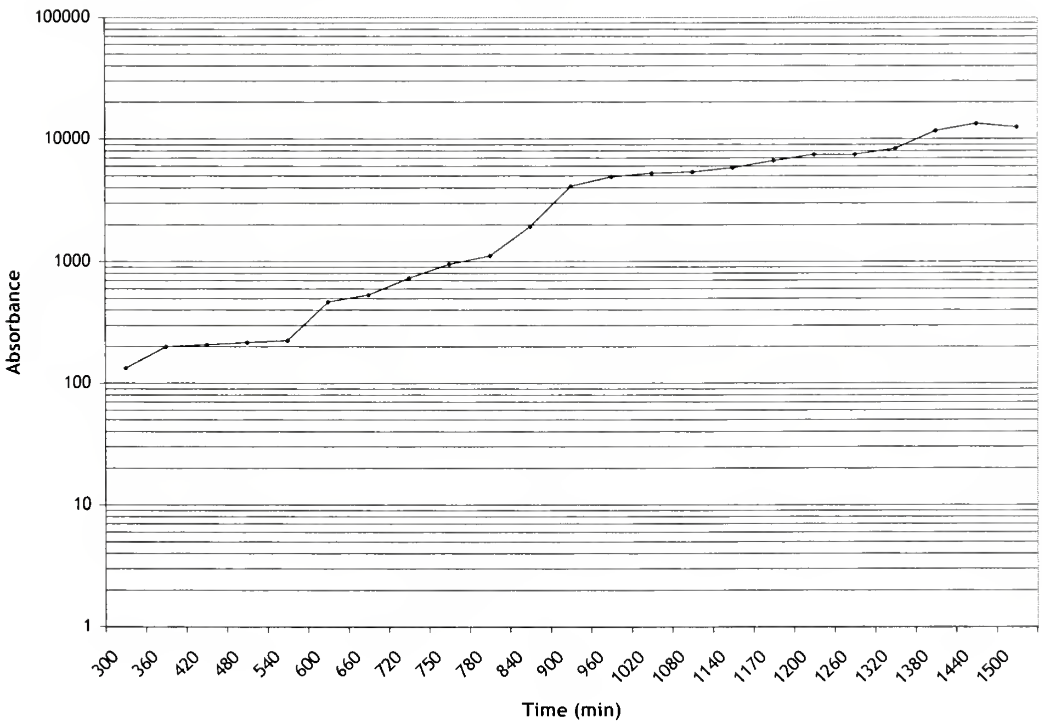
Figure A-97. Glucose With Cd Cell Growth Curve 3



Cell Count increases from 5000 to 10000

Actual growth rate = (1320min -1020min) = 300 min

Figure A-98. Average Glucose With Cd Cell Growth Curve



Cell Count increases from 6667 to 13333

Actual growth rate = (1440min -1170min) = 270 min

GLOSSARY

GLUCOSE WITH Pb CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Average
300	200	200	200	200
360	275	250	250	258
420	325	250	325	300
480	400	500	400	433
540	425	500	500	475
600	650	500	650	600
660	850	500	850	733
720	900	500	900	767
750	1000	1000	1000	1000
780	1000	1000	1050	1017
840	2500	3000	2500	2667
900	3900	4500	5000	4467
960	5650	7000	5000	5883
1020	7150	8000	7500	7550
1080	7450	8500	7500	7817
1140	7500	7500	7500	7500
1170	10000	7500	7500	8333
1200	10000	10000	10000	10000
1260	10000	10000	10000	10000
1320	12500	12500	12500	12500
1380	15000	15000	15000	15000
1440	17500	17500	17500	17500
1500	12500	12500	12500	12500

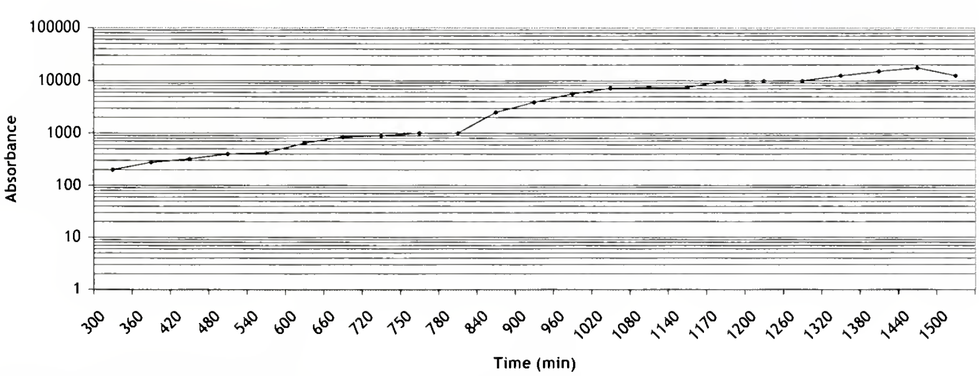


Figure A-99. Glucose With Pb Cell Growth Curve 1

Cell Count increases from 425 to 850

Actual growth rate = (660min -540min) = 120 min

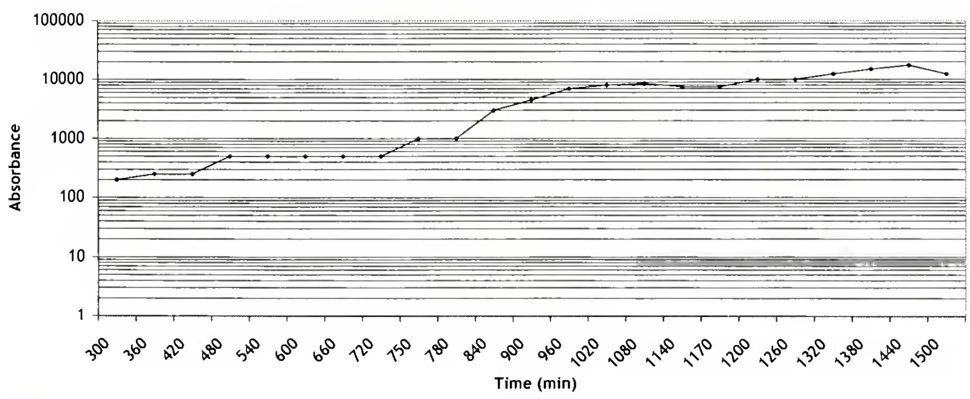
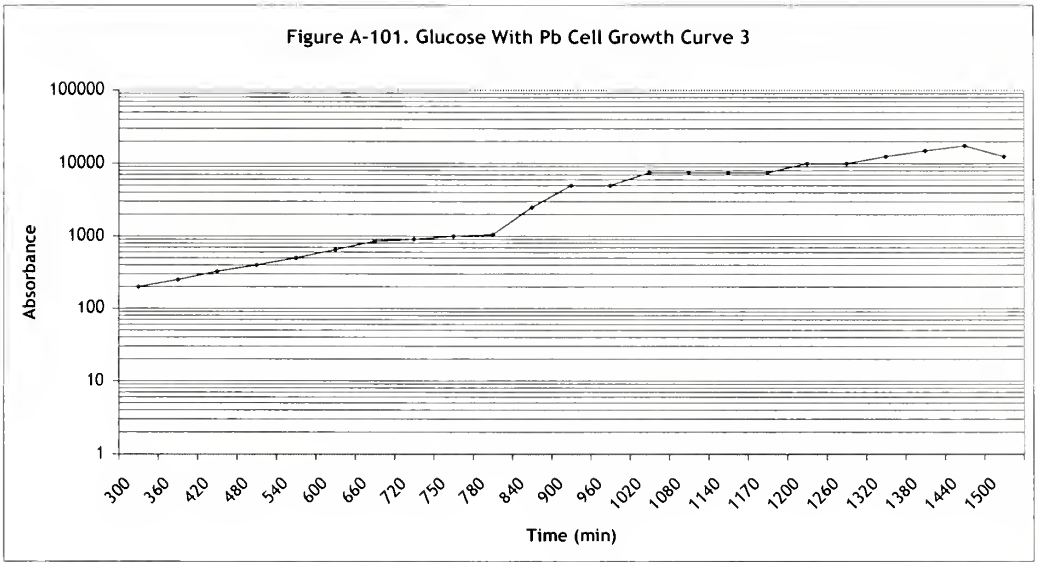


Figure A-100. Glucose With Pb Cell Growth Curve 2

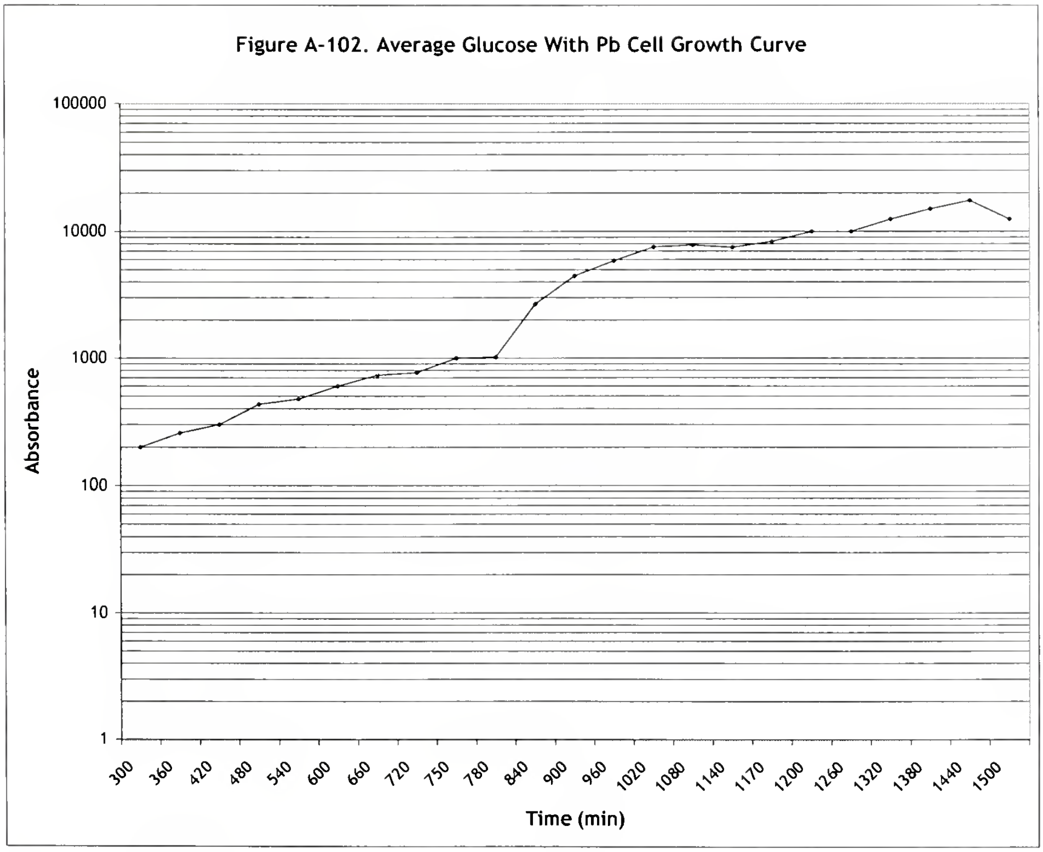
Cell Count increases from 7500 to 15000

Actual growth rate = (1380min -1170min) = 210 min



Cell Count increases from 7500 to 15000

Actual growth rate = (1380min -1170min) = 210 min



Cell Count increases from 300 to 600

Actual growth rate = (600min -420min) = 180 min

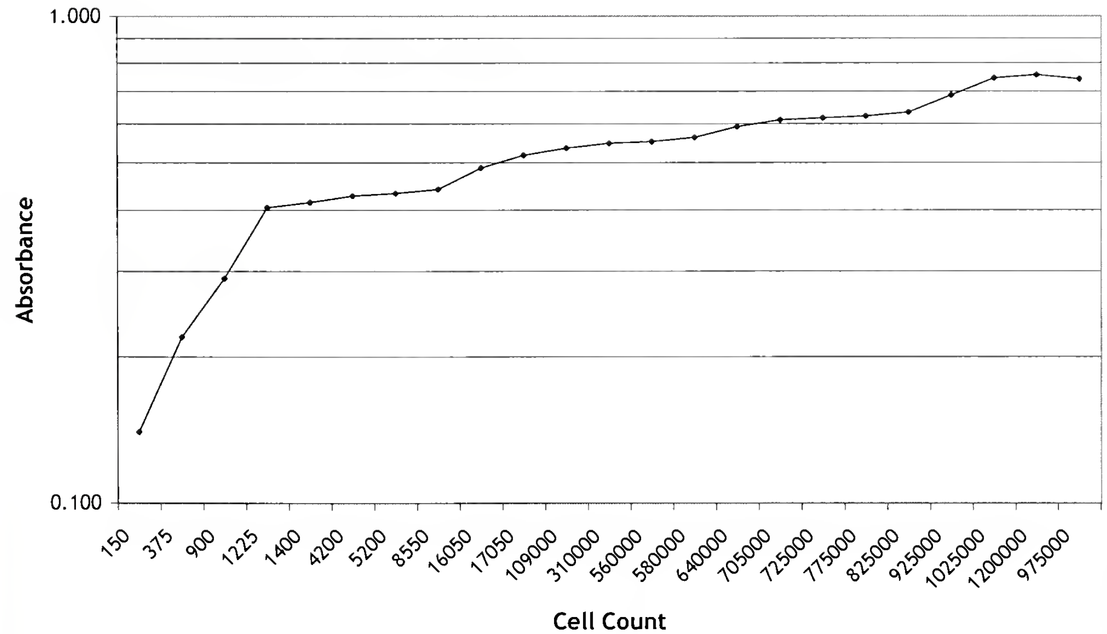
E.coli Cell growth vs. Absorbance

LACTOSE ONLY CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
300	150	0.140	1.38	0.724
360	375	0.220	1.66	0.603
420	900	0.290	1.95	0.513
480	1225	0.405	2.54	0.394
540	1400	0.415	2.60	0.385
600	4200	0.428	2.68	0.373
660	5200	0.433	2.71	0.369
720	8550	0.441	2.76	0.362
750	16050	0.488	3.08	0.325
780	17050	0.518	3.30	0.303
840	109000	0.535	3.43	0.292
900	310000	0.548	3.53	0.283
960	560000	0.552	3.56	0.281
1020	580000	0.563	3.66	0.274
1080	640000	0.592	3.91	0.256
1140	705000	0.611	4.08	0.245
1170	725000	0.617	4.14	0.242
1200	775000	0.623	4.20	0.238
1260	825000	0.634	4.31	0.232
1320	925000	0.687	4.86	0.206
1380	1025000	0.745	5.56	0.180
1440	1200000	0.757	5.71	0.175
1500	975000	0.742	5.52	0.181

Cell Count	Absorbance
150	0.140
375	0.220
900	0.290
1225	0.405
1400	0.415
4200	0.428
5200	0.433
8550	0.441
16050	0.488
17050	0.518
109000	0.535
310000	0.548
560000	0.552
580000	0.563
640000	0.592
705000	0.611
725000	0.617
775000	0.623
825000	0.634
925000	0.687
1025000	0.745
1200000	0.757
975000	0.742

Figure A-103. Lactose Control Absorbance vs.Cell Count 1



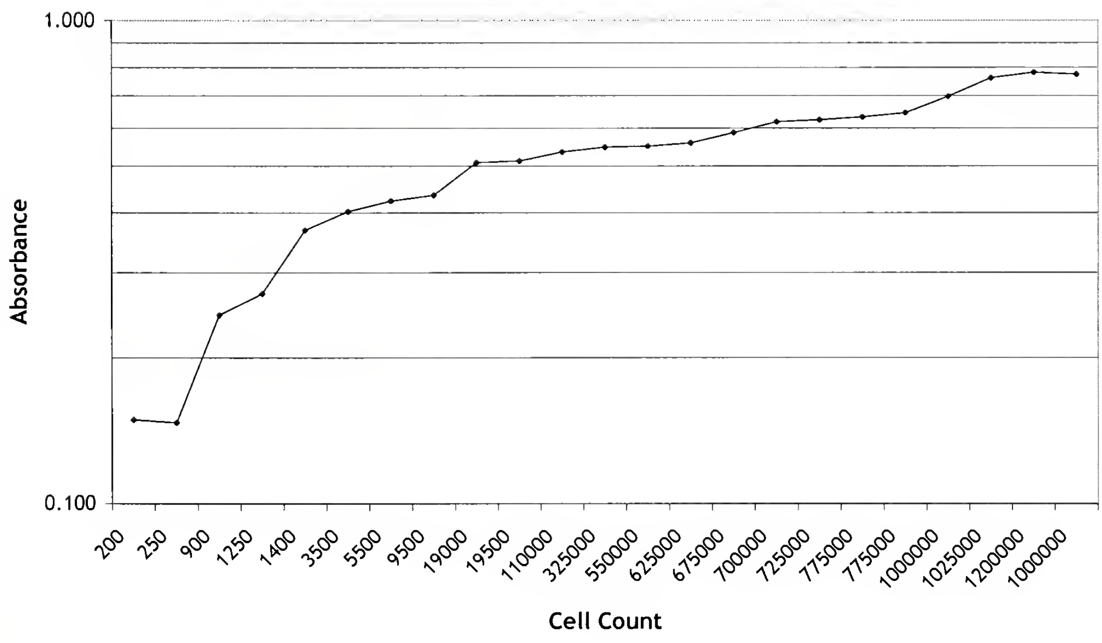
Cell Count increases from 8550 to 17050

Difference in absorbance = (0.518 -0.441) = 0.077

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
300	200	0.149	1.41	0.710
360	250	0.147	1.40	0.713
420	900	0.245	1.76	0.569
480	1250	0.271	1.87	0.536
540	1400	0.368	2.33	0.429
600	3500	0.402	2.52	0.396
660	5500	0.423	2.65	0.378
720	9500	0.435	2.72	0.367
750	19000	0.509	3.23	0.310
780	19500	0.513	3.26	0.307
840	110000	0.535	3.43	0.292
900	325000	0.548	3.53	0.283
960	550000	0.550	3.55	0.282
1020	625000	0.559	3.62	0.276
1080	675000	0.587	3.86	0.259
1140	700000	0.618	4.15	0.241
1170	725000	0.624	4.21	0.238
1200	775000	0.633	4.30	0.233
1260	775000	0.645	4.42	0.226
1320	1000000	0.698	4.99	0.200
1380	1025000	0.762	5.78	0.173
1440	1200000	0.782	6.05	0.165
1500	1000000	0.775	5.96	0.168

Cell Count	Absorbance
200	0.149
250	0.147
900	0.245
1250	0.271
1400	0.368
3500	0.402
5500	0.423
9500	0.435
19000	0.509
19500	0.513
110000	0.535
325000	0.548
550000	0.550
625000	0.559
675000	0.587
700000	0.618
725000	0.624
775000	0.633
775000	0.645
1000000	0.698
1025000	0.762
1200000	0.782
1000000	0.775

Figure A-104. Lactose Control Absorbance vs.Cell Count 2



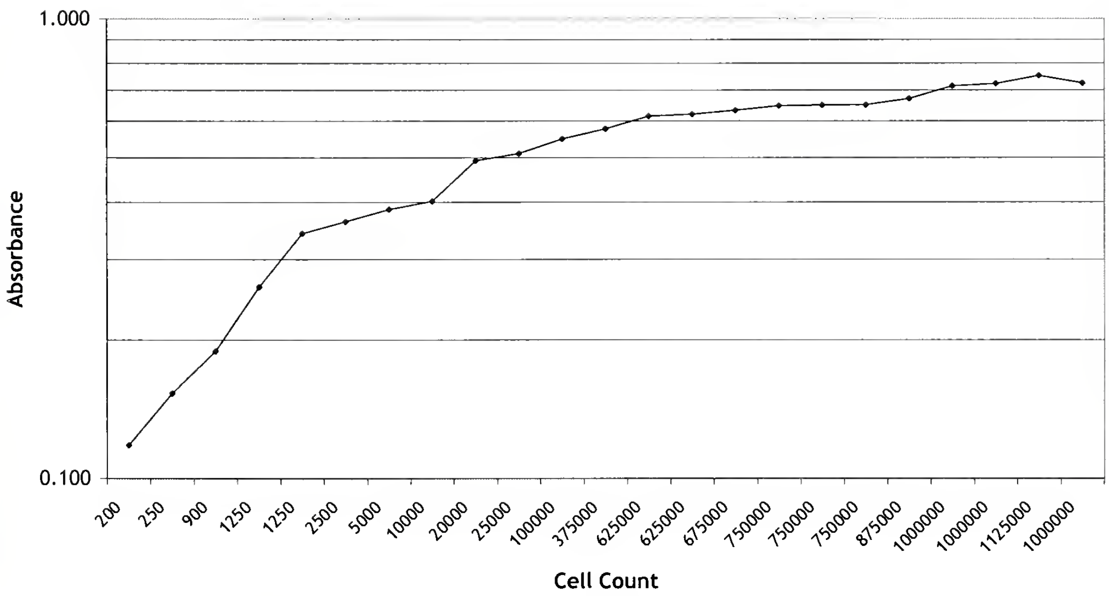
Cell Count increases from 9500 to 19000

Difference in absorbance = (0.509 -0.435) = 0.074

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
300	200	0.118	1.31	0.762
360	250	0.153	1.42	0.703
420	900	0.189	1.55	0.647
480	1250	0.261	1.82	0.548
540	1250	0.342	2.20	0.455
600	2500	0.363	2.31	0.434
660	5000	0.386	2.43	0.411
720	10000	0.402	2.52	0.396
750	20000	0.493	3.11	0.321
780	25000	0.511	3.24	0.308
840	100000	0.549	3.54	0.282
900	375000	0.577	3.78	0.265
960	625000	0.615	4.12	0.243
1020	625000	0.621	4.18	0.239
1080	675000	0.633	4.30	0.233
1140	750000	0.648	4.45	0.225
1170	750000	0.650	4.47	0.224
1200	750000	0.651	4.48	0.223
1260	875000	0.671	4.69	0.213
1320	1000000	0.715	5.19	0.193
1380	1000000	0.723	5.28	0.189
1440	1125000	0.752	5.65	0.177
1500	1000000	0.725	5.31	0.188

Cell Count	Absorbance
200	0.118
250	0.153
900	0.189
1250	0.261
1250	0.342
2500	0.363
5000	0.386
10000	0.402
20000	0.493
25000	0.511
100000	0.549
375000	0.577
625000	0.615
625000	0.621
675000	0.633
750000	0.648
750000	0.650
750000	0.651
875000	0.671
1000000	0.715
1000000	0.723
1125000	0.752
1000000	0.725

Figure A-105. Lactose Control Absorbance vs.Cell Count 3



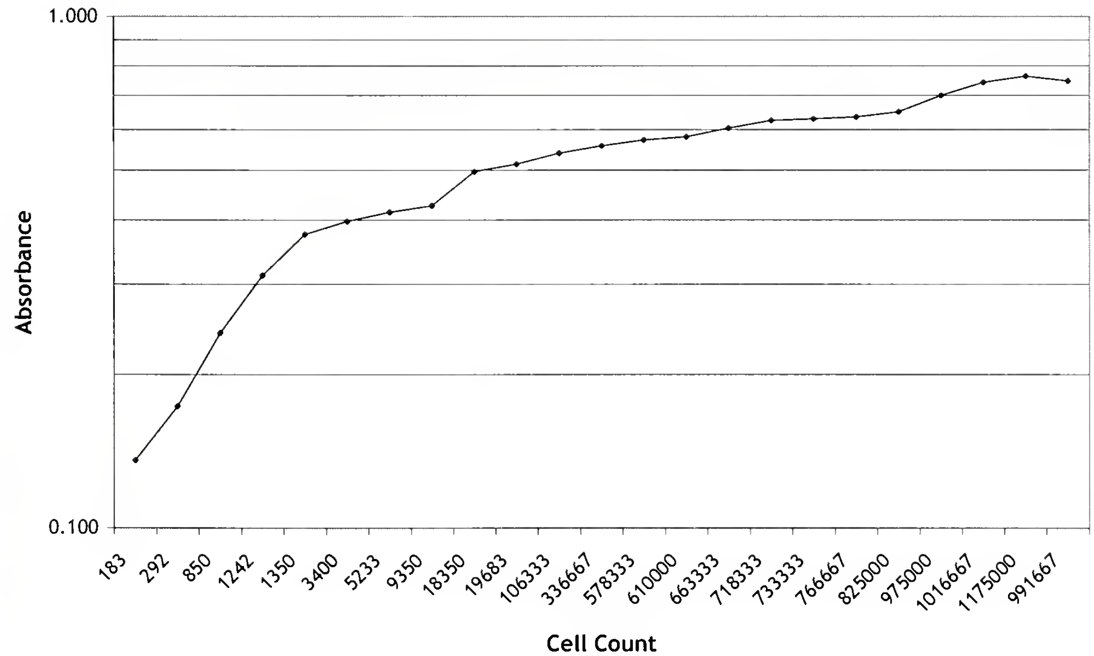
Cell Count increases from 10000 to 20000

Difference in absorbance = (0.493 -0.402) = 0.091

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
300	183	0.136	1.37	0.732
360	292	0.173	1.49	0.671
420	850	0.241	1.74	0.574
480	1242	0.312	2.05	0.487
540	1350	0.375	2.37	0.422
600	3400	0.398	2.50	0.400
660	5233	0.414	2.59	0.385
720	9350	0.426	2.67	0.375
750	18350	0.497	3.14	0.319
780	19683	0.514	3.27	0.306
840	106333	0.540	3.46	0.289
900	336667	0.558	3.61	0.277
960	578333	0.572	3.74	0.268
1020	610000	0.581	3.81	0.262
1080	663333	0.604	4.02	0.249
1140	718333	0.626	4.22	0.237
1170	733333	0.630	4.27	0.234
1200	766667	0.636	4.32	0.231
1260	825000	0.650	4.47	0.224
1320	975000	0.700	5.01	0.200
1380	1016667	0.743	5.54	0.181
1440	1175000	0.764	5.80	0.172
1500	991667	0.747	5.59	0.179

Cell Count	Absorbance
183	0.136
292	0.173
850	0.241
1242	0.312
1350	0.375
3400	0.398
5233	0.414
9350	0.426
18350	0.497
19683	0.514
106333	0.540
336667	0.558
578333	0.572
610000	0.581
663333	0.604
718333	0.626
733333	0.630
766667	0.636
825000	0.650
975000	0.700
1016667	0.743
1175000	0.764
991667	0.747

Figure A-106. Average Lactose Control Absorbance vs.Cell Count



Cell Count increases from 9350 to 18350

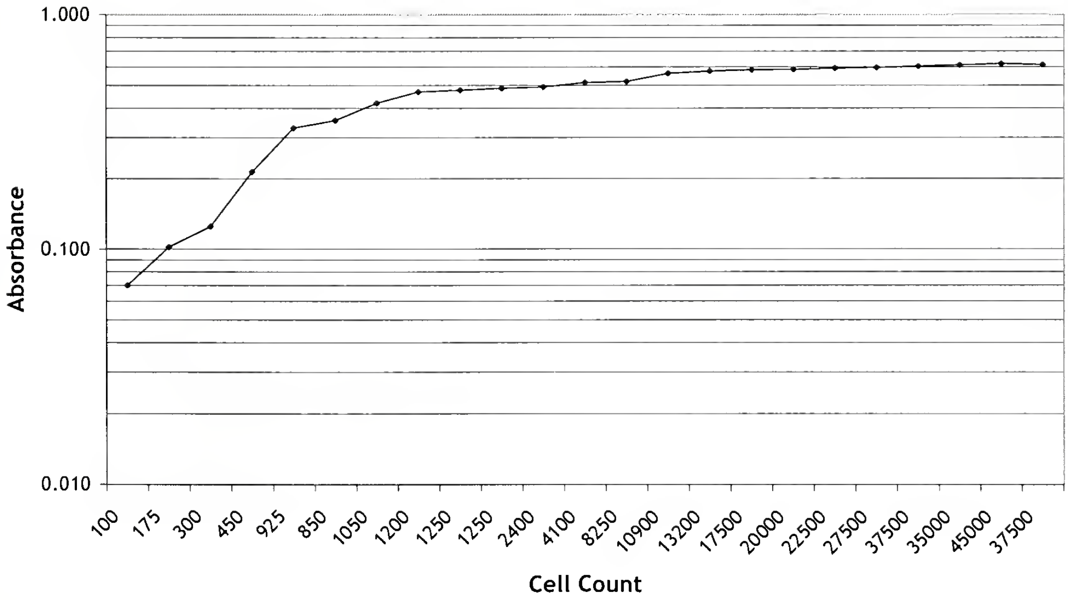
Difference in absorbance = (0.497 -0.426) = **0.071**

LACTOSE WITH Cd CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
300	100	0.070	1.17	0.851
360	175	0.102	1.26	0.791
420	300	0.125	1.33	0.750
480	450	0.214	1.64	0.611
540	925	0.330	2.14	0.468
600	850	0.355	2.26	0.442
660	1050	0.420	2.63	0.380
720	1200	0.470	2.95	0.339
750	1250	0.478	3.01	0.333
780	1250	0.486	3.06	0.327
840	2400	0.492	3.10	0.322
900	4100	0.515	3.27	0.305
960	8250	0.520	3.31	0.302
1020	10900	0.562	3.65	0.274
1080	13200	0.575	3.76	0.266
1140	17500	0.582	3.82	0.262
1170	20000	0.585	3.85	0.260
1200	22500	0.591	3.90	0.256
1260	27500	0.596	3.94	0.254
1320	37500	0.603	4.01	0.249
1380	35000	0.610	4.07	0.245
1440	45000	0.618	4.15	0.241
1500	37500	0.612	4.09	0.244

Cell Count	Absorbance
100	0.070
175	0.102
300	0.125
450	0.214
925	0.330
850	0.355
1050	0.420
1200	0.470
1250	0.478
1250	0.486
2400	0.492
4100	0.515
8250	0.520
10900	0.562
13200	0.575
17500	0.582
20000	0.585
22500	0.591
27500	0.596
37500	0.603
35000	0.610
45000	0.618
37500	0.612

Figure A-107. Lactose With Cd Absorbance vs. Cell Count 1

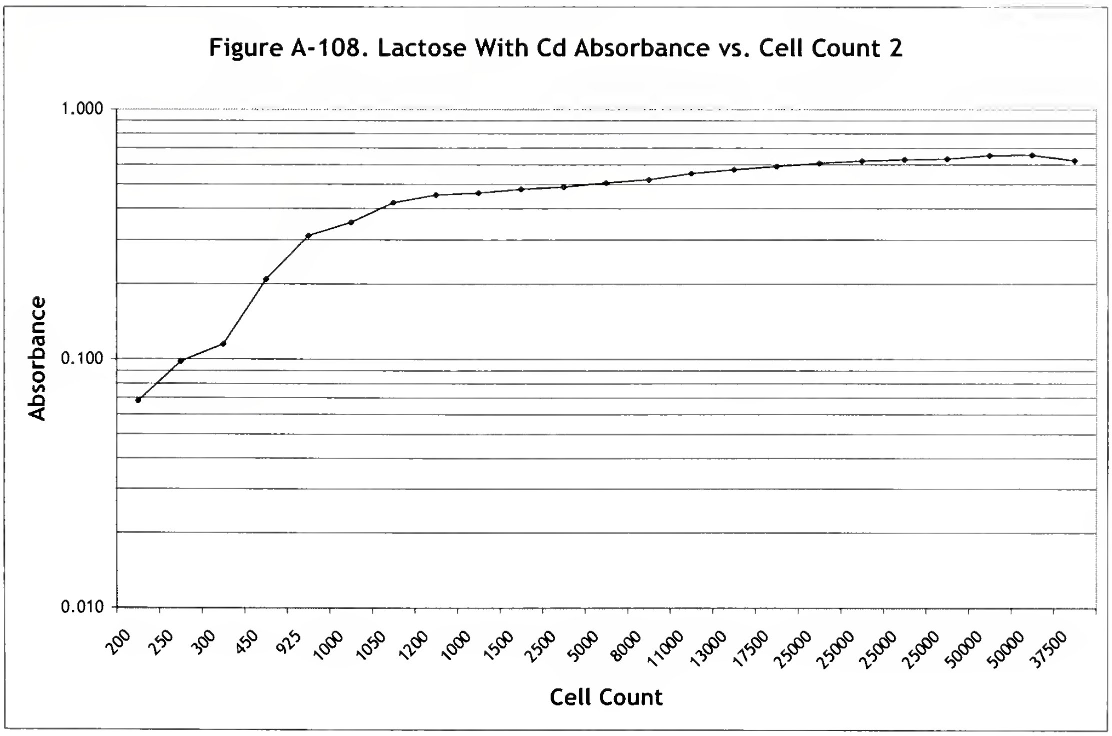


Cell Count increases from 10900 to 22500

Difference in absorbance = **(0.591 -0.562) = 0.029**

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
300	200	0.068	1.17	0.855
360	250	0.098	1.25	0.798
420	300	0.115	1.30	0.767
480	450	0.209	1.62	0.618
540	925	0.312	2.05	0.488
600	1000	0.352	2.25	0.445
660	1050	0.422	2.64	0.378
720	1200	0.453	2.84	0.352
750	1000	0.461	2.89	0.346
780	1500	0.479	3.01	0.332
840	2500	0.489	3.08	0.324
900	5000	0.508	3.22	0.310
960	8000	0.523	3.33	0.300
1020	11000	0.554	3.58	0.279
1080	13000	0.574	3.75	0.267
1140	17500	0.591	3.90	0.256
1170	25000	0.608	4.06	0.247
1200	25000	0.622	4.19	0.239
1260	25000	0.629	4.26	0.235
1320	25000	0.632	4.29	0.233
1380	50000	0.654	4.51	0.222
1440	50000	0.655	4.52	0.221
1500	37500	0.623	4.20	0.238

Cell Count	Absorbance
200	0.068
250	0.098
300	0.115
450	0.209
925	0.312
1000	0.352
1050	0.422
1200	0.453
1000	0.461
1500	0.479
2500	0.489
5000	0.508
8000	0.523
11000	0.554
13000	0.574
17500	0.591
25000	0.608
25000	0.622
25000	0.629
25000	0.632
50000	0.654
50000	0.655
37500	0.623



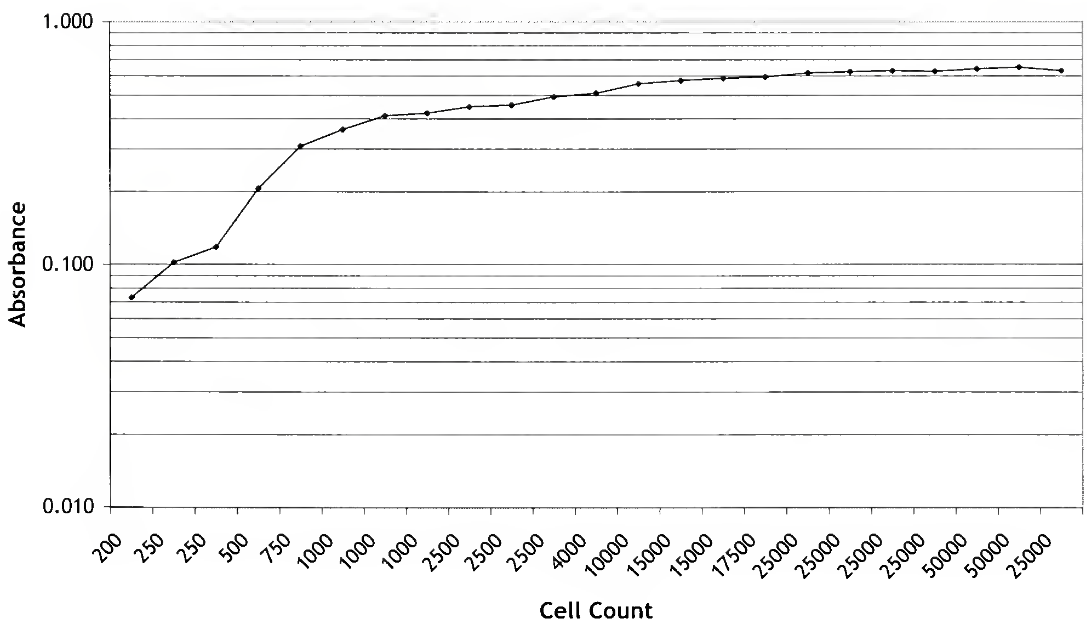
Cell Count increases from 25000 to 50000

Difference in absorbance = (0.654 -0.632) = 0.022

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
300	200	0.073	1.18	0.845
360	250	0.102	1.26	0.791
420	250	0.118	1.31	0.762
480	500	0.206	1.61	0.622
540	750	0.308	2.03	0.492
600	1000	0.362	2.30	0.435
660	1000	0.412	2.58	0.387
720	1000	0.422	2.64	0.378
750	2500	0.449	2.81	0.356
780	2500	0.456	2.86	0.350
840	2500	0.493	3.11	0.321
900	4000	0.511	3.24	0.308
960	10000	0.558	3.61	0.277
1020	15000	0.576	3.77	0.265
1080	15000	0.588	3.87	0.258
1140	17500	0.595	3.94	0.254
1170	25000	0.617	4.14	0.242
1200	25000	0.624	4.21	0.238
1260	25000	0.631	4.28	0.234
1320	25000	0.627	4.24	0.236
1380	50000	0.643	4.40	0.228
1440	50000	0.652	4.49	0.223
1500	25000	0.631	4.28	0.234

Cell Count	Absorbance
200	0.073
250	0.102
250	0.118
500	0.206
750	0.308
1000	0.362
1000	0.412
1000	0.422
2500	0.449
2500	0.456
2500	0.493
4000	0.511
10000	0.558
15000	0.576
15000	0.588
17500	0.595
25000	0.617
25000	0.624
25000	0.631
25000	0.627
50000	0.643
50000	0.652
25000	0.631

Figure A-109. Lactose With Cd Absorbance vs. Cell Count 3



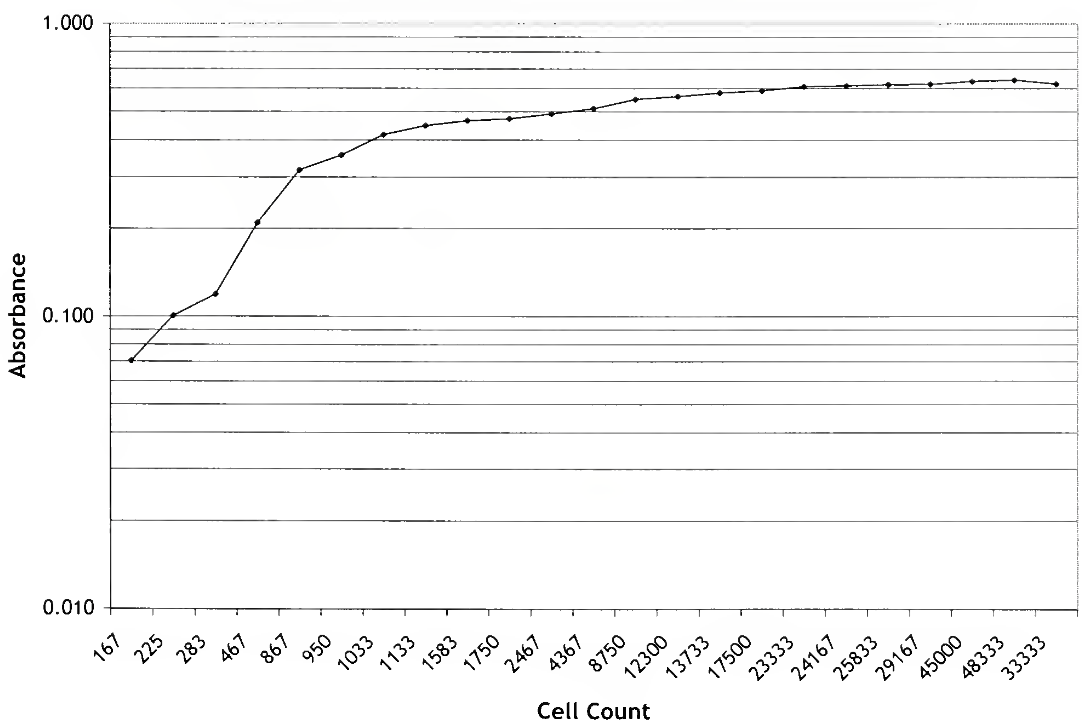
Cell Count increases from 25000 to 50000

Difference in absorbance = (0.643 -0.617) = 0.026

Time(min)	Average Cell Count	Absorbance	Antilog	Transmitance
300	167	0.070	1.18	0.850
360	225	0.101	1.26	0.793
420	283	0.119	1.32	0.760
480	467	0.210	1.62	0.617
540	867	0.317	2.07	0.482
600	950	0.356	2.27	0.440
660	1033	0.418	2.62	0.382
720	1133	0.448	2.81	0.356
750	1583	0.466	2.92	0.342
780	1750	0.474	2.98	0.336
840	2467	0.491	3.10	0.323
900	4367	0.511	3.25	0.308
960	8750	0.551	3.56	0.281
1020	12300	0.564	3.66	0.273
1080	13733	0.579	3.79	0.264
1140	17500	0.589	3.88	0.257
1170	23333	0.609	4.06	0.246
1200	24167	0.612	4.10	0.244
1260	25833	0.619	4.16	0.241
1320	29167	0.621	4.18	0.240
1380	45000	0.636	4.32	0.231
1440	48333	0.642	4.38	0.228
1500	33333	0.622	4.19	0.239

Cell Count	Absorbance
167	0.070
225	0.101
283	0.119
467	0.210
867	0.317
950	0.356
1033	0.418
1133	0.448
1583	0.466
1750	0.474
2467	0.491
4367	0.511
8750	0.551
12300	0.564
13733	0.579
17500	0.589
23333	0.609
24167	0.612
25833	0.619
29167	0.621
45000	0.636
48333	0.642
33333	0.622

Figure A-110. Average Lactose With Cd Absorbance vs. Cell Count



Cell Count increases from 8750 to 17500

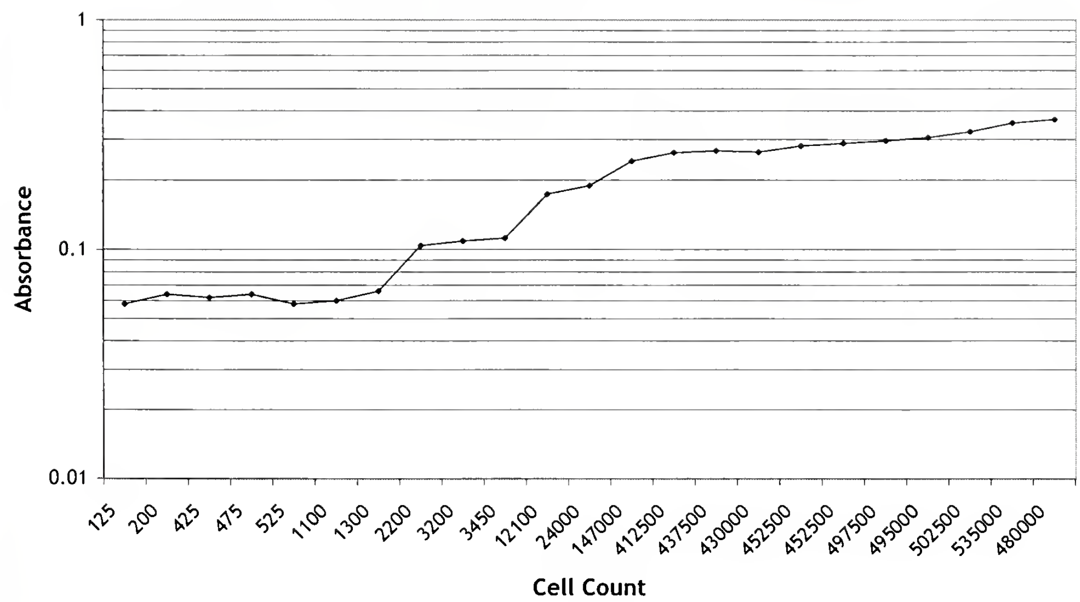
Difference in absorbance = (0.589 -0.551) = 0.038

LACTOSE WITH Pb CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
300	125	0.058	1.14	0.875
360	200	0.064	1.16	0.863
420	425	0.062	1.15	0.867
480	475	0.064	1.16	0.863
540	525	0.058	1.14	0.875
600	1100	0.060	1.15	0.871
660	1300	0.066	1.16	0.859
720	2200	0.104	1.27	0.787
750	3200	0.109	1.29	0.778
780	3450	0.112	1.29	0.773
840	12100	0.174	1.49	0.670
900	24000	0.189	1.55	0.647
960	147000	0.242	1.75	0.573
1020	412500	0.263	1.83	0.546
1080	437500	0.268	1.85	0.540
1140	430000	0.265	1.84	0.543
1170	452500	0.281	1.91	0.524
1200	452500	0.289	1.95	0.514
1260	497500	0.296	1.98	0.506
1320	495000	0.306	2.02	0.494
1380	502500	0.324	2.11	0.474
1440	535000	0.354	2.26	0.443
1500	480000	0.366	2.32	0.431

Cell Count	Absorbance
125	0.058
200	0.064
425	0.062
475	0.064
525	0.058
1100	0.060
1300	0.066
2200	0.104
3200	0.109
3450	0.112
12100	0.174
24000	0.189
147000	0.242
412500	0.263
437500	0.268
430000	0.265
452500	0.281
452500	0.289
497500	0.296
495000	0.306
502500	0.324
535000	0.354
480000	0.366

Figure A-111. Lactose With Pb Absorbance vs. Cell Count 1



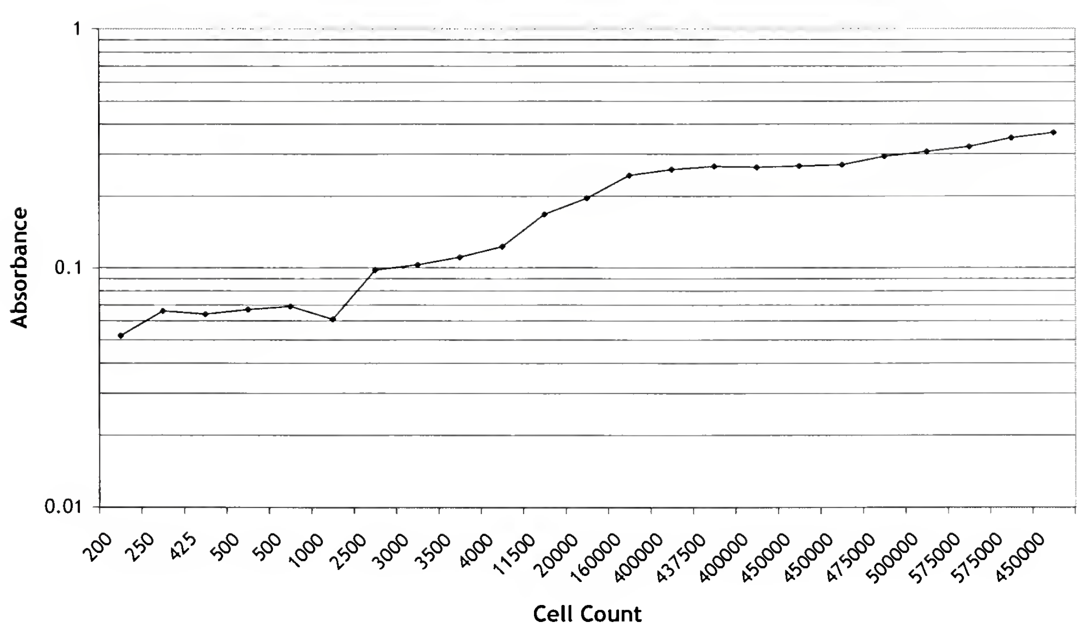
Cell Count increases from 1100 to 2200

Difference in absorbance = (0.104 -0.060) = 0.044

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
300	200	0.052	1.13	0.887
360	250	0.066	1.16	0.859
420	425	0.064	1.16	0.863
480	500	0.067	1.17	0.857
540	500	0.069	1.17	0.853
600	1000	0.061	1.15	0.869
660	2500	0.098	1.25	0.798
720	3000	0.103	1.27	0.789
750	3500	0.111	1.29	0.774
780	4000	0.123	1.33	0.753
840	11500	0.168	1.47	0.679
900	20000	0.196	1.57	0.637
960	160000	0.245	1.76	0.569
1020	400000	0.259	1.82	0.551
1080	437500	0.267	1.85	0.541
1140	400000	0.264	1.84	0.545
1170	450000	0.268	1.85	0.540
1200	450000	0.271	1.87	0.536
1260	475000	0.294	1.97	0.508
1320	500000	0.308	2.03	0.492
1380	575000	0.322	2.10	0.476
1440	575000	0.351	2.24	0.446
1500	450000	0.368	2.33	0.429

Cell Count	Absorbance
200	0.052
250	0.066
425	0.064
500	0.067
500	0.069
1000	0.061
2500	0.098
3000	0.103
3500	0.111
4000	0.123
11500	0.168
20000	0.196
160000	0.245
400000	0.259
437500	0.267
400000	0.264
450000	0.268
450000	0.271
475000	0.294
500000	0.308
575000	0.322
575000	0.351
450000	0.368

Figure A-112. Lactose With Pb Absorbance vs. Cell Count 2



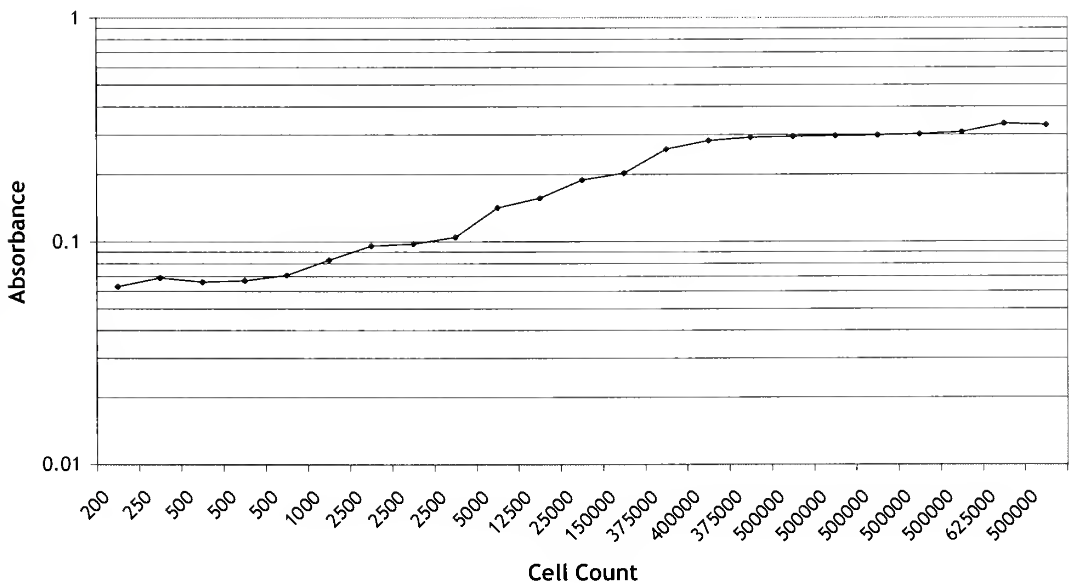
Cell Count increases from 1000 to 2500

Difference in absorbance = (0.098 -0.061) = 0.037

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
300	200	0.063	1.16	0.865
360	250	0.069	1.17	0.853
420	500	0.066	1.16	0.859
480	500	0.067	1.17	0.857
540	500	0.071	1.18	0.849
600	1000	0.083	1.21	0.826
660	2500	0.096	1.25	0.802
720	2500	0.098	1.25	0.798
750	2500	0.105	1.27	0.785
780	5000	0.142	1.39	0.721
840	12500	0.157	1.44	0.697
900	25000	0.189	1.55	0.647
960	150000	0.203	1.60	0.627
1020	375000	0.259	1.82	0.551
1080	400000	0.283	1.92	0.521
1140	375000	0.292	1.96	0.511
1170	500000	0.295	1.97	0.507
1200	500000	0.297	1.98	0.505
1260	500000	0.299	1.99	0.502
1320	500000	0.302	2.00	0.499
1380	500000	0.308	2.03	0.492
1440	625000	0.336	2.17	0.461
1500	500000	0.332	2.15	0.466

Cell Count	Absorbance
200	0.063
250	0.069
500	0.066
500	0.067
500	0.071
1000	0.083
2500	0.096
2500	0.098
2500	0.105
5000	0.142
12500	0.157
25000	0.189
150000	0.203
375000	0.259
400000	0.283
375000	0.292
500000	0.295
500000	0.297
500000	0.299
500000	0.302
500000	0.308
625000	0.336
500000	0.332

Figure A-113. Lactose With Pb Absorbance vs. Cell Count 3



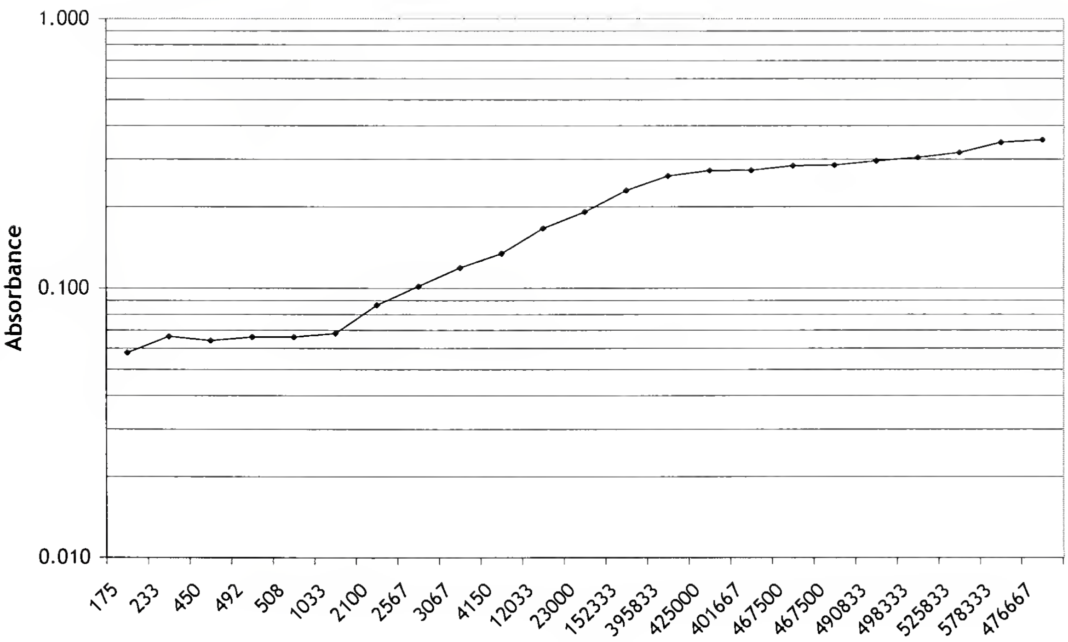
Cell Count increases from 2500 to 5000

Difference in absorbance = (0.142 -0.096) = 0.046

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
300	175	0.058	1.14	0.876
360	233	0.066	1.17	0.858
420	450	0.064	1.16	0.863
480	492	0.066	1.16	0.859
540	508	0.066	1.16	0.859
600	1033	0.068	1.17	0.855
660	2100	0.087	1.22	0.819
720	2567	0.102	1.26	0.791
750	3067	0.119	1.32	0.760
780	4150	0.134	1.36	0.735
840	12033	0.166	1.47	0.682
900	23000	0.191	1.55	0.644
960	152333	0.230	1.70	0.589
1020	395833	0.260	1.82	0.549
1080	425000	0.273	1.87	0.534
1140	401667	0.274	1.88	0.533
1170	467500	0.284	1.92	0.520
1200	467500	0.286	1.93	0.518
1260	490833	0.296	1.98	0.505
1320	498333	0.305	2.02	0.495
1380	525833	0.318	2.08	0.481
1440	578333	0.347	2.22	0.450
1500	476667	0.355	2.27	0.441

Cell Count	Absorbance
175	0.058
233	0.066
450	0.064
492	0.066
508	0.066
1033	0.068
2100	0.087
2567	0.102
3067	0.119
4150	0.134
12033	0.166
23000	0.191
152333	0.230
395833	0.260
425000	0.273
401667	0.274
467500	0.284
467500	0.286
490833	0.296
498333	0.305
525833	0.318
578333	0.347
476667	0.355

Figure A-114. Average Lactose With Pb Absorbance vs. Cell Count



Cell Count increases from 2100 to 4150

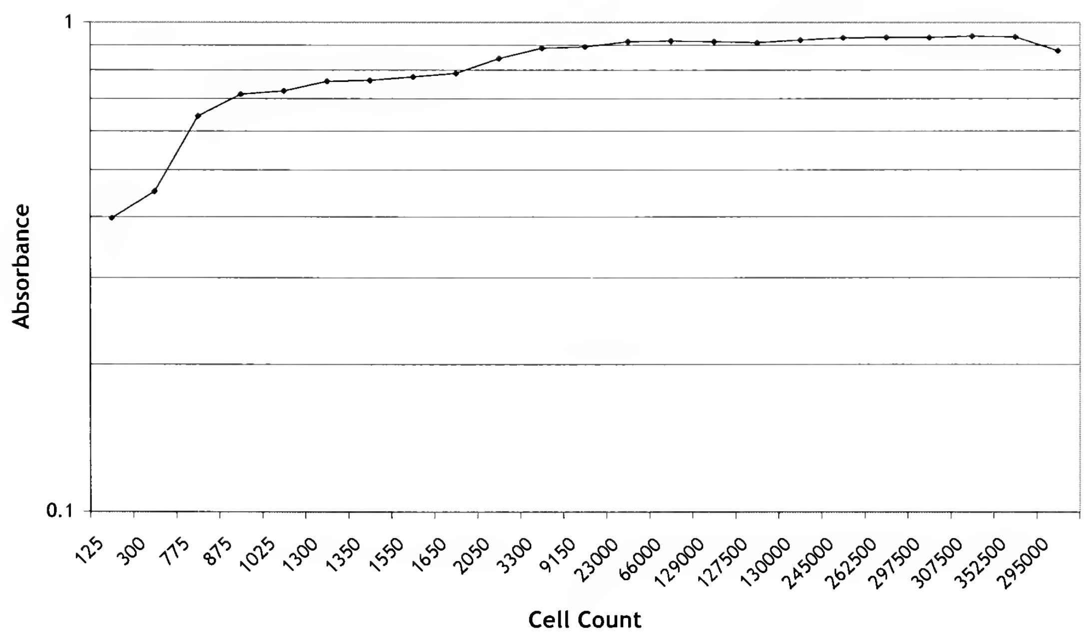
Difference in absorbance = (0.134 -0.087) = 0.047

GLUCOSE ONLY CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
300	125	0.398	2.50	0.400
360	300	0.452	2.83	0.353
420	775	0.645	4.42	0.226
480	875	0.715	5.19	0.193
540	1025	0.726	5.32	0.188
600	1300	0.759	5.74	0.174
660	1350	0.763	5.79	0.173
720	1550	0.775	5.96	0.168
750	1650	0.788	6.14	0.163
780	2050	0.845	7.00	0.143
840	3300	0.887	7.71	0.130
900	9150	0.892	7.80	0.128
960	23000	0.915	8.22	0.122
1020	66000	0.918	8.28	0.121
1080	129000	0.915	8.22	0.122
1140	127500	0.911	8.15	0.123
1170	130000	0.921	8.34	0.120
1200	245000	0.932	8.55	0.117
1260	262500	0.933	8.57	0.117
1320	297500	0.933	8.57	0.117
1380	307500	0.938	8.67	0.115
1440	352500	0.935	8.61	0.116
1500	295000	0.877	7.53	0.133

Cell Count	Absorbance
125	0.398
300	0.452
775	0.645
875	0.715
1025	0.726
1300	0.759
1350	0.763
1550	0.775
1650	0.788
2050	0.845
3300	0.887
9150	0.892
23000	0.915
66000	0.918
129000	0.915
127500	0.911
130000	0.921
245000	0.932
262500	0.933
297500	0.933
307500	0.938
352500	0.935
295000	0.877

Figure A-115. Glucose Control Absorbance vs. Cell Count 1



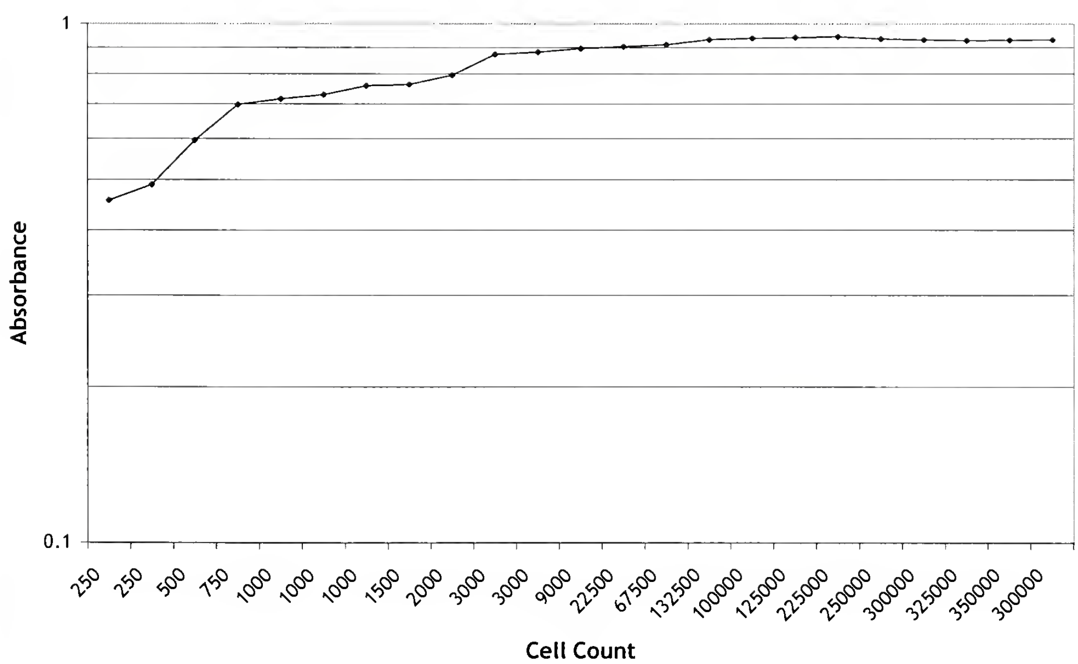
Cell Count increases from 1650 to 3300

Difference in absorbance = (0.887 -0.788) = 0.099

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
300	250	0.456	2.86	0.350
360	250	0.489	3.08	0.324
420	500	0.596	3.94	0.254
480	750	0.698	4.99	0.200
540	1000	0.716	5.20	0.192
600	1000	0.729	5.36	0.187
660	1000	0.759	5.74	0.174
720	1500	0.764	5.81	0.172
750	2000	0.796	6.25	0.160
780	3000	0.873	7.46	0.134
840	3000	0.882	7.62	0.131
900	9000	0.896	7.87	0.127
960	22500	0.903	8.00	0.125
1020	67500	0.912	8.17	0.122
1080	132500	0.933	8.57	0.117
1140	100000	0.938	8.67	0.115
1170	125000	0.941	8.73	0.115
1200	225000	0.945	8.81	0.114
1260	250000	0.935	8.61	0.116
1320	300000	0.931	8.53	0.117
1380	325000	0.928	8.47	0.118
1440	350000	0.930	8.51	0.117
1500	300000	0.931	8.53	0.117

Cell Count	Absorbance
250	0.456
250	0.489
500	0.596
750	0.698
1000	0.716
1000	0.729
1000	0.759
1500	0.764
2000	0.796
3000	0.873
3000	0.882
9000	0.896
22500	0.903
67500	0.912
132500	0.933
100000	0.938
125000	0.941
225000	0.945
250000	0.935
300000	0.931
325000	0.928
350000	0.930
300000	0.931

Figure A-116. Glucose Control Absorbance vs. Cell Count 2



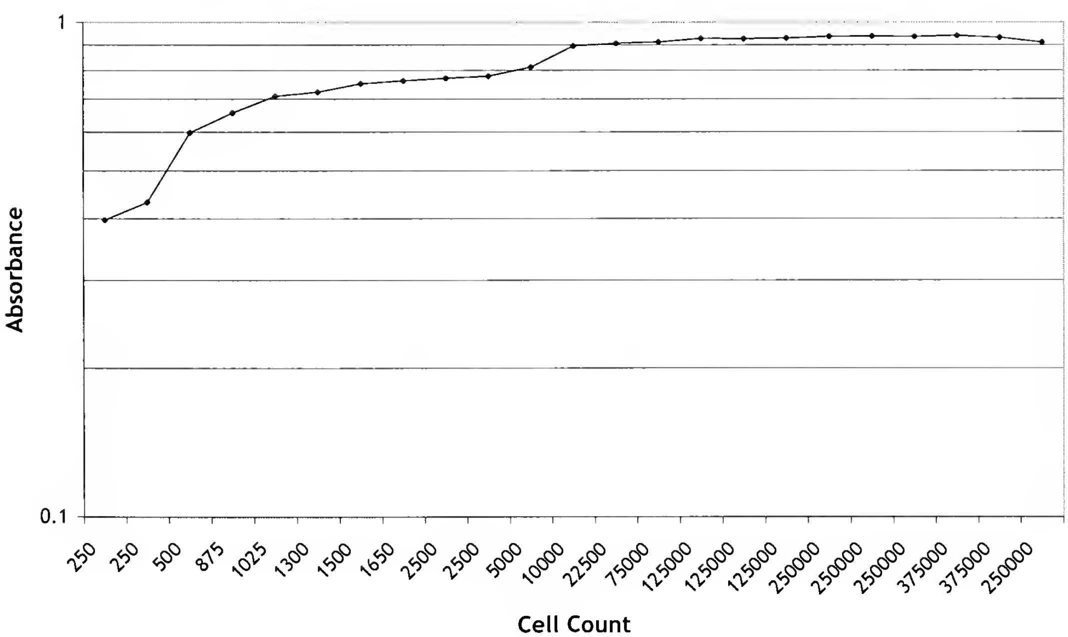
Cell Count increases from 1500 to 3000

Difference in absorbance = (0.873 -0.764) = 0.109

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
300	250	0.398	2.50	0.400
360	250	0.432	2.70	0.370
420	500	0.598	3.96	0.252
480	875	0.656	4.53	0.221
540	1025	0.709	5.12	0.195
600	1300	0.723	5.28	0.189
660	1500	0.752	5.65	0.177
720	1650	0.762	5.78	0.173
750	2500	0.771	5.90	0.169
780	2500	0.779	6.01	0.166
840	5000	0.812	6.49	0.154
900	10000	0.896	7.87	0.127
960	22500	0.907	8.07	0.124
1020	75000	0.912	8.17	0.122
1080	125000	0.928	8.47	0.118
1140	125000	0.927	8.45	0.118
1170	125000	0.929	8.49	0.118
1200	250000	0.936	8.63	0.116
1260	250000	0.938	8.67	0.115
1320	250000	0.936	8.63	0.116
1380	375000	0.941	8.73	0.115
1440	375000	0.932	8.55	0.117
1500	250000	0.911	8.15	0.123

Cell Count	Absorbance
250	0.398
250	0.432
500	0.598
875	0.656
1025	0.709
1300	0.723
1500	0.752
1650	0.762
2500	0.771
2500	0.779
5000	0.812
10000	0.896
22500	0.907
75000	0.912
125000	0.928
125000	0.927
125000	0.929
250000	0.936
250000	0.938
250000	0.936
375000	0.941
375000	0.932
250000	0.911

Figure A-117. Glucose Control Absorbance vs. Cell Count 3

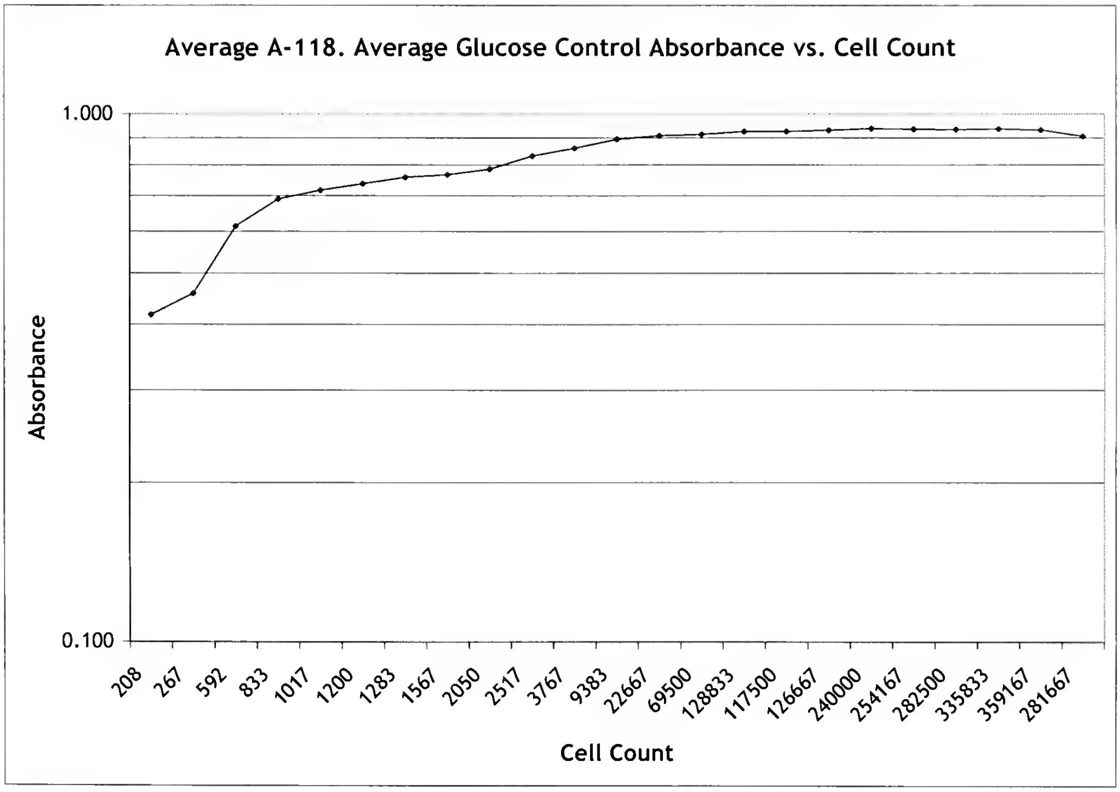


Cell Count increases from 5000 to 10000

Difference in absorbance = (0.896 -0.812) = 0.084

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
300	208	0.417	2.61	0.383
360	267	0.458	2.87	0.349
420	592	0.613	4.10	0.244
480	833	0.690	4.89	0.204
540	1017	0.717	5.21	0.192
600	1200	0.737	5.46	0.183
660	1283	0.758	5.73	0.175
720	1567	0.767	5.85	0.171
750	2050	0.785	6.10	0.164
780	2517	0.832	6.80	0.147
840	3767	0.860	7.25	0.138
900	9383	0.895	7.85	0.127
960	22667	0.908	8.10	0.123
1020	69500	0.914	8.20	0.122
1080	128833	0.925	8.42	0.119
1140	117500	0.925	8.42	0.119
1170	126667	0.930	8.52	0.117
1200	240000	0.938	8.66	0.115
1260	254167	0.935	8.62	0.116
1320	282500	0.933	8.58	0.117
1380	335833	0.936	8.62	0.116
1440	359167	0.932	8.56	0.117
1500	281667	0.906	8.06	0.124

Cell Count	Absorbance
208	0.417
267	0.458
592	0.613
833	0.690
1017	0.717
1200	0.737
1283	0.758
1567	0.767
2050	0.785
2517	0.832
3767	0.860
9383	0.895
22667	0.908
69500	0.914
128833	0.925
117500	0.925
126667	0.930
240000	0.938
254167	0.935
282500	0.933
335833	0.936
359167	0.932
281667	0.906



Cell Count increases from 592 to 1200

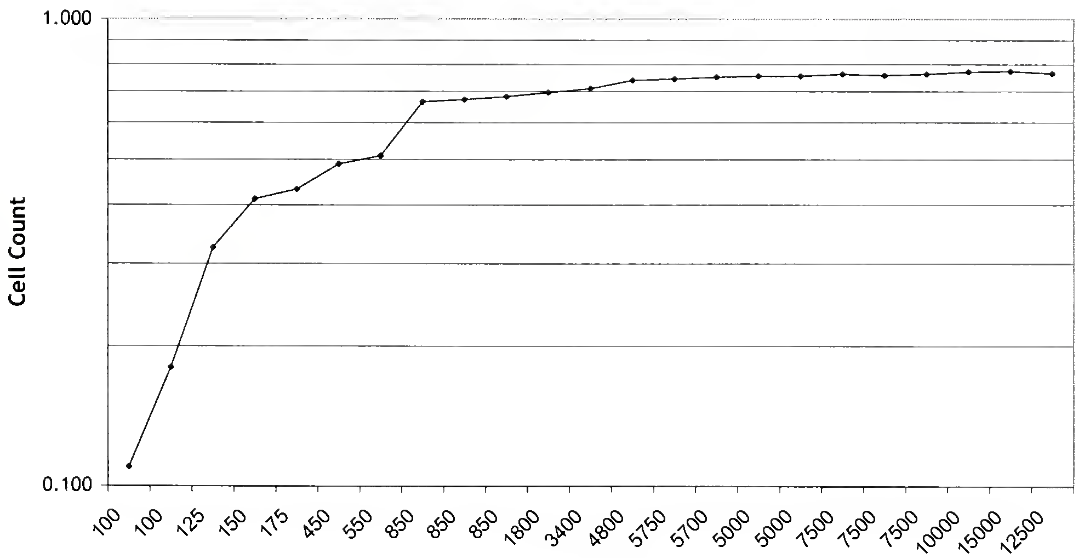
Difference in absorbance = (0.737 -0.613) = 0.124

GLUCOSE WITH Cd CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
300	100	0.110	1.29	0.776
360	100	0.180	1.51	0.661
420	125	0.325	2.11	0.473
480	150	0.412	2.58	0.387
540	175	0.432	2.70	0.370
600	450	0.489	3.08	0.324
660	550	0.510	3.24	0.309
720	850	0.665	4.62	0.216
750	850	0.673	4.71	0.212
780	850	0.682	4.81	0.208
840	1800	0.697	4.98	0.201
900	3400	0.710	5.13	0.195
960	4800	0.740	5.50	0.182
1020	5750	0.745	5.56	0.180
1080	5700	0.752	5.65	0.177
1140	5000	0.755	5.69	0.176
1170	5000	0.755	5.69	0.176
1200	7500	0.762	5.78	0.173
1260	7500	0.758	5.73	0.175
1320	7500	0.763	5.79	0.173
1380	10000	0.771	5.90	0.169
1440	15000	0.774	5.94	0.168
1500	12500	0.765	5.82	0.172

Cell Count	Absorbance
100	0.110
100	0.180
125	0.325
150	0.412
175	0.432
450	0.489
550	0.510
850	0.665
850	0.673
850	0.682
1800	0.697
3400	0.710
4800	0.740
5750	0.745
5700	0.752
5000	0.755
5000	0.755
7500	0.762
7500	0.758
7500	0.763
10000	0.771
15000	0.774
12500	0.765

Figure A-119. Glucose With Cd Absorbance vs. Cell Count 1



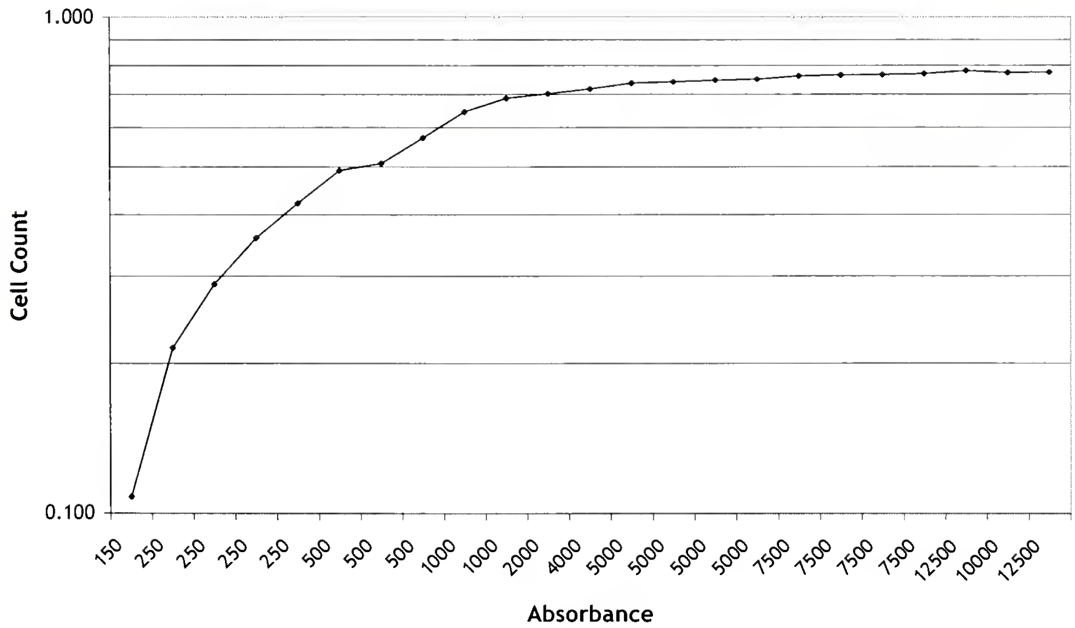
Cell Count increases from 5000 to 10000

Difference in absorbance = (0.771 -0.755) = 0.016

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
300	150	0.108	1.28	0.780
360	250	0.215	1.64	0.610
420	250	0.289	1.95	0.514
480	250	0.359	2.29	0.438
540	250	0.422	2.64	0.378
600	500	0.492	3.10	0.322
660	500	0.508	3.22	0.310
720	500	0.572	3.73	0.268
750	1000	0.646	4.43	0.226
780	1000	0.688	4.88	0.205
840	2000	0.702	5.04	0.199
900	4000	0.718	5.22	0.191
960	5000	0.738	5.47	0.183
1020	5000	0.742	5.52	0.181
1080	5000	0.748	5.60	0.179
1140	5000	0.751	5.64	0.177
1170	7500	0.763	5.79	0.173
1200	7500	0.766	5.83	0.171
1260	7500	0.767	5.85	0.171
1320	7500	0.771	5.90	0.169
1380	12500	0.781	6.04	0.166
1440	10000	0.774	5.94	0.168
1500	12500	0.775	5.96	0.168

Cell Count	Absorbance
150	0.108
250	0.215
250	0.289
250	0.359
250	0.422
500	0.492
500	0.508
500	0.572
1000	0.646
1000	0.688
2000	0.702
4000	0.718
5000	0.738
5000	0.742
5000	0.748
5000	0.751
7500	0.763
7500	0.766
7500	0.767
7500	0.771
12500	0.781
10000	0.774
12500	0.775

Figure A-120. Glucose With Cd Absorbance vs. Cell Count 2



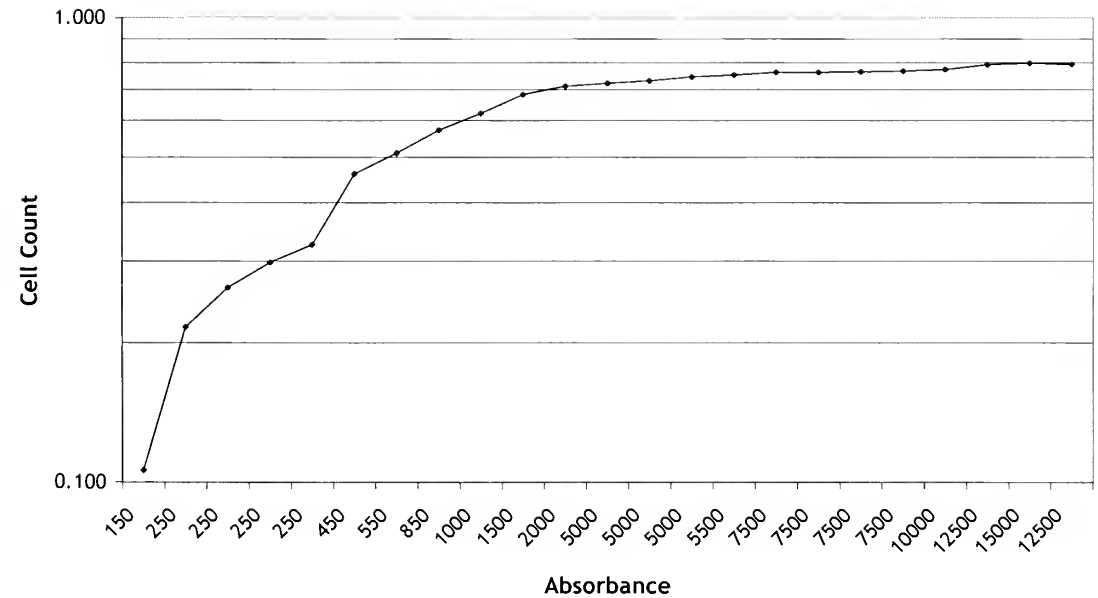
Cell Count increases from 5000 to 10000

Difference in absorbance = (0.774 -0.751) = 0.023

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
300	150	0.106	1.28	0.783
360	250	0.216	1.64	0.608
420	250	0.263	1.83	0.546
480	250	0.298	1.99	0.504
540	250	0.325	2.11	0.473
600	450	0.461	2.89	0.346
660	550	0.511	3.24	0.308
720	850	0.572	3.73	0.268
750	1000	0.622	4.19	0.239
780	1500	0.683	4.82	0.207
840	2000	0.712	5.15	0.194
900	5000	0.722	5.27	0.190
960	5000	0.731	5.38	0.186
1020	5000	0.746	5.57	0.179
1080	5500	0.753	5.66	0.177
1140	7500	0.762	5.78	0.173
1170	7500	0.763	5.79	0.173
1200	7500	0.765	5.82	0.172
1260	7500	0.767	5.85	0.171
1320	10000	0.773	5.93	0.169
1380	12500	0.792	6.19	0.161
1440	15000	0.799	6.30	0.159
1500	12500	0.794	6.22	0.161

Cell Count	Absorbance
150	0.106
250	0.216
250	0.263
250	0.298
250	0.325
450	0.461
550	0.511
850	0.572
1000	0.622
1500	0.683
2000	0.712
5000	0.722
5000	0.731
5000	0.746
5500	0.753
7500	0.762
7500	0.763
7500	0.765
7500	0.767
10000	0.773
12500	0.792
15000	0.799
12500	0.794

Figure A-121. Glucose With Cd Absorbance vs. Cell Count 3



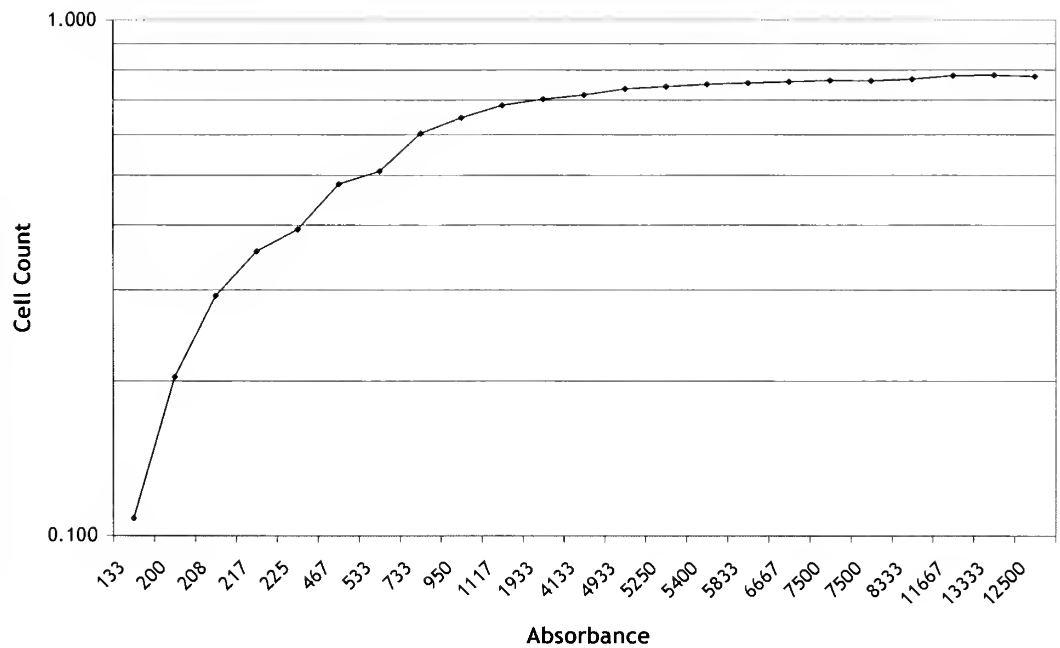
Cell Count increases from 5000 to 10000

Difference in absorbance = (0.773 -0.746) = 0.027

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
300	133	0.108	1.28	0.780
360	200	0.204	1.60	0.626
420	208	0.292	1.96	0.510
480	217	0.356	2.27	0.440
540	225	0.393	2.47	0.405
600	467	0.481	3.02	0.331
660	533	0.510	3.23	0.309
720	733	0.603	4.01	0.249
750	950	0.647	4.44	0.225
780	1117	0.684	4.83	0.207
840	1933	0.704	5.05	0.198
900	4133	0.717	5.21	0.192
960	4933	0.736	5.45	0.184
1020	5250	0.744	5.55	0.180
1080	5400	0.751	5.64	0.177
1140	5833	0.756	5.70	0.175
1170	6667	0.760	5.76	0.174
1200	7500	0.764	5.81	0.172
1260	7500	0.764	5.81	0.172
1320	8333	0.769	5.87	0.170
1380	11667	0.781	6.04	0.165
1440	13333	0.782	6.06	0.165
1500	12500	0.778	6.00	0.167

Cell Count	Absorbance
133	0.108
200	0.204
208	0.292
217	0.356
225	0.393
467	0.481
533	0.510
733	0.603
950	0.647
1117	0.684
1933	0.704
4133	0.717
4933	0.736
5250	0.744
5400	0.751
5833	0.756
6667	0.760
7500	0.764
7500	0.764
8333	0.769
11667	0.781
13333	0.782
12500	0.778

Figure A-122. Average Glucose With Cd Absorbance vs. Cell Count



Cell Count increases from 6667 to 13333

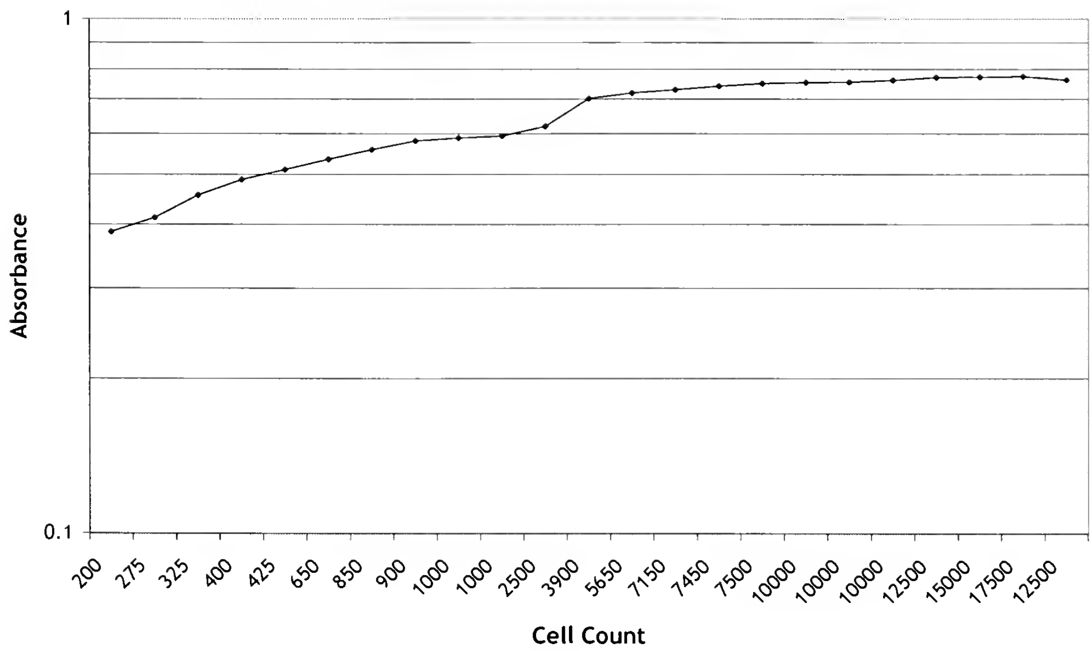
Difference in absorbance = (0.782 -0.760) = 0.022

GLUCOSE WITH Pb CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
300	200	0.387	2.44	0.410
360	275	0.412	2.58	0.387
420	325	0.456	2.86	0.350
480	400	0.489	3.08	0.324
540	425	0.511	3.24	0.308
600	650	0.535	3.43	0.292
660	850	0.559	3.62	0.276
720	900	0.581	3.81	0.262
750	1000	0.589	3.88	0.258
780	1000	0.594	3.93	0.255
840	2500	0.620	4.17	0.240
900	3900	0.702	5.04	0.198
960	5650	0.719	5.24	0.191
1020	7150	0.730	5.37	0.186
1080	7450	0.742	5.52	0.181
1140	7500	0.751	5.63	0.178
1170	10000	0.754	5.68	0.176
1200	10000	0.755	5.69	0.176
1260	10000	0.761	5.76	0.174
1320	12500	0.771	5.90	0.170
1380	15000	0.772	5.92	0.169
1440	17500	0.774	5.94	0.168
1500	12500	0.762	5.78	0.173

Cell Count	Absorbance
200	0.387
275	0.412
325	0.456
400	0.489
425	0.511
650	0.535
850	0.559
900	0.581
1000	0.589
1000	0.594
2500	0.620
3900	0.702
5650	0.719
7150	0.730
7450	0.742
7500	0.751
10000	0.754
10000	0.755
10000	0.761
12500	0.771
15000	0.772
17500	0.774
12500	0.762

Figure A-123. Glucose With Pb Absorbance vs. Cell Count 1



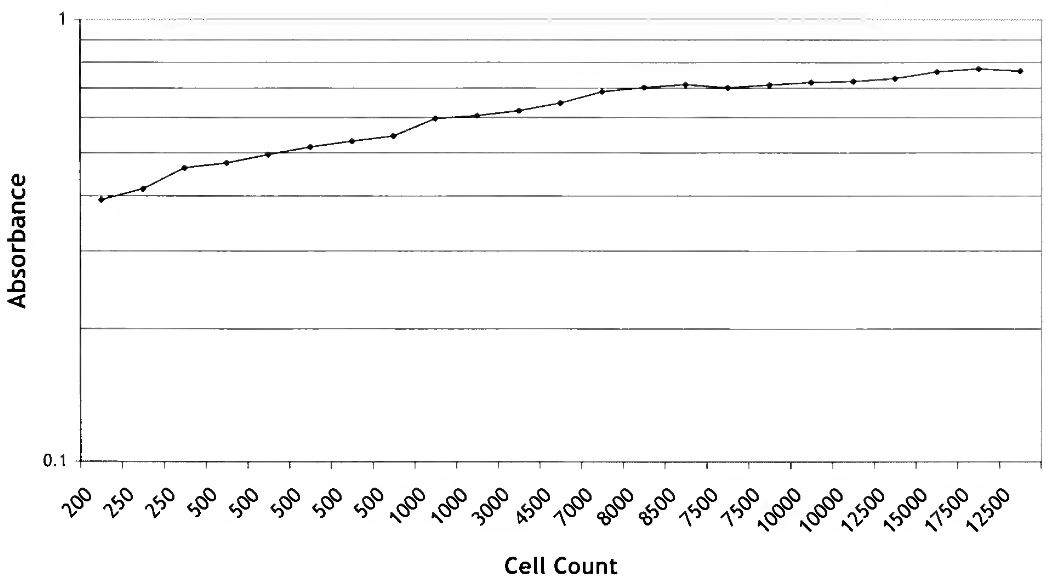
Cell Count increases from 425 to 850

Difference in absorbance = (0.559 -0.511) = 0.048

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
300	200	0.392	2.47	0.406
360	250	0.415	2.60	0.385
420	250	0.463	2.90	0.344
480	500	0.475	2.99	0.335
540	500	0.496	3.13	0.319
600	500	0.515	3.27	0.305
660	500	0.531	3.40	0.294
720	500	0.546	3.52	0.284
750	1000	0.597	3.95	0.253
780	1000	0.606	4.04	0.248
840	3000	0.622	4.19	0.239
900	4500	0.648	4.45	0.225
960	7000	0.687	4.86	0.206
1020	8000	0.702	5.04	0.199
1080	8500	0.712	5.15	0.194
1140	7500	0.701	5.02	0.199
1170	7500	0.711	5.14	0.195
1200	10000	0.721	5.26	0.190
1260	10000	0.724	5.30	0.189
1320	12500	0.736	5.45	0.184
1380	15000	0.762	5.78	0.173
1440	17500	0.774	5.94	0.168
1500	12500	0.765	5.82	0.172

Cell Count	Absorbance
200	0.392
250	0.415
250	0.463
500	0.475
500	0.496
500	0.515
500	0.531
500	0.546
1000	0.597
1000	0.606
3000	0.622
4500	0.648
7000	0.687
8000	0.702
8500	0.712
7500	0.701
7500	0.711
10000	0.721
10000	0.724
12500	0.736
15000	0.762
17500	0.774
12500	0.765

Figure A-124. Glucose With Pb Absorbance vs.Cell Count2



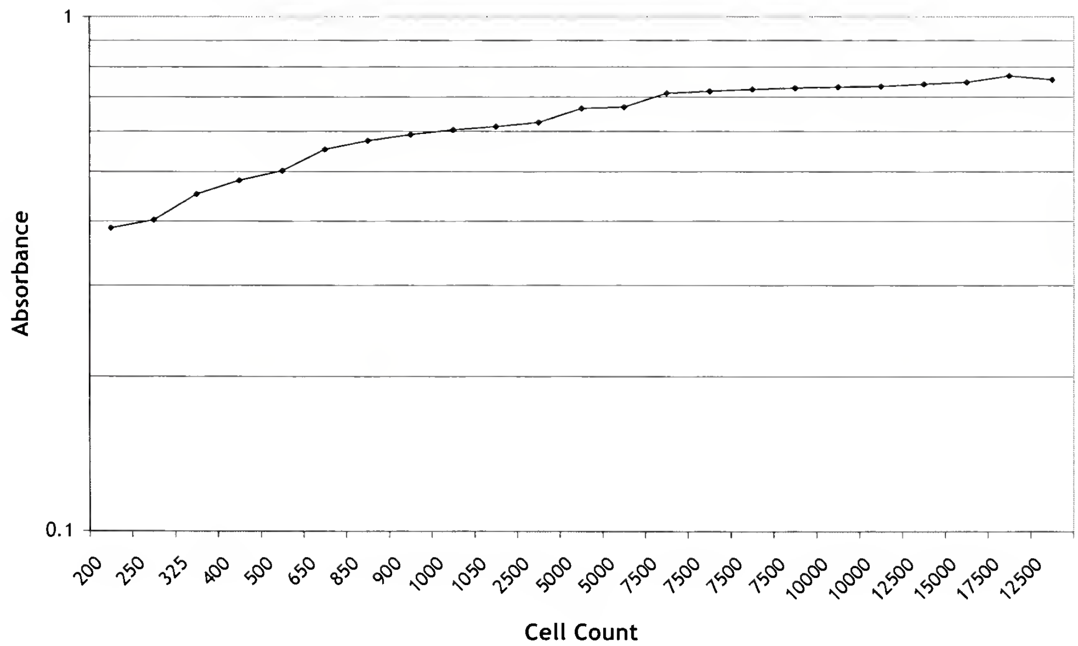
Cell Count increases from 7500 to 15000

Difference in absorbance = (0.762 -0.711) = 0.051

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
300	200	0.388	2.44	0.409
360	250	0.402	2.52	0.396
420	325	0.452	2.83	0.353
480	400	0.481	3.03	0.330
540	500	0.502	3.18	0.315
600	650	0.553	3.57	0.280
660	850	0.575	3.76	0.266
720	900	0.591	3.90	0.256
750	1000	0.604	4.02	0.249
780	1050	0.613	4.10	0.244
840	2500	0.624	4.21	0.238
900	5000	0.665	4.62	0.216
960	5000	0.669	4.67	0.214
1020	7500	0.712	5.15	0.194
1080	7500	0.718	5.22	0.191
1140	7500	0.724	5.30	0.189
1170	7500	0.728	5.35	0.187
1200	10000	0.732	5.40	0.185
1260	10000	0.734	5.42	0.185
1320	12500	0.741	5.51	0.182
1380	15000	0.748	5.60	0.179
1440	17500	0.769	5.87	0.170
1500	12500	0.756	5.70	0.175

Cell Count	Absorbance
200	0.388
250	0.402
325	0.452
400	0.481
500	0.502
650	0.553
850	0.575
900	0.591
1000	0.604
1050	0.613
2500	0.624
5000	0.665
5000	0.669
7500	0.712
7500	0.718
7500	0.724
7500	0.728
10000	0.732
10000	0.734
12500	0.741
15000	0.748
17500	0.769
12500	0.756

Figure A-125. Glucose With Pb Absorbance vs. Cell Count 3



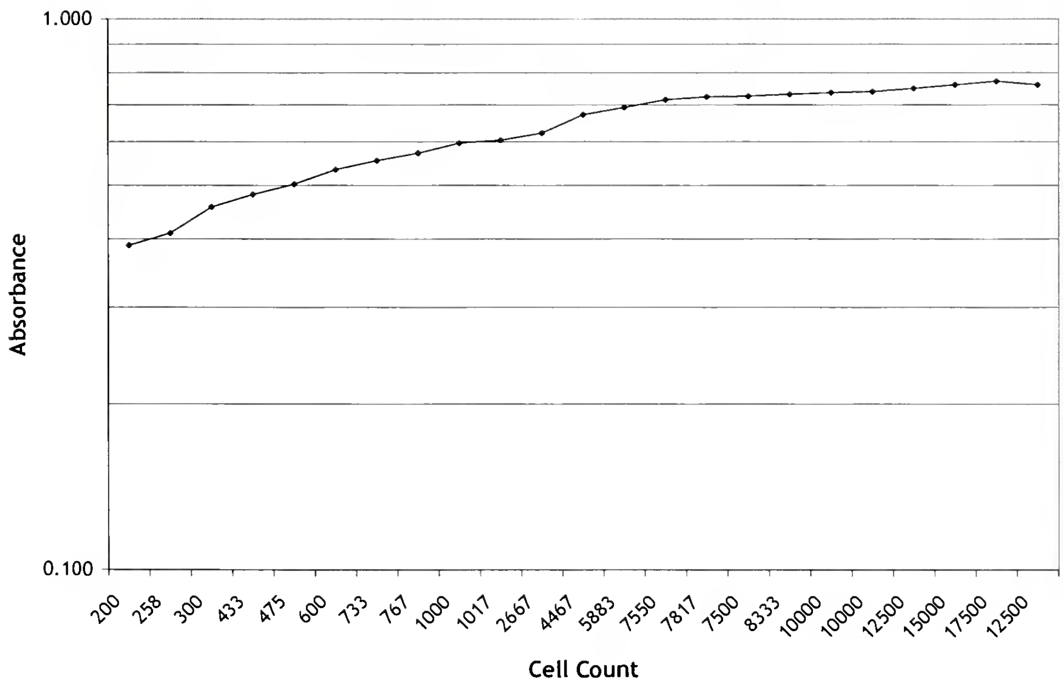
Cell Count increases from 5000 to 10000

Difference in absorbance = (0.732 -0.669) = 0.063

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
300	200	0.389	2.45	0.408
360	258	0.410	2.57	0.389
420	300	0.457	2.86	0.349
480	433	0.482	3.03	0.330
540	475	0.503	3.18	0.314
600	600	0.534	3.42	0.292
660	733	0.555	3.59	0.279
720	767	0.573	3.74	0.268
750	1000	0.597	3.95	0.253
780	1017	0.604	4.02	0.249
840	2667	0.622	4.19	0.239
900	4467	0.672	4.70	0.213
960	5883	0.692	4.92	0.203
1020	7550	0.715	5.19	0.193
1080	7817	0.724	5.30	0.189
1140	7500	0.725	5.31	0.188
1170	8333	0.731	5.38	0.186
1200	10000	0.736	5.45	0.184
1260	10000	0.740	5.49	0.182
1320	12500	0.749	5.61	0.178
1380	15000	0.761	5.76	0.173
1440	17500	0.772	5.92	0.169
1500	12500	0.761	5.77	0.173

Cell Count	Absorbance
200	0.389
258	0.410
300	0.457
433	0.482
475	0.503
600	0.534
733	0.555
767	0.573
1000	0.597
1017	0.604
2667	0.622
4467	0.672
5883	0.692
7550	0.715
7817	0.724
7500	0.725
8333	0.731
10000	0.736
10000	0.740
12500	0.749
15000	0.761
17500	0.772
12500	0.761

Figure A-126. Average Glucose With Pb Absorbance vs. Cell Count



Cell Count increases from 300 to 600

Difference in absorbance = (0.534 -0.457) = 0.077

APPENDIX B

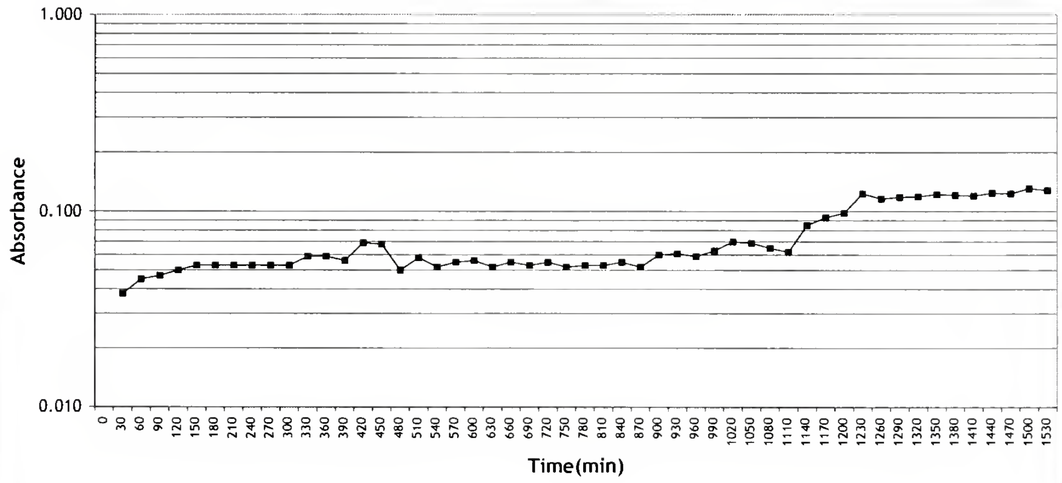
All of the doubling times for *M.roseus* are presented in APPENDIX B

Micrococus roseus

Lactose control

Time	Run1	Run2	Run3	Average
0	0.000	0.000	0.000	0.000
30	0.038	0.039	0.037	0.038
60	0.045	0.043	0.046	0.045
90	0.047	0.043	0.043	0.044
120	0.050	0.043	0.046	0.046
150	0.053	0.044	0.045	0.047
180	0.053	0.049	0.048	0.050
210	0.053	0.065	0.049	0.056
240	0.053	0.066	0.051	0.057
270	0.053	0.065	0.053	0.057
300	0.053	0.065	0.052	0.057
330	0.059	0.066	0.056	0.060
360	0.059	0.065	0.055	0.060
390	0.056	0.071	0.059	0.062
420	0.069	0.076	0.058	0.068
450	0.068	0.075	0.061	0.068
480	0.050	0.072	0.058	0.060
510	0.058	0.057	0.054	0.056
540	0.052	0.053	0.056	0.054
570	0.055	0.059	0.059	0.058
600	0.056	0.068	0.061	0.062
630	0.052	0.065	0.063	0.060
660	0.055	0.066	0.064	0.062
690	0.053	0.069	0.059	0.060
720	0.055	0.065	0.062	0.061
750	0.052	0.060	0.060	0.057
780	0.053	0.059	0.059	0.057
810	0.053	0.060	0.058	0.057
840	0.055	0.061	0.061	0.059
870	0.052	0.060	0.060	0.057
900	0.060	0.065	0.063	0.063
930	0.061	0.060	0.061	0.061
960	0.059	0.067	0.058	0.061
990	0.063	0.065	0.059	0.062
1020	0.070	0.068	0.055	0.064
1050	0.069	0.067	0.058	0.065
1080	0.065	0.073	0.061	0.066
1110	0.062	0.066	0.065	0.064
1140	0.085	0.063	0.069	0.072
1170	0.093	0.086	0.076	0.085
1200	0.098	0.097	0.088	0.094
1230	0.123	0.128	0.126	0.126
1260	0.116	0.127	0.130	0.124
1290	0.118	0.121	0.132	0.124
1320	0.119	0.127	0.134	0.127
1350	0.122	0.125	0.135	0.127
1380	0.121	0.123	0.137	0.127
1410	0.120	0.125	0.136	0.127
1440	0.124	0.135	0.135	0.131
1470	0.123	0.131	0.139	0.131
1500	0.131	0.133	0.140	0.135
1530	0.128	0.129	0.142	0.133

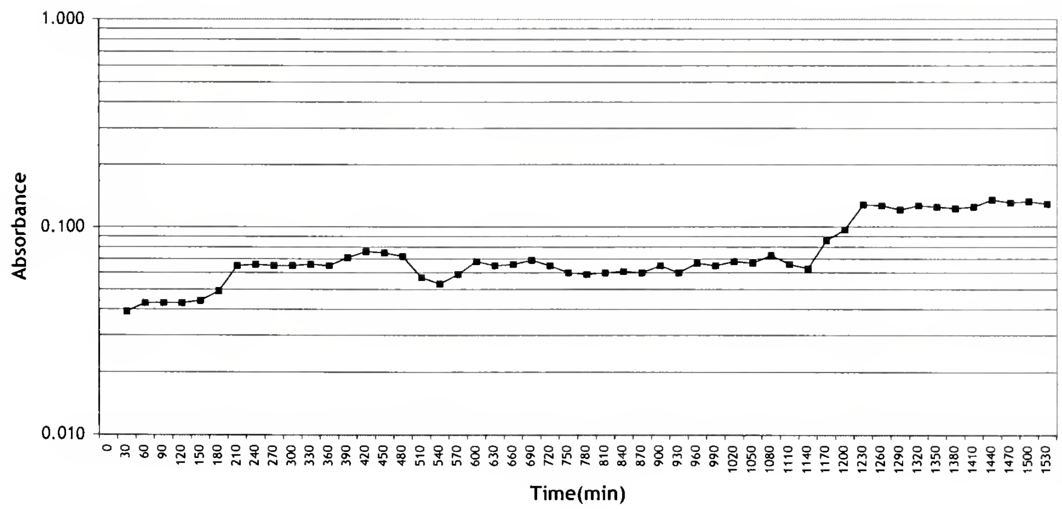
Figure B-1. Lactose Control (first set of data)



Absorbance increases from 0.062 to 0.123

Actual growth rate = (1230 min -1100 min) = 120 min

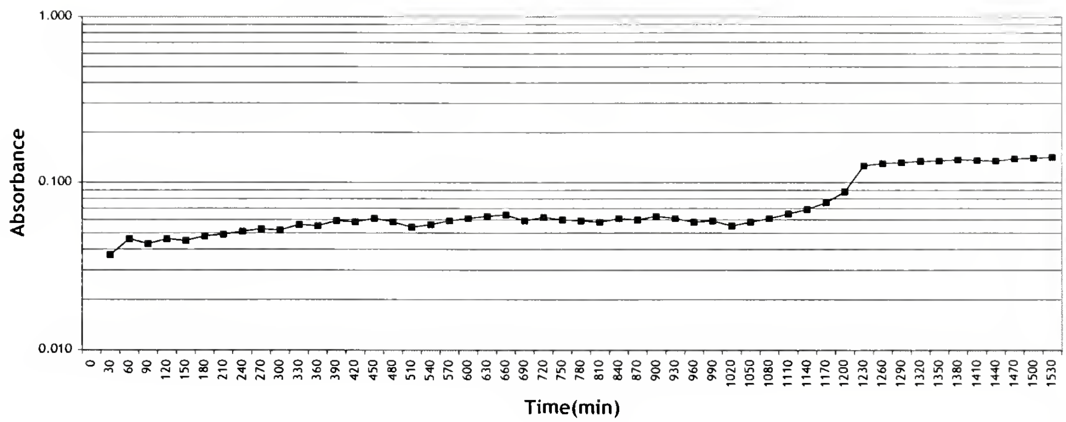
Figure B-2. Lactose Control (second set of data)



Absorbance increases from 0.063 to 0.127

Actual growth rate = (1260 min -1140 min) = 120 min

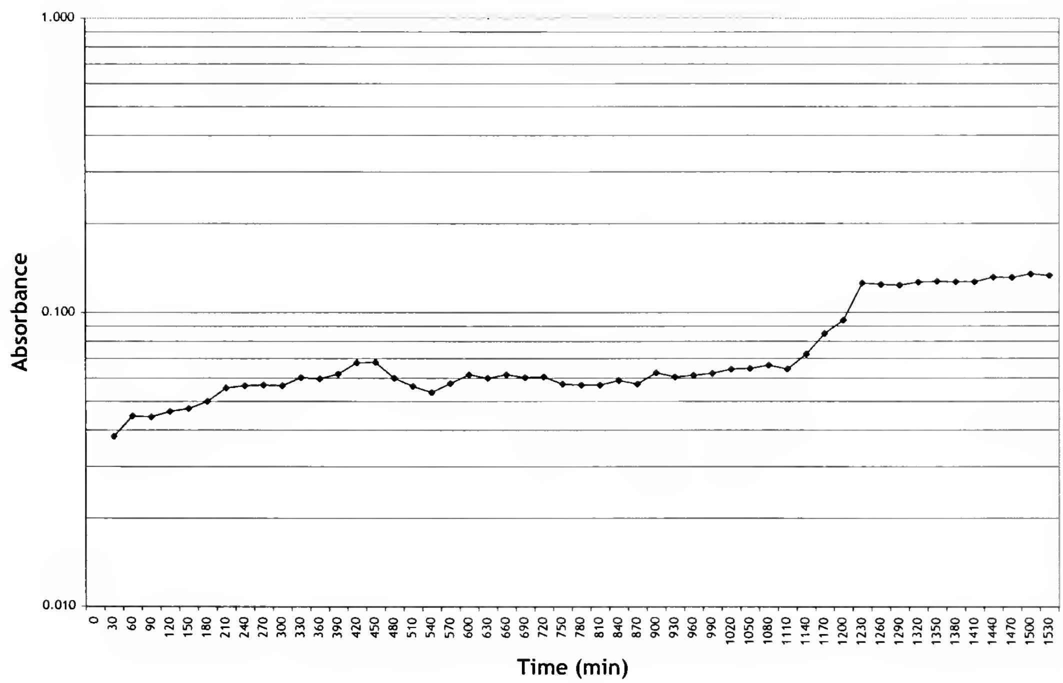
Figure B-3. Lactose Control (third set of data)



Absorbance increases from 0.065 to 0.130

Actual growth rate = (1260 min -1110 min) = 150 min

Figure B-4. Average Lactose Control



Absorbance increases from 0.064 to 0.126

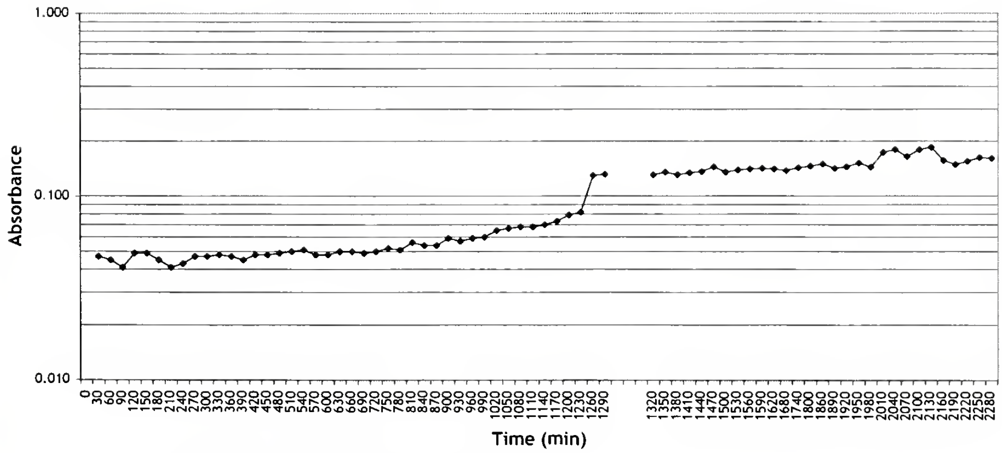
Actual growth rate = (1230 min -1110 min) = 120 min

Micrococus roseus
Lactose With Cadmium

Time (min)	Run 1	Run 2	Average
0	0.000	0.000	0.000
30	0.047	0.032	0.040
60	0.045	0.039	0.042
90	0.041	0.030	0.036
120	0.049	0.032	0.041
150	0.049	0.040	0.045
180	0.045	0.039	0.042
210	0.041	0.041	0.041
240	0.043	0.044	0.044
270	0.047	0.044	0.046
300	0.047	0.047	0.047
330	0.048	0.047	0.048
360	0.047	0.047	0.047
390	0.045	0.035	0.040
420	0.048	0.038	0.043
450	0.048	0.038	0.043
480	0.049	0.061	0.055
510	0.050	0.067	0.059
540	0.051	0.067	0.059
570	0.048	0.065	0.057
600	0.048	0.067	0.058
630	0.050	0.066	0.058
660	0.050	0.069	0.060
690	0.049	0.061	0.055
720	0.050	0.063	0.057
750	0.052	0.060	0.056
780	0.051	0.063	0.057
810	0.056	0.060	0.058
840	0.054	0.060	0.057
870	0.054	0.061	0.058
900	0.059	0.065	0.062
930	0.057	0.061	0.059
960	0.059	0.065	0.062
990	0.060	0.068	0.064
1020	0.065	0.065	0.065
1050	0.067	0.064	0.066
1080	0.068	0.075	0.072
1110	0.068	0.073	0.071
1140	0.070	0.078	0.074
1170	0.073	0.078	0.076
1200	0.079	0.080	0.080
1230	0.082	0.083	0.083
1260	0.130	0.100	0.115
1290	0.132	0.128	0.130

1320	0.131	0.123	0.127
1350	0.135	0.121	0.128
1380	0.131	0.124	0.128
1410	0.134	0.128	0.131
1440	0.136	0.126	0.131
1470	0.145	0.126	0.136
1500	0.135	0.128	0.132
1530	0.139	0.132	0.136
1560	0.141	0.128	0.135
1590	0.142	0.127	0.135
1620	0.141	0.125	0.133
1680	0.138	0.131	0.135
1740	0.143	0.128	0.136
1800	0.146	0.130	0.138
1860	0.150	0.131	0.141
1890	0.142	0.148	0.145
1920	0.145	0.142	0.144
1950	0.152	0.157	0.155
1980	0.144	0.164	0.154
2010	0.174	0.174	0.174
2040	0.180	0.147	0.164
2070	0.165	0.153	0.159
2100	0.180	0.164	0.172
2130	0.185	0.161	0.173
2160	0.157	0.167	0.162
2190	0.149	0.171	0.160
2220	0.155	0.209	0.182
2250	0.163	0.202	0.183
2280	0.161	0.176	0.169

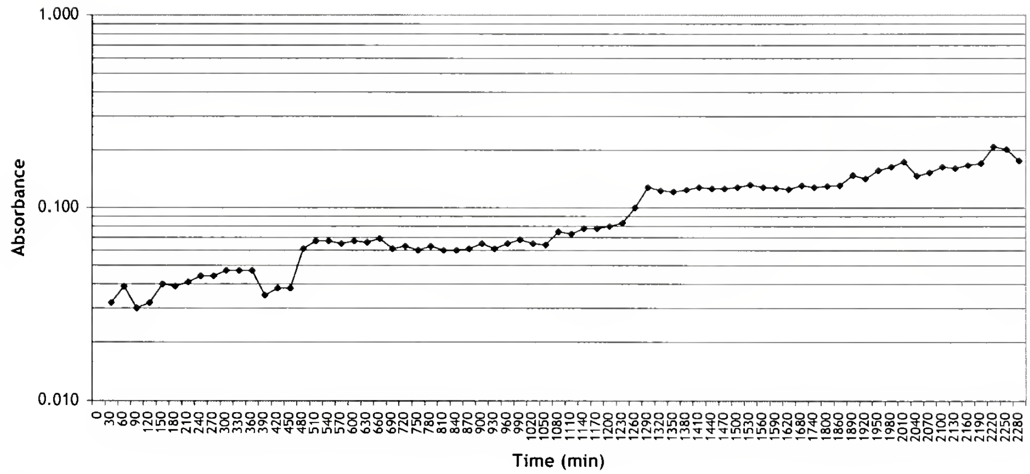
Figure B-5. Lactose With Cd 1



Absorbance increases from 0.065 to 0.130

Actual growth = **(1260 min -1020 min) = 240 min**

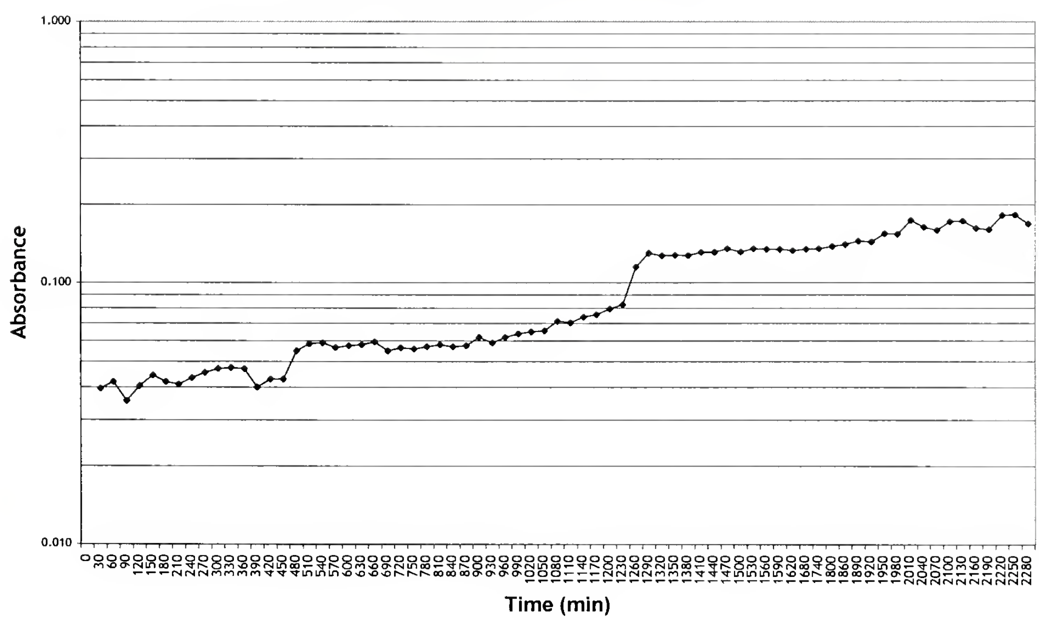
Figure B-6. Lactose With Cd 2



Absorbance increases from 0.064 to 0.128

Actual growth = (1290 min -1050 min) = 240 min

Figure B-7. Average Lactose With Cd

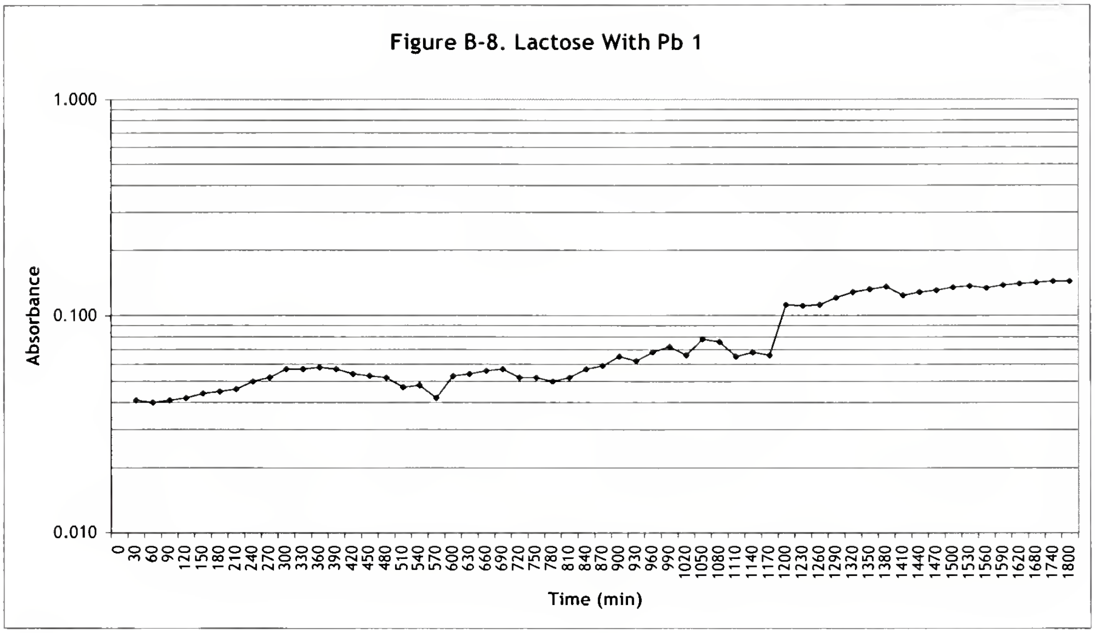


Absorbance increases from 0.065 to 0.130

Actual growth = (1290 min -1020 min) = 270 min

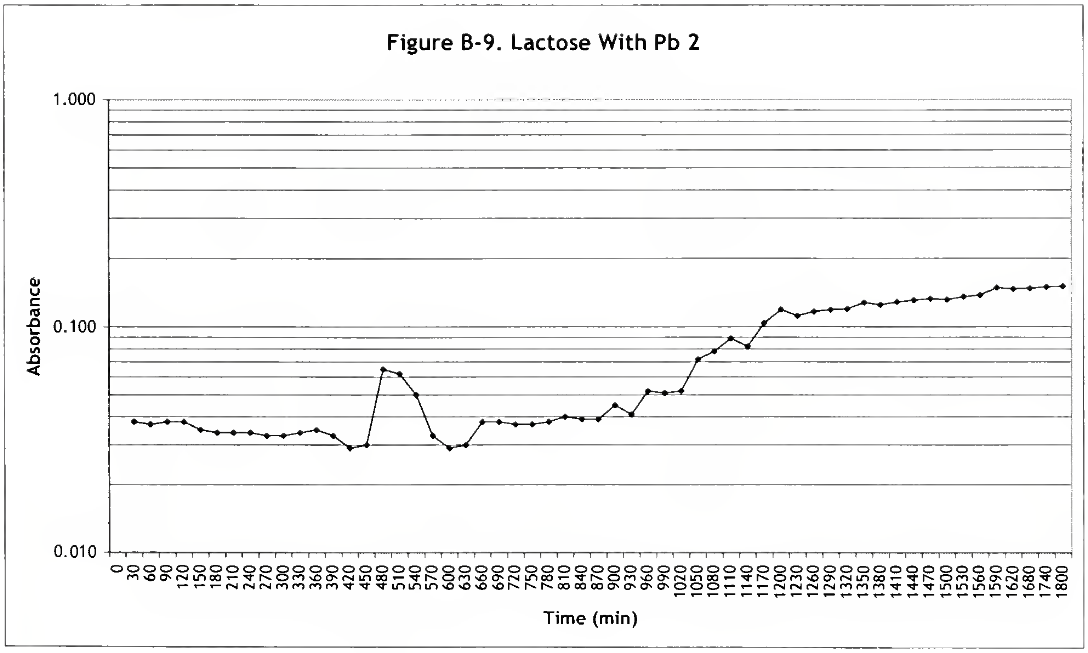
Lactose With Lead (*Micrococus roseus*)

Time (min)	Run 1	Run 2	Run 3	Average
0	0.000	0.000	0.000	0.000
30	0.041	0.038	0.036	0.038
60	0.040	0.037	0.037	0.038
90	0.041	0.038	0.038	0.039
120	0.042	0.038	0.036	0.039
150	0.044	0.035	0.035	0.038
180	0.045	0.034	0.033	0.037
210	0.046	0.034	0.034	0.038
240	0.050	0.034	0.036	0.040
270	0.052	0.033	0.038	0.041
300	0.057	0.033	0.035	0.042
330	0.057	0.034	0.033	0.041
360	0.058	0.035	0.035	0.043
390	0.057	0.033	0.033	0.041
420	0.054	0.029	0.038	0.040
450	0.053	0.030	0.046	0.043
480	0.052	0.065	0.053	0.057
510	0.047	0.062	0.059	0.056
540	0.048	0.050	0.051	0.050
570	0.042	0.033	0.048	0.041
600	0.053	0.029	0.045	0.042
630	0.054	0.030	0.043	0.042
660	0.056	0.038	0.041	0.045
690	0.057	0.038	0.048	0.048
720	0.052	0.037	0.045	0.045
750	0.052	0.037	0.041	0.043
780	0.050	0.038	0.043	0.044
810	0.052	0.040	0.044	0.045
840	0.057	0.039	0.046	0.047
870	0.059	0.039	0.041	0.046
900	0.065	0.045	0.045	0.052
930	0.062	0.041	0.049	0.051
960	0.068	0.052	0.051	0.057
990	0.072	0.051	0.052	0.058
1020	0.066	0.052	0.051	0.056
1050	0.078	0.072	0.073	0.074
1080	0.076	0.078	0.075	0.076
1110	0.065	0.089	0.084	0.079
1140	0.068	0.082	0.091	0.080
1170	0.066	0.104	0.102	0.091
1200	0.112	0.119	0.116	0.116
1230	0.111	0.112	0.118	0.114
1260	0.112	0.117	0.117	0.115
1290	0.121	0.119	0.119	0.120
1320	0.128	0.120	0.120	0.123
1350	0.132	0.128	0.122	0.127
1380	0.136	0.125	0.124	0.128
1410	0.124	0.129	0.129	0.127
1440	0.128	0.131	0.131	0.130
1470	0.131	0.133	0.133	0.132
1500	0.135	0.132	0.132	0.133
1530	0.137	0.136	0.135	0.136
1560	0.134	0.138	0.138	0.137
1590	0.138	0.149	0.141	0.143
1620	0.140	0.147	0.144	0.144
1680	0.142	0.148	0.146	0.145
1740	0.144	0.150	0.149	0.148
1800	0.144	0.151	0.151	0.149



Absorbance increases from 0.068 to 0.136

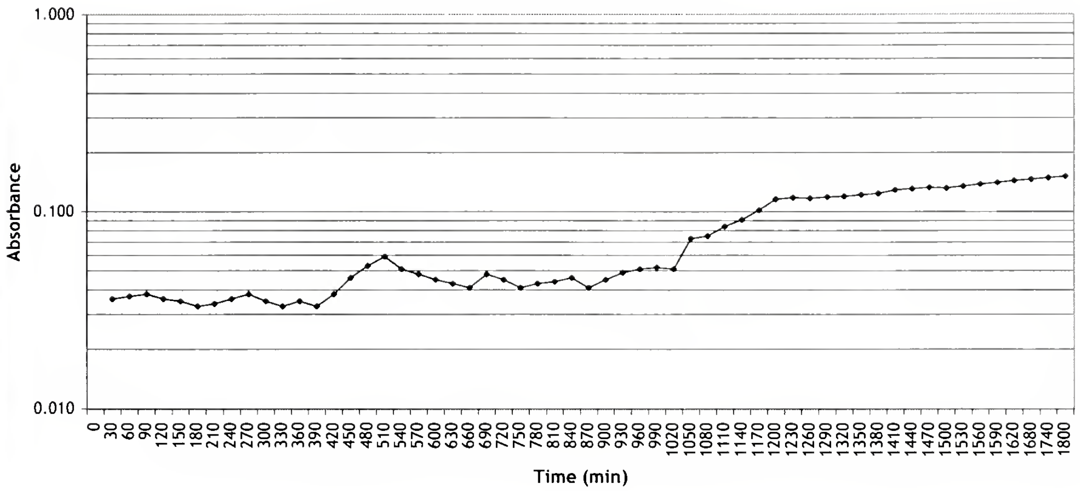
Actual growth rate = **(1380 min -1140 min) = 240 min**



Absorbance increases from 0.052 to 0.104

Actual growth rate = **(1170 min -960 min) = 210 min**

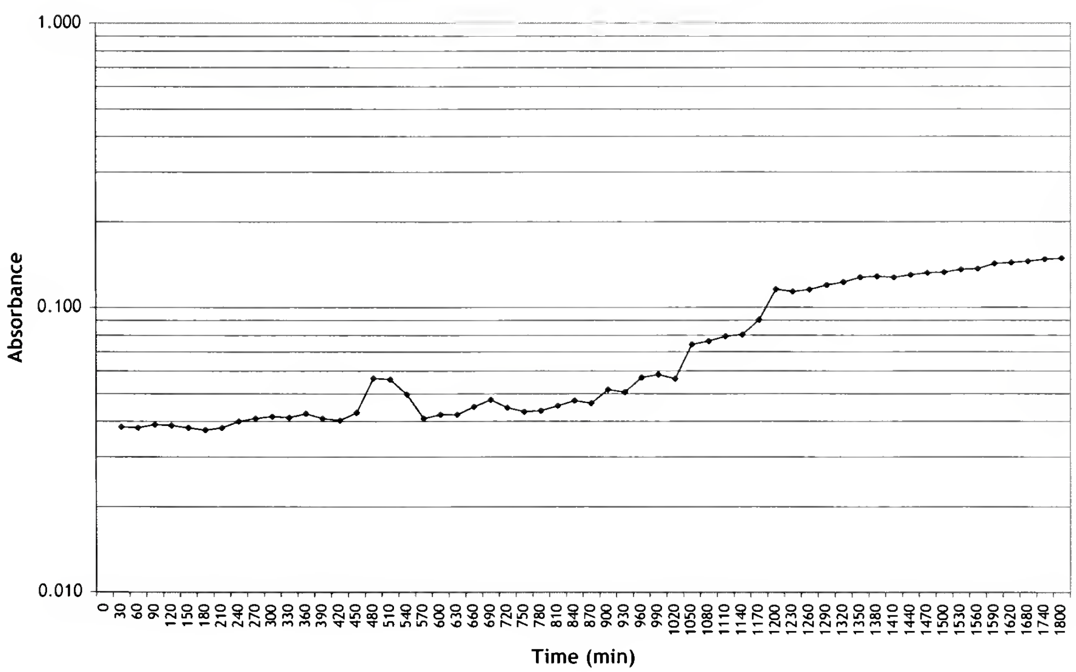
Figure B-10. Lactose With Pb 3



Absorbance increases from 0.051 to 0.102

Actual growth rate = (1170 min -960 min) = 210 min

Figure B-11. Average Lactose With Pb



Absorbance increases from 0.058 to 0.116

Actual growth rate = (1200 min -990 min) = 210 min

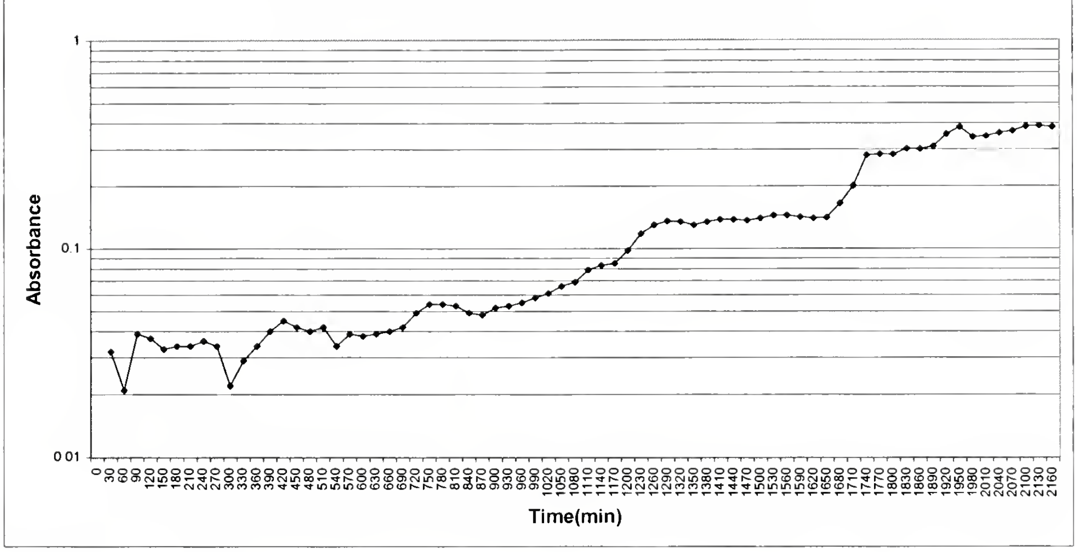
Micrococus roseus

Glucose control

Time(min)	Run1	Run2	Run3	Average
0	0	0	0	0.000
30	0.032	0.028	0.026	0.029
60	0.021	0.029	0.038	0.029
90	0.039	0.037	0.041	0.039
120	0.037	0.038	0.044	0.040
150	0.033	0.043	0.046	0.041
180	0.034	0.045	0.044	0.041
210	0.034	0.047	0.049	0.043
240	0.036	0.030	0.047	0.038
270	0.034	0.031	0.042	0.036
300	0.022	0.032	0.041	0.032
330	0.029	0.042	0.044	0.038
360	0.034	0.045	0.046	0.042
390	0.040	0.051	0.049	0.047
420	0.045	0.041	0.051	0.046
450	0.042	0.041	0.05	0.044
480	0.040	0.043	0.052	0.045
510	0.042	0.048	0.049	0.046
540	0.034	0.046	0.053	0.044
570	0.039	0.046	0.051	0.045
600	0.038	0.045	0.049	0.044
630	0.039	0.052	0.054	0.048
660	0.040	0.062	0.056	0.053
690	0.042	0.061	0.059	0.054
720	0.049	0.066	0.066	0.060
750	0.054	0.064	0.068	0.062
780	0.054	0.060	0.066	0.060
810	0.053	0.062	0.067	0.061
840	0.049	0.060	0.071	0.060
870	0.048	0.059	0.068	0.058
900	0.052	0.065	0.072	0.063
930	0.053	0.063	0.071	0.062
960	0.055	0.068	0.074	0.066
990	0.058	0.069	0.076	0.068
1020	0.061	0.072	0.078	0.070
1050	0.066	0.071	0.08	0.072
1080	0.069	0.078	0.081	0.076
1110	0.079	0.085	0.085	0.083
1140	0.083	0.095	0.096	0.091
1170	0.085	0.097	0.092	0.091
1200	0.098	0.111	0.108	0.106
1230	0.118	0.115	0.119	0.117
1260	0.130	0.111	0.124	0.122
1290	0.136	0.125	0.131	0.131
1320	0.135	0.123	0.134	0.131
1350	0.130	0.122	0.138	0.130
1380	0.135	0.129	0.141	0.135
1410	0.138	0.125	0.143	0.135
1440	0.138	0.125	0.144	0.136
1470	0.137	0.121	0.148	0.135

1500	0.140	0.122	0.146	0.136
1530	0.145	0.121	0.151	0.139
1560	0.145	0.129	0.152	0.142
1590	0.142	0.125	0.154	0.140
1620	0.140	0.135	0.156	0.144
1650	0.141	0.130	0.152	0.141
1680	0.165	0.136	0.158	0.153
1710	0.201	0.190	0.211	0.201
1740	0.251	0.222	0.248	0.240
1770	0.265	0.249	0.267	0.260
1800	0.282	0.261	0.302	0.282
1830	0.303	0.276	0.314	0.298
1860	0.301	0.278	0.321	0.300
1890	0.310	0.274	0.326	0.303
1920	0.355	0.283	0.348	0.329
1950	0.384	0.294	0.361	0.346
1980	0.344	0.297	0.365	0.335
2010	0.347	0.290	0.361	0.333
2040	0.360	0.288	0.369	0.339
2070	0.368	0.285	0.372	0.342
2100	0.386	0.297	0.383	0.355
2130	0.390	0.304	0.392	0.362
2160	0.384	0.284	0.391	0.353

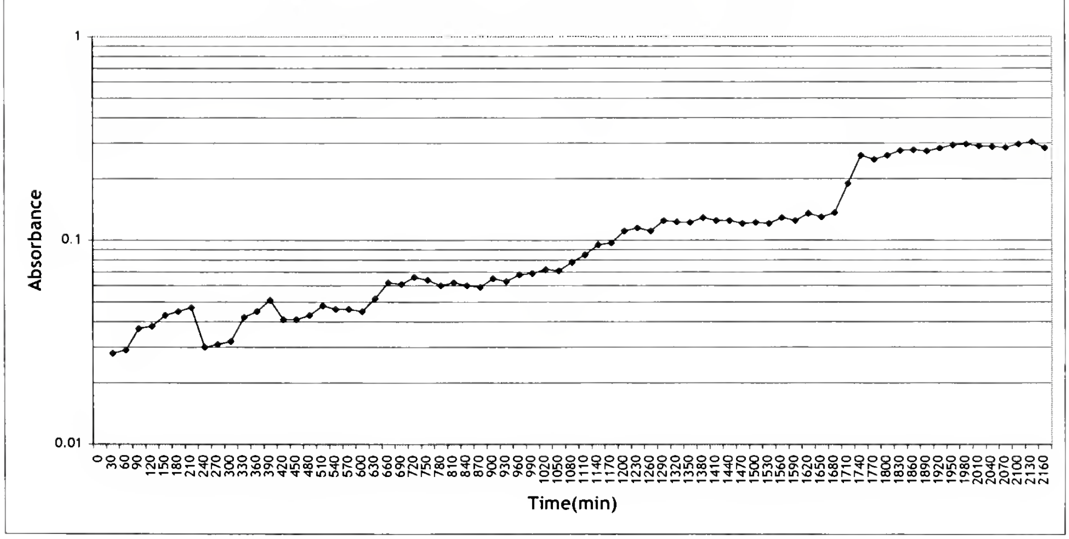
Figure B-12. Glucose Control (first set of data)



Absorbance increases from 0.141 to 0.282

Actual growth rate = (1800 min -1650 min) = 150 min

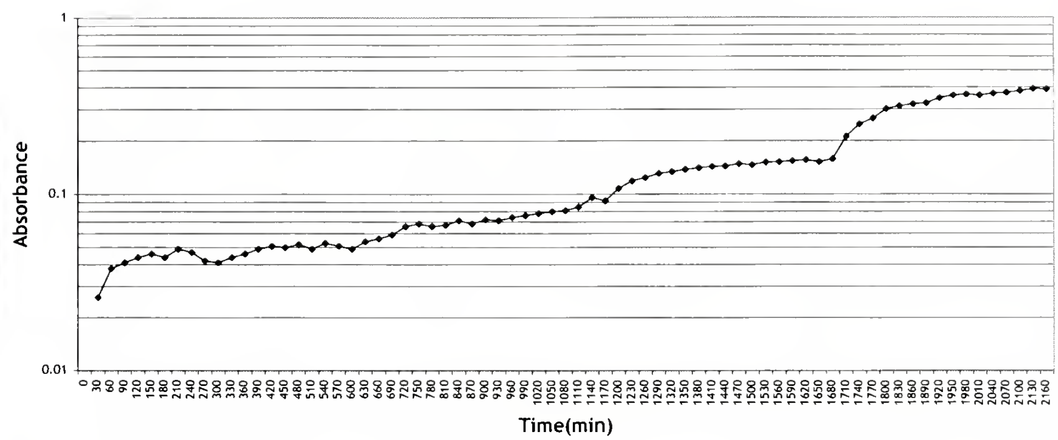
Figure B-13. Glucose Control (second set of data)



Absorbance increases from 0.130 to 0.261

Actual growth rate = (1800 min -1650 min) = 150 min

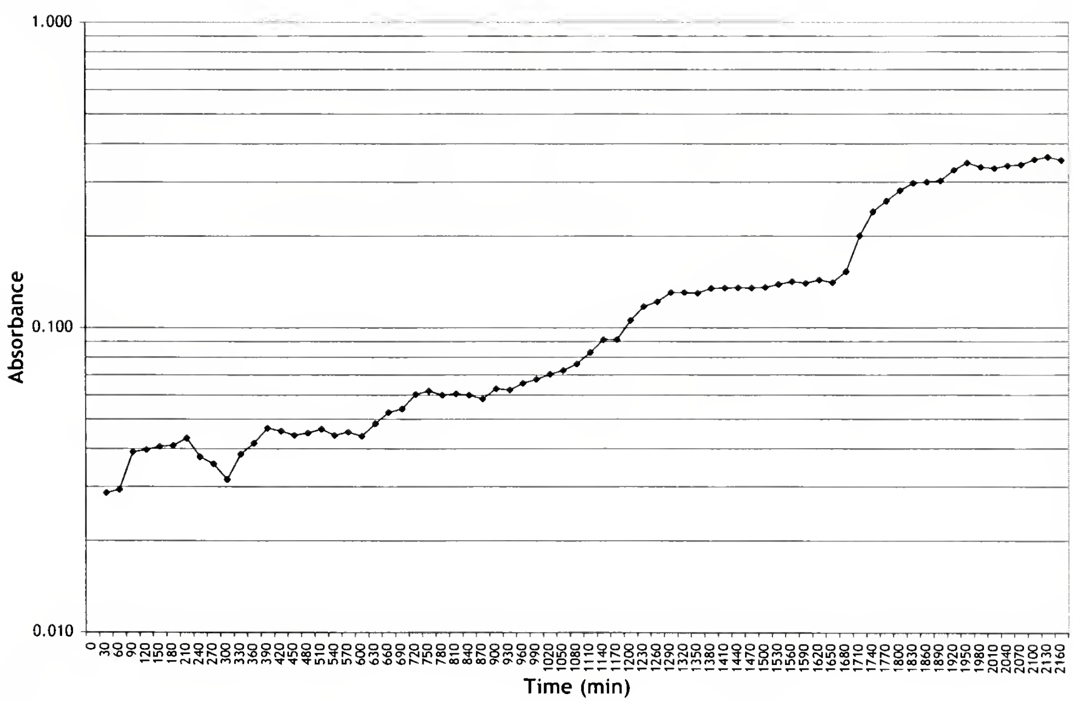
Figure B-14. Glucose Control (third set of data)



Absorbance increases from 0.158 to 0.314

Actual growth rate = (1830 min -1680 min) = 150 min

Figure B-15. Average Glucose Control



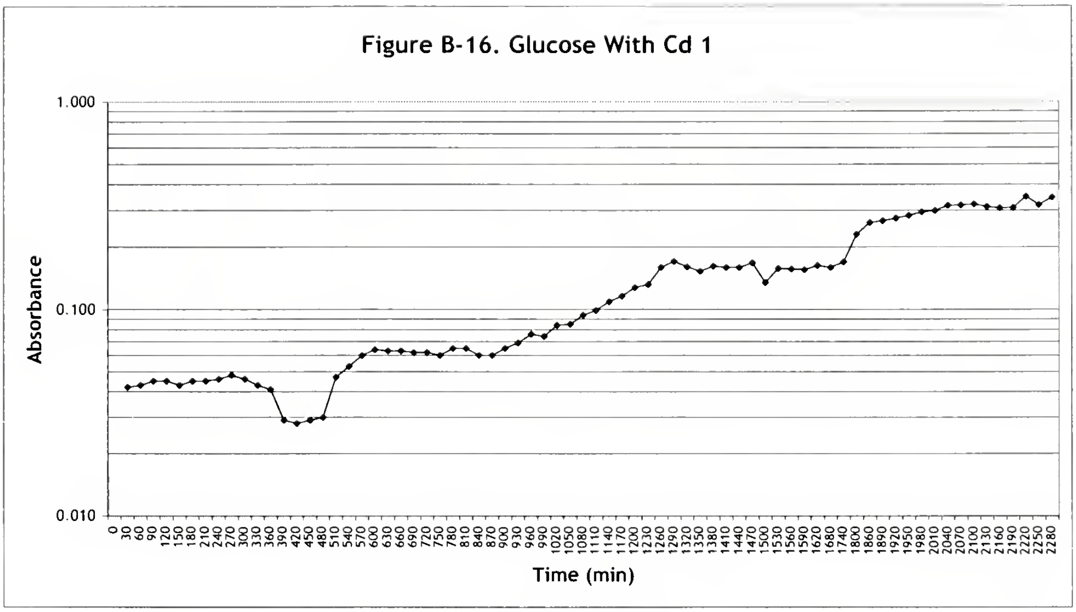
Absorbance increases from 0.141 to 0.282

Actual growth rate = (1800 min -1650 min) = 150 min

Glucose and Cd at concentration of $1*10^{-3}M$

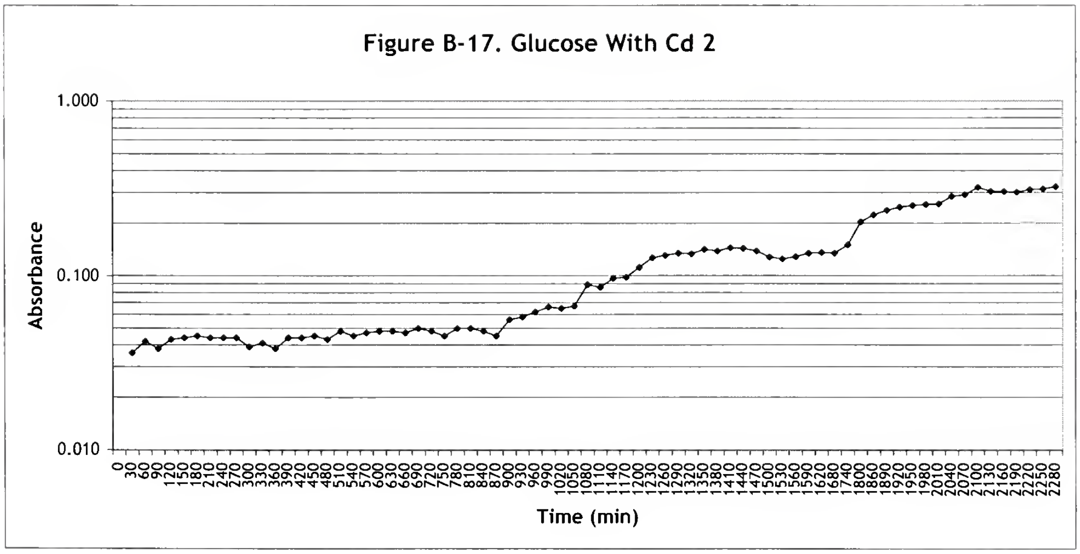
Time (min)	Run 1	Run 2	Run3	Average
0	0.000	0.000	0.000	0.000
30	0.042	0.036	0.032	0.037
60	0.043	0.042	0.038	0.041
90	0.045	0.038	0.041	0.041
120	0.045	0.043	0.044	0.044
150	0.043	0.044	0.046	0.044
180	0.045	0.045	0.047	0.046
210	0.045	0.044	0.045	0.045
240	0.046	0.044	0.046	0.045
270	0.048	0.044	0.043	0.045
300	0.046	0.039	0.049	0.045
330	0.043	0.041	0.046	0.043
360	0.041	0.038	0.044	0.041
390	0.029	0.044	0.042	0.038
420	0.028	0.044	0.046	0.039
450	0.029	0.045	0.048	0.041
480	0.030	0.043	0.045	0.039
510	0.047	0.048	0.051	0.049
540	0.053	0.045	0.053	0.050
570	0.060	0.047	0.056	0.054
600	0.064	0.048	0.059	0.057
630	0.063	0.048	0.061	0.057
660	0.063	0.047	0.063	0.058
690	0.062	0.050	0.065	0.059
720	0.062	0.048	0.068	0.059
750	0.060	0.045	0.071	0.059
780	0.065	0.050	0.069	0.061
810	0.065	0.050	0.073	0.063
840	0.060	0.048	0.071	0.060
870	0.060	0.045	0.075	0.060
900	0.065	0.056	0.078	0.066
930	0.069	0.058	0.075	0.067
960	0.076	0.062	0.078	0.072
990	0.074	0.066	0.076	0.072
1020	0.084	0.065	0.078	0.076
1050	0.085	0.067	0.081	0.078
1080	0.094	0.089	0.088	0.090
1110	0.099	0.086	0.097	0.094
1140	0.109	0.097	0.106	0.104
1170	0.116	0.098	0.112	0.109
1200	0.128	0.112	0.122	0.121
1230	0.132	0.127	0.128	0.129
1260	0.160	0.131	0.148	0.146
1290	0.171	0.135	0.161	0.156
1320	0.161	0.134	0.168	0.154
1350	0.153	0.142	0.171	0.155
1380	0.162	0.139	0.174	0.158
1410	0.160	0.145	0.176	0.160
1440	0.160	0.144	0.174	0.159
1470	0.168	0.139	0.178	0.162
1500	0.135	0.128	0.174	0.146
1530	0.158	0.125	0.176	0.153
1560	0.157	0.129	0.178	0.155

1590	0.156	0.135	0.181	0.157
1620	0.163	0.136	0.183	0.161
1680	0.160	0.135	0.185	0.160
1740	0.170	0.151	0.181	0.167
1800	0.230	0.204	0.218	0.217
1860	0.263	0.224	0.242	0.243
1890	0.268	0.238	0.261	0.256
1920	0.276	0.248	0.272	0.265
1950	0.284	0.253	0.281	0.273
1980	0.295	0.256	0.294	0.282
2010	0.299	0.259	0.296	0.285
2040	0.317	0.285	0.312	0.305
2070	0.318	0.291	0.317	0.309
2100	0.322	0.321	0.324	0.322
2130	0.312	0.305	0.321	0.313
2160	0.308	0.304	0.319	0.310
2190	0.309	0.301	0.325	0.312
2220	0.351	0.312	0.341	0.335
2250	0.319	0.315	0.348	0.327
2280	0.347	0.324	0.346	0.339



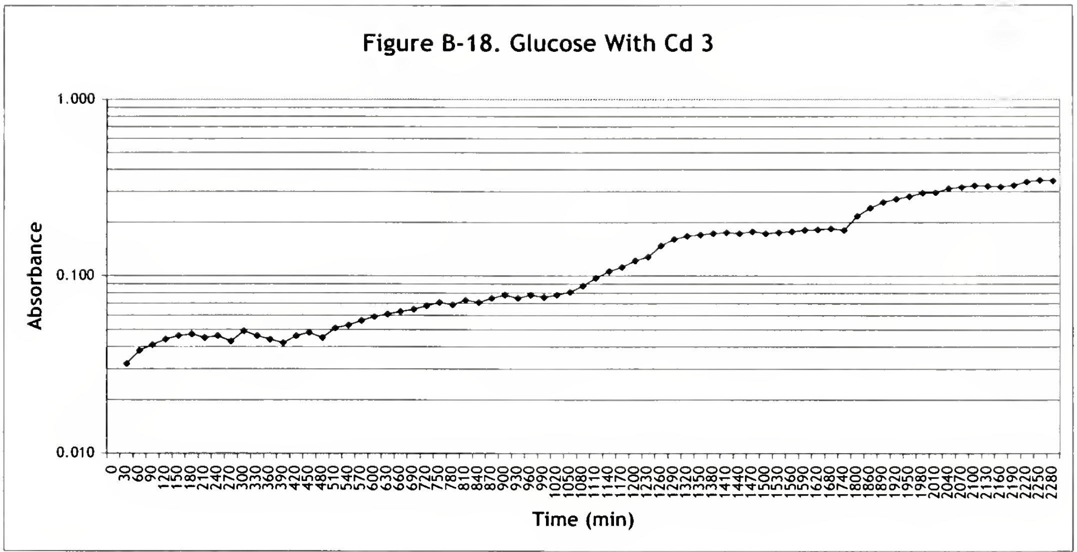
Absorbance increases from 0.085 to 0.171

Actual growth rate = (1290 min -1050 min) = 240 min



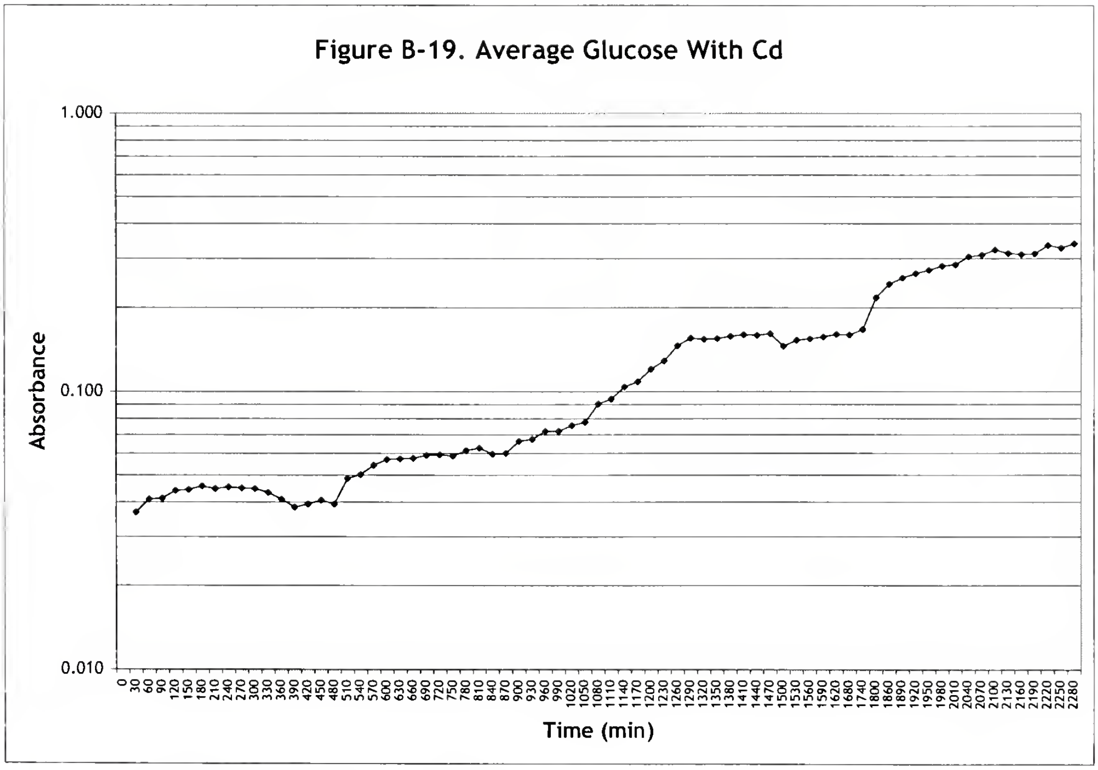
Absorbance increases from 0.065 to 0.131

Actual growth rate = (1260 min -1020 min) = 240 min



Absorbance increases from 0.081 to 0.161

Actual growth rate = (1290 min -1050 min) = 240 min



Absorbance increases from 0.078 to 0.156

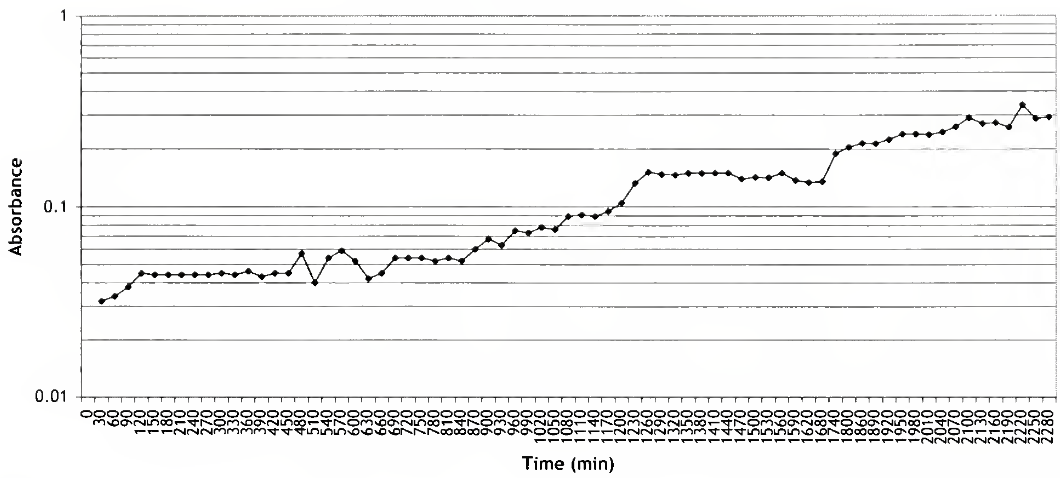
Actual growth rate = (1290 min -1050 min) = 240 min

Glucose and Pb at concentration of $1*10^{-3}M$

Time (min	Run 1	Run 2	Run3	Average
0	0	0	0	0.000
30	0.032	0.038	0.032	0.034
60	0.034	0.031	0.036	0.034
90	0.038	0.042	0.041	0.040
120	0.045	0.043	0.043	0.044
150	0.044	0.045	0.045	0.045
180	0.044	0.045	0.044	0.044
210	0.044	0.047	0.046	0.046
240	0.044	0.046	0.043	0.044
270	0.044	0.045	0.044	0.044
300	0.045	0.045	0.045	0.045
330	0.044	0.045	0.044	0.044
360	0.046	0.040	0.046	0.044
390	0.043	0.054	0.043	0.047
420	0.045	0.048	0.047	0.047
450	0.045	0.048	0.051	0.048
480	0.057	0.045	0.057	0.053
510	0.040	0.045	0.053	0.046
540	0.054	0.051	0.056	0.054
570	0.059	0.053	0.059	0.057
600	0.052	0.048	0.054	0.051
630	0.042	0.052	0.056	0.050
660	0.045	0.054	0.057	0.052
690	0.054	0.050	0.054	0.053
720	0.054	0.052	0.056	0.054
750	0.054	0.054	0.057	0.055
780	0.052	0.050	0.059	0.054
810	0.054	0.055	0.058	0.056
840	0.052	0.054	0.057	0.054
870	0.060	0.050	0.060	0.057
900	0.068	0.058	0.068	0.065
930	0.063	0.068	0.067	0.066
960	0.075	0.065	0.075	0.072
990	0.073	0.066	0.078	0.072
1020	0.078	0.075	0.079	0.077
1050	0.076	0.078	0.081	0.078
1080	0.089	0.088	0.089	0.089
1110	0.091	0.086	0.091	0.089
1140	0.089	0.092	0.092	0.091
1170	0.095	0.112	0.095	0.101
1200	0.105	0.131	0.108	0.115
1230	0.133	0.134	0.141	0.136
1260	0.152	0.155	0.161	0.156
1290	0.148	0.153	0.162	0.154
1320	0.147	0.149	0.159	0.152
1350	0.150	0.152	0.163	0.155
1380	0.150	0.148	0.165	0.154
1410	0.150	0.156	0.161	0.156
1440	0.150	0.152	0.167	0.156
1470	0.140	0.162	0.165	0.156
1500	0.143	0.158	0.163	0.155
1530	0.142	0.169	0.171	0.161

1560	0.150	0.175	0.169	0.165
1590	0.138	0.178	0.173	0.163
1620	0.134	0.186	0.182	0.167
1680	0.136	0.189	0.186	0.170
1740	0.190	0.200	0.191	0.194
1800	0.205	0.240	0.211	0.219
1860	0.215	0.256	0.215	0.229
1890	0.214	0.262	0.217	0.231
1920	0.224	0.282	0.218	0.241
1950	0.240	0.287	0.242	0.256
1980	0.240	0.280	0.261	0.260
2010	0.238	0.311	0.293	0.281
2040	0.245	0.300	0.301	0.282
2070	0.261	0.299	0.308	0.289
2100	0.291	0.308	0.311	0.303
2130	0.271	0.305	0.312	0.296
2160	0.275	0.299	0.308	0.294
2190	0.260	0.302	0.311	0.291
2220	0.340	0.309	0.326	0.325
2250	0.288	0.320	0.336	0.315
2280	0.293	0.315	0.332	0.313

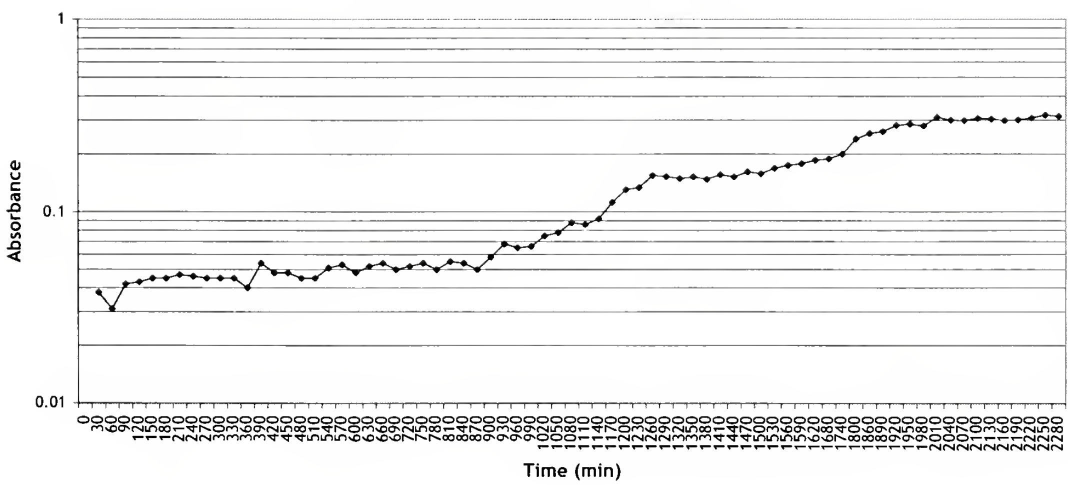
Figure B-20. Glucose With Pb 1



Absorbance increases from 0.076 to 0.152

Actual growth rate = **(1260 min -1050 min) = 210 min**

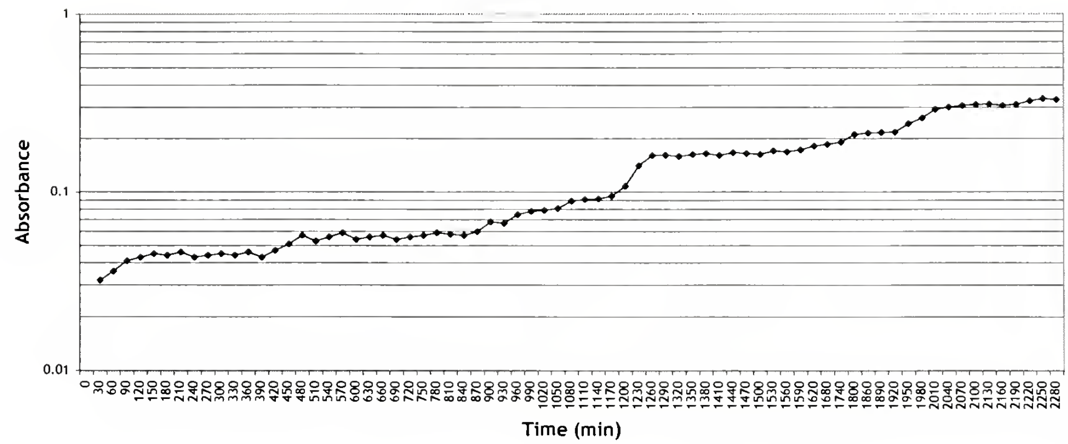
Figure B-21. Glucose With Pb 2



Absorbance increases from 0.076 to 0.152

Actual growth rate = **(1260 min -1050 min) = 210 min**

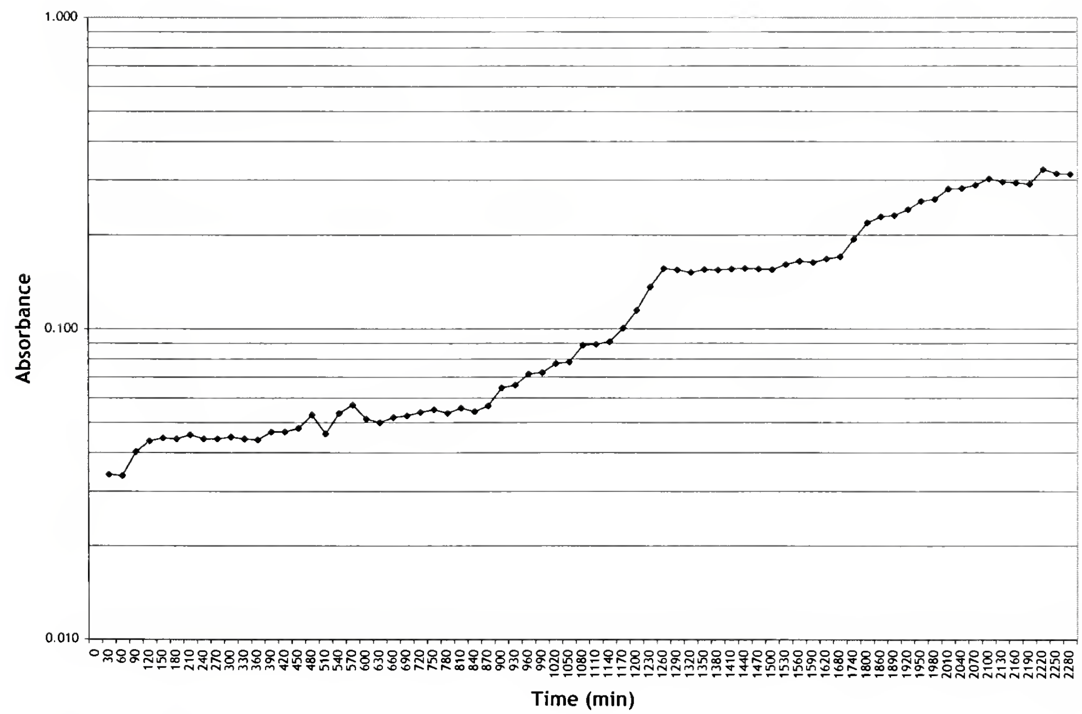
Figure B-22. Glucose with Pb 3



Absorbance increases from 0.081 to 0.161

Actual growth rate = (1260 min -1050 min) = 210 min

Figure B-23. Average Glucose With Pb



Absorbance increases from 0.078 to 0.156

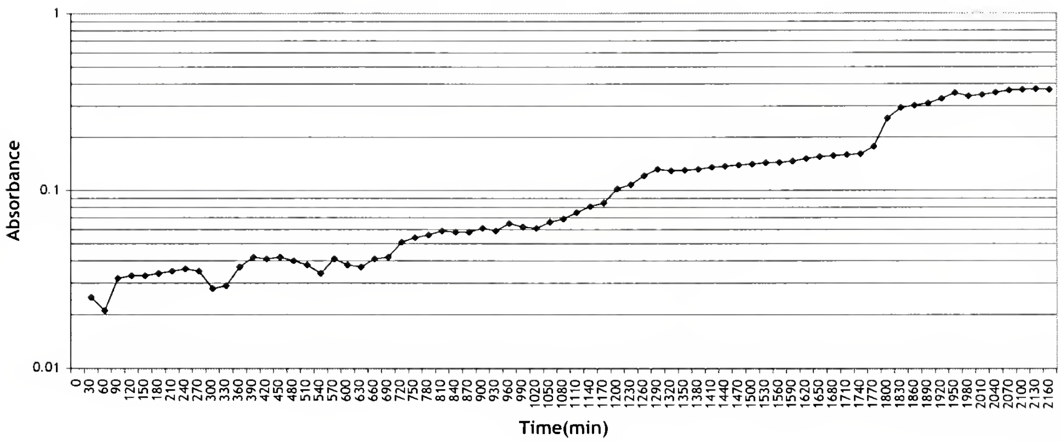
Actual growth rate = (1260 min -1050 min) = 210 min

Glucose and Cd at concentration of $1*10^{-5}$M

Time(min)	Run1	Run2	Run3	Average
0	0	0	0	0.000
30	0.025	0.021	0.024	0.023
60	0.021	0.029	0.023	0.024
90	0.032	0.037	0.028	0.032
120	0.033	0.038	0.033	0.035
150	0.033	0.036	0.032	0.034
180	0.034	0.041	0.034	0.036
210	0.035	0.045	0.036	0.039
240	0.036	0.047	0.038	0.040
270	0.035	0.044	0.041	0.040
300	0.028	0.045	0.046	0.040
330	0.029	0.042	0.048	0.040
360	0.037	0.045	0.044	0.042
390	0.042	0.048	0.045	0.045
420	0.041	0.044	0.046	0.044
450	0.042	0.044	0.048	0.045
480	0.040	0.043	0.044	0.042
510	0.038	0.048	0.042	0.043
540	0.034	0.046	0.046	0.042
570	0.041	0.046	0.048	0.045
600	0.038	0.045	0.046	0.043
630	0.037	0.055	0.051	0.048
660	0.041	0.058	0.054	0.051
690	0.042	0.059	0.051	0.051
720	0.051	0.061	0.052	0.055
750	0.054	0.064	0.054	0.057
780	0.056	0.065	0.056	0.059
810	0.059	0.062	0.058	0.060
840	0.058	0.064	0.059	0.060
870	0.058	0.061	0.058	0.059
900	0.061	0.065	0.062	0.063
930	0.059	0.063	0.063	0.062
960	0.065	0.068	0.065	0.066
990	0.062	0.069	0.064	0.065
1020	0.061	0.072	0.065	0.066
1050	0.066	0.071	0.066	0.068
1080	0.069	0.078	0.068	0.072
1110	0.075	0.085	0.078	0.079
1140	0.081	0.089	0.081	0.084
1170	0.085	0.091	0.092	0.089
1200	0.102	0.098	0.104	0.101
1230	0.108	0.103	0.114	0.108
1260	0.121	0.111	0.123	0.118
1290	0.132	0.128	0.128	0.129
1320	0.129	0.127	0.129	0.128
1350	0.130	0.128	0.130	0.129
1380	0.132	0.129	0.134	0.132
1410	0.135	0.125	0.138	0.133
1440	0.137	0.125	0.141	0.134
1470	0.139	0.126	0.144	0.136
1500	0.141	0.128	0.146	0.138

1530	0.143	0.127	0.148	0.139
1560	0.144	0.129	0.151	0.141
1590	0.146	0.131	0.154	0.144
1620	0.151	0.129	0.158	0.146
1650	0.155	0.131	0.156	0.147
1680	0.157	0.133	0.161	0.150
1710	0.159	0.138	0.163	0.153
1740	0.161	0.146	0.168	0.158
1770	0.177	0.168	0.186	0.177
1800	0.256	0.226	0.248	0.243
1830	0.294	0.248	0.297	0.280
1860	0.303	0.252	0.312	0.289
1890	0.311	0.268	0.325	0.301
1920	0.331	0.291	0.333	0.318
1950	0.356	0.294	0.361	0.337
1980	0.342	0.296	0.363	0.334
2010	0.347	0.290	0.368	0.335
2040	0.357	0.297	0.371	0.342
2070	0.368	0.291	0.374	0.344
2100	0.371	0.298	0.379	0.349
2130	0.375	0.302	0.381	0.353
2160	0.372	0.294	0.379	0.348

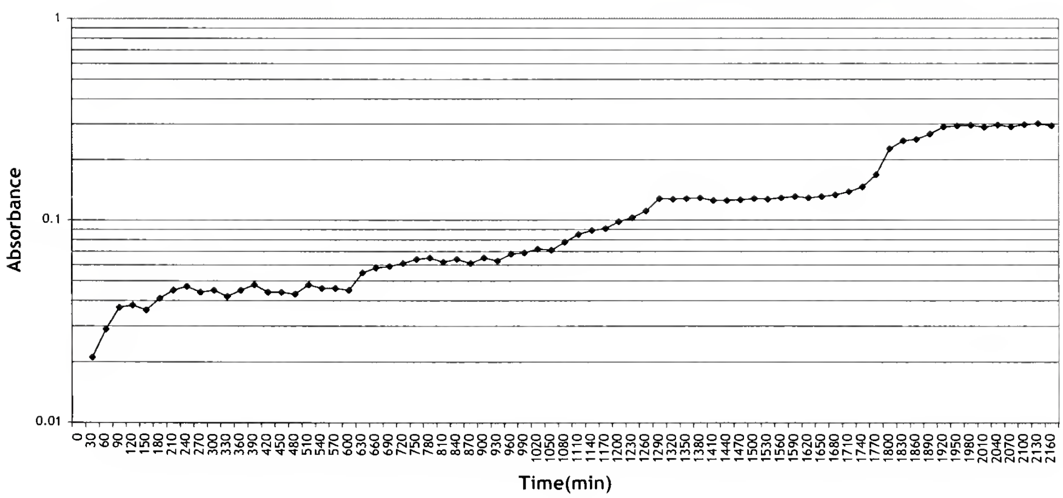
Figure B-24. Glucose With Lower Concentration of Cd (first set of data)



Absorbance increases from 0.177 to 0.356

Actual growth rate = (1950 min -1770 min) = 180 min

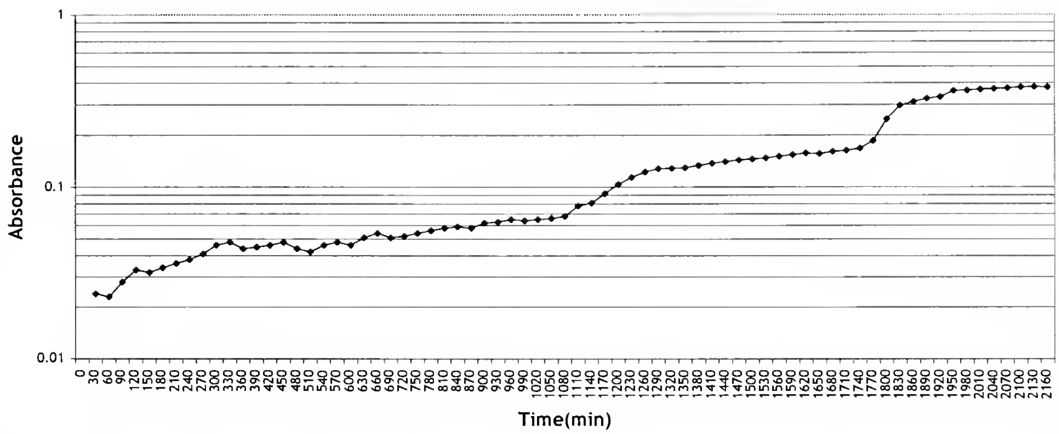
Figure B-25. Glucose With Lower Concentration of Cd (second set of data)



Absorbance increases from 0.146 to 0.291

Actual growth rate = (1920 min -1740 min) = 180 min

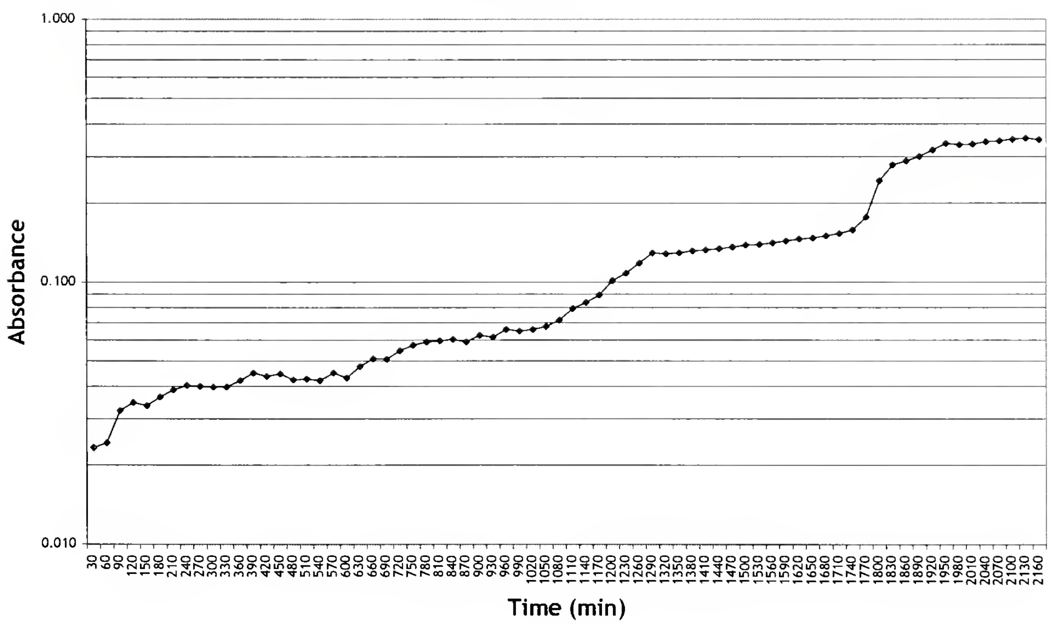
Figure B-26. Glucose With Lower Concentration of Cd (third set of data)



Absorbance increases from 0.156 to 0.312

Actual growth rate = (1860 min -1650 min) = 210 min

Figure B-27. Average Glucose With Cd At Lower Concentration



Absorbance increases from 0.158 to 0.318

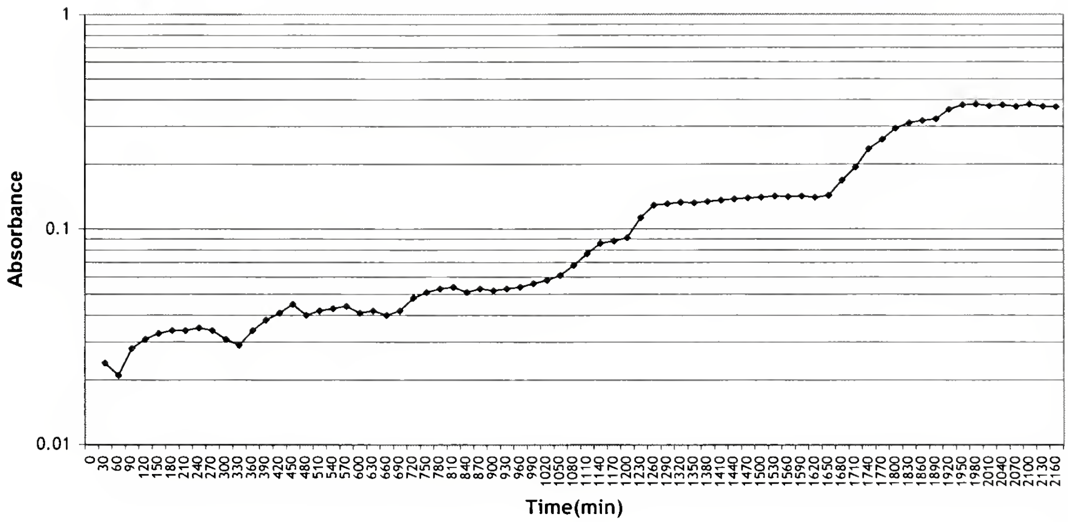
Actual growth rate = (1920 min -1740 min) = 180 min

Glucose and Pb at concentration of $1*10^{-5}M$

Time(min)	Run1	Run2	Run3	Average
0	0	0	0	0.000
30	0.024	0.021	0.025	0.023
60	0.021	0.023	0.021	0.022
90	0.028	0.031	0.032	0.030
120	0.031	0.038	0.033	0.034
150	0.033	0.037	0.034	0.035
180	0.034	0.041	0.033	0.036
210	0.034	0.042	0.035	0.037
240	0.035	0.030	0.036	0.034
270	0.034	0.031	0.035	0.033
300	0.031	0.032	0.032	0.032
330	0.029	0.037	0.036	0.034
360	0.034	0.038	0.037	0.036
390	0.038	0.039	0.042	0.040
420	0.041	0.039	0.041	0.040
450	0.045	0.041	0.042	0.043
480	0.040	0.043	0.041	0.041
510	0.042	0.044	0.043	0.043
540	0.043	0.045	0.046	0.045
570	0.044	0.046	0.045	0.045
600	0.041	0.044	0.047	0.044
630	0.042	0.051	0.05	0.048
660	0.040	0.059	0.052	0.050
690	0.042	0.061	0.058	0.054
720	0.048	0.062	0.061	0.057
750	0.051	0.063	0.062	0.059
780	0.053	0.061	0.064	0.059
810	0.054	0.064	0.065	0.061
840	0.051	0.063	0.067	0.060
870	0.053	0.062	0.065	0.060
900	0.052	0.065	0.061	0.059
930	0.053	0.064	0.064	0.060
960	0.054	0.065	0.063	0.061
990	0.056	0.067	0.065	0.063
1020	0.058	0.071	0.068	0.066
1050	0.061	0.073	0.071	0.068
1080	0.068	0.079	0.075	0.074
1110	0.077	0.083	0.078	0.079
1140	0.086	0.096	0.081	0.088
1170	0.088	0.097	0.089	0.091
1200	0.091	0.103	0.102	0.099
1230	0.113	0.118	0.114	0.115
1260	0.129	0.121	0.126	0.125
1290	0.131	0.125	0.132	0.129
1320	0.133	0.127	0.134	0.131
1350	0.132	0.127	0.135	0.131
1380	0.134	0.13	0.134	0.133
1410	0.136	0.129	0.137	0.134

1440	0.138	0.131	0.137	0.135
1470	0.139	0.131	0.139	0.136
1500	0.140	0.132	0.141	0.138
1530	0.142	0.134	0.143	0.140
1560	0.141	0.136	0.144	0.140
1590	0.142	0.129	0.146	0.139
1620	0.140	0.135	0.149	0.141
1650	0.143	0.137	0.146	0.142
1680	0.168	0.146	0.157	0.157
1710	0.194	0.168	0.172	0.178
1740	0.236	0.209	0.211	0.219
1770	0.261	0.244	0.251	0.252
1800	0.294	0.263	0.291	0.283
1830	0.312	0.278	0.301	0.297
1860	0.319	0.281	0.312	0.304
1890	0.325	0.295	0.323	0.314
1920	0.361	0.291	0.358	0.337
1950	0.378	0.294	0.372	0.348
1980	0.381	0.297	0.379	0.352
2010	0.375	0.299	0.381	0.352
2040	0.378	0.301	0.382	0.354
2070	0.372	0.305	0.379	0.352
2100	0.381	0.299	0.385	0.355
2130	0.372	0.294	0.381	0.349
2160	0.371	0.299	0.382	0.351

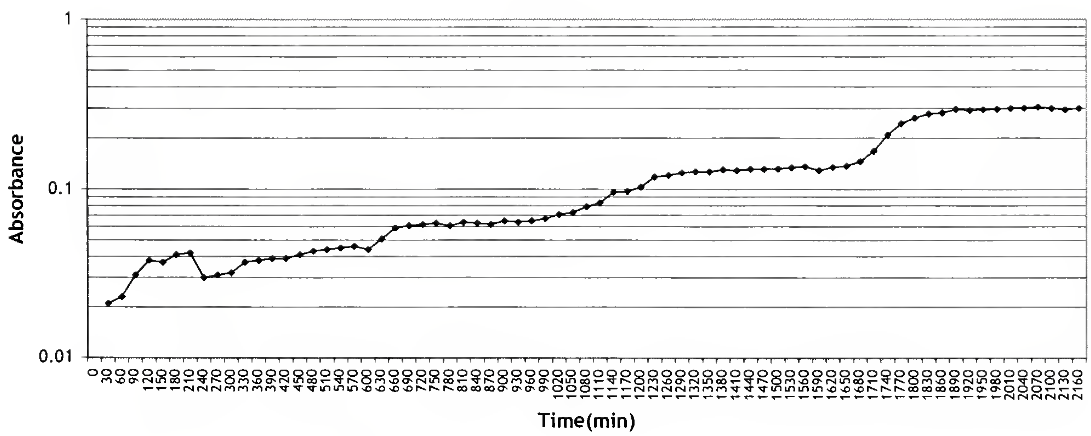
Figure B-28. Glucose With Lower Concentration of Pb (first set of data)



Absorbance increases from 0.143 to 0.294

Actual growth rate = (1800 min -1650 min) = 150 min

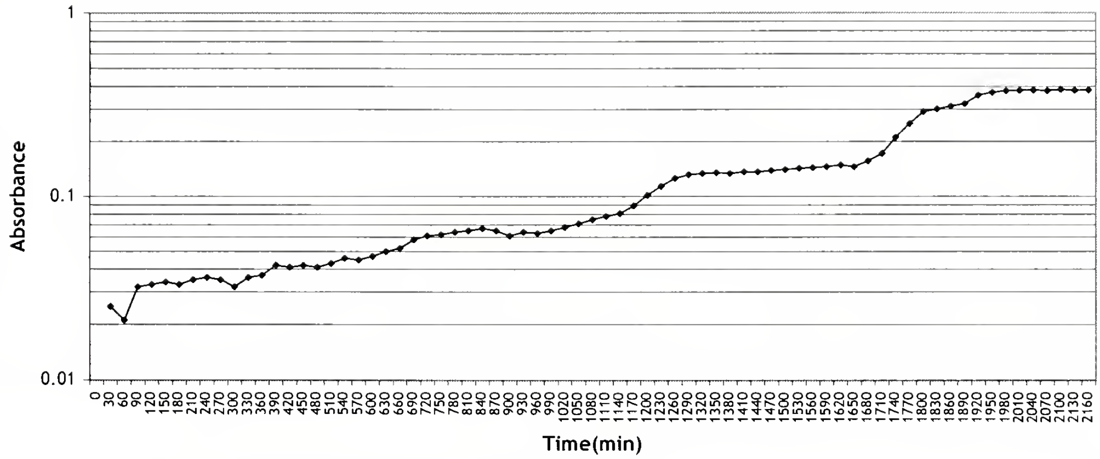
Figure B-29. Glucose With Lower Concentration of Pb (second set of data)



Absorbance increases from 0.146 to 0.278

Actual growth rate = (1830 min -1680 min) = 150 min

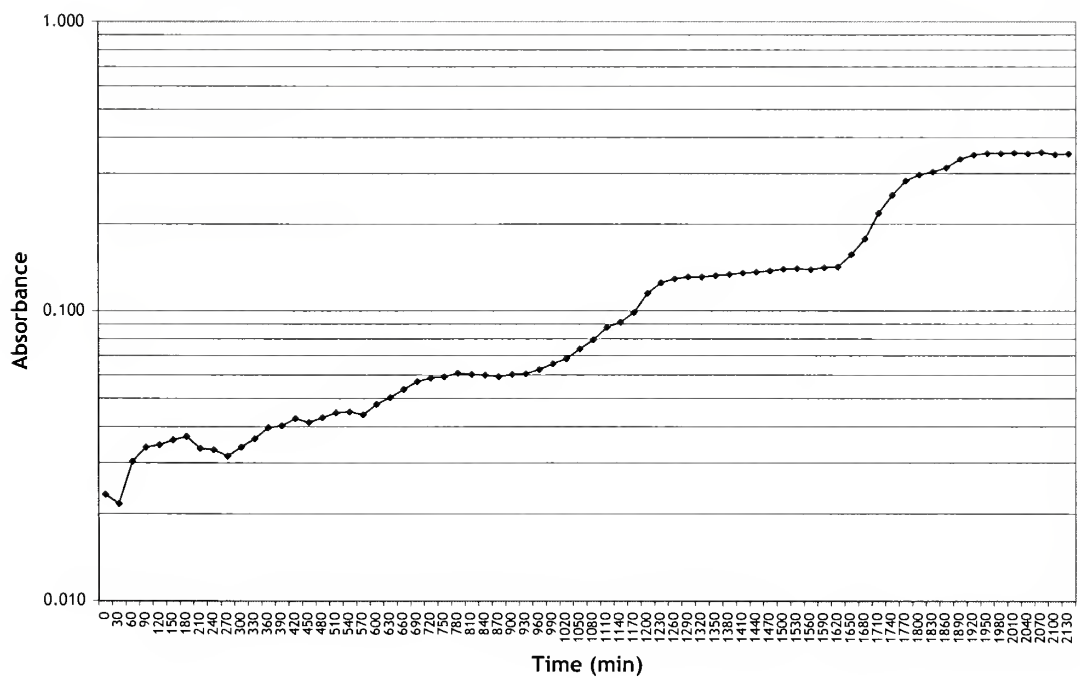
Figure B-30. Glucose With Lower Concentration of Cd (third set of data)



Absorbance increases from 0.157 to 0.312

Actual growth rate = (1860 min -1680 min) = 180 min

Figure B-31. Average Glucose With Pb At Lower Concentration



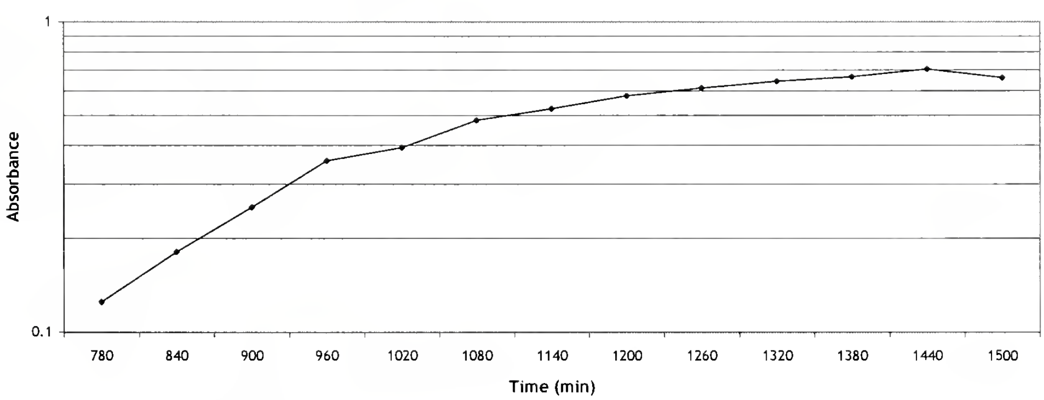
Absorbance increases from 0.142 to 0.283

Actual growth rate = (1800 min -1650 min) = 150 min

Data for *M.roseus* from absorbance vs. time in LB Broth- Rich Media

Glucose control				
Time(min)	Run1	Run2	Run3	Average
0				
780	0.125	0.050	0.091	0.089
840	0.181	0.085	0.132	0.133
900	0.252	0.117	0.175	0.181
960	0.357	0.173	0.266	0.265
1020	0.394	0.188	0.301	0.294
1080	0.482	0.201	0.358	0.347
1140	0.525	0.236	0.412	0.391
1200	0.578	0.242	0.475	0.432
1260	0.612	0.281	0.512	0.468
1320	0.644	0.401	0.556	0.534
1380	0.666	0.466	0.608	0.580
1440	0.706	0.479	0.644	0.610
1500	0.661	0.487	0.689	0.612

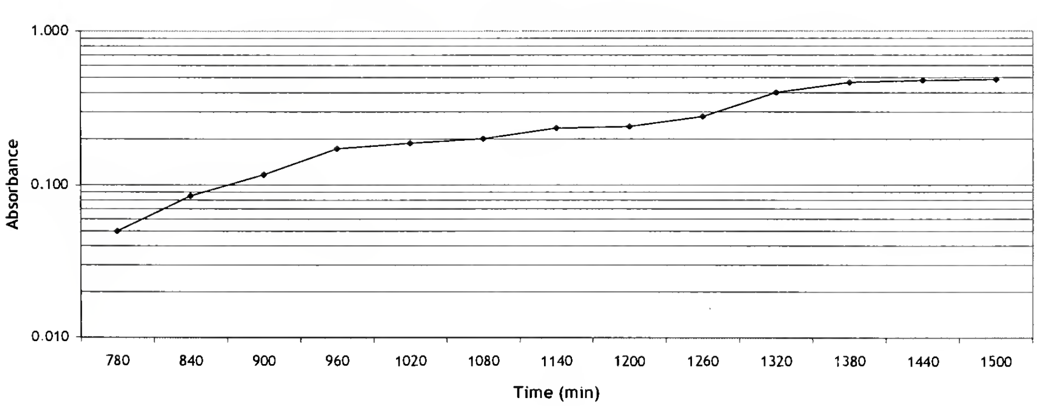
Figure B-32. Glucose Control Run 1



Absorbance increases from 0.125 to .0.252

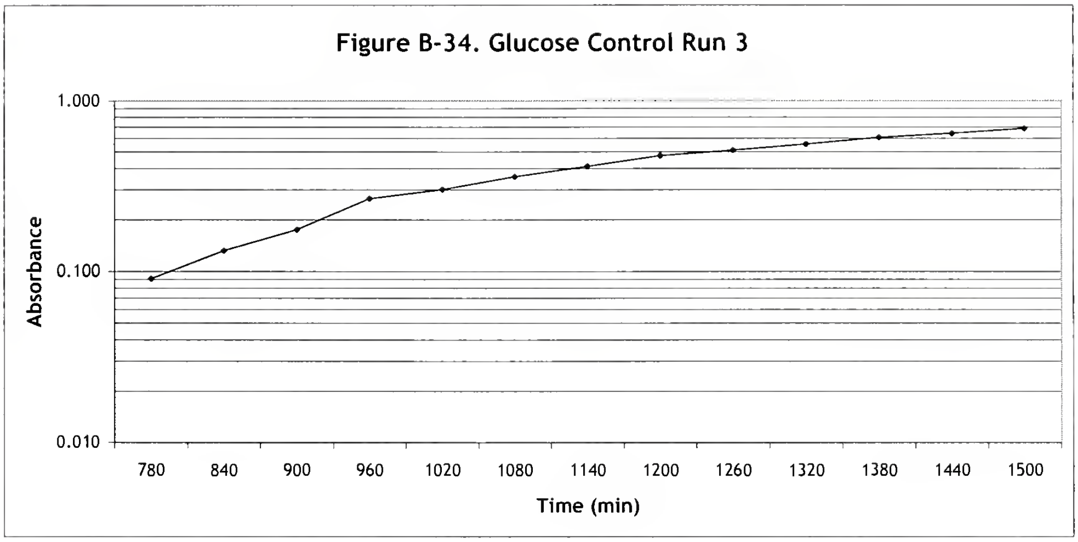
Actual growth rate = (900min -780 min) = 120 min

Figure B-33. Glucose Control Run 2



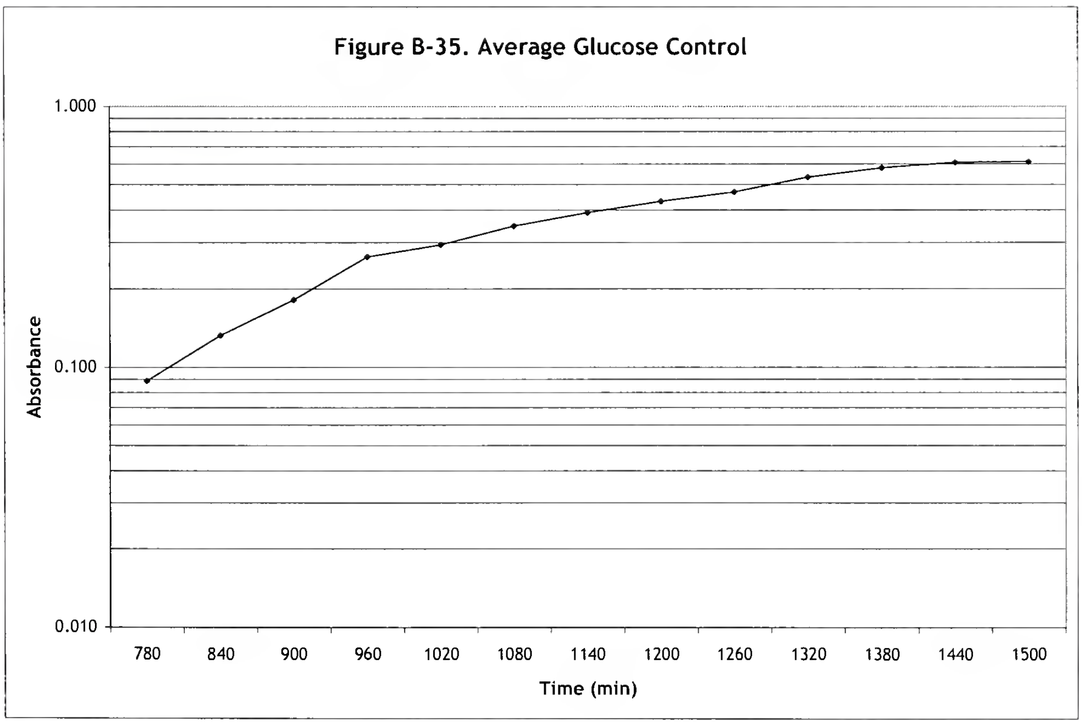
Absorbance increases from 0.085 to .0.173

Actual growth rate = (960min -840 min) = 120 min



Absorbance increases from 0.132 to .0.266

Actual growth rate = **(960min -840 min) = 120 min**



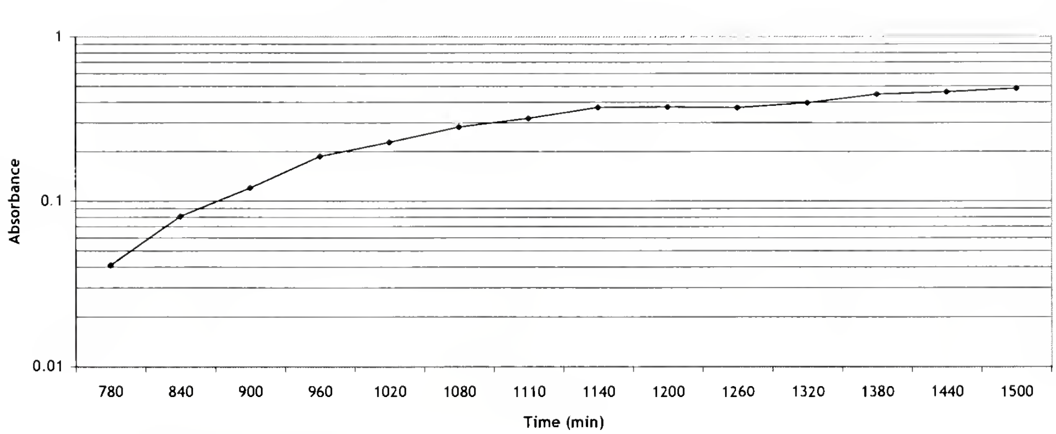
Absorbance increases from 0.133 to .0.265

Actual growth rate = **(960min -840 min) = 120 min**

Data for *M.roseus* from absorbance vs. time in LB Broth- Rich Media

Glucose With $1*10^{-5}$ M Cd				
Time(min)	Run1	Run2	Run3	Average
0				
780	0.041	0.043	0.032	0.039
840	0.081	0.052	0.059	0.064
900	0.120	0.065	0.063	0.083
960	0.187	0.078	0.092	0.119
1020	0.228	0.138	0.107	0.158
1080	0.282	0.179	0.158	0.206
1110	0.319	0.209	0.228	0.252
1140	0.371	0.246	0.264	0.294
1200	0.374	0.278	0.298	0.317
1260	0.370	0.339	0.378	0.362
1320	0.396	0.358	0.454	0.403
1380	0.448	0.41	0.474	0.444
1440	0.462	0.421	0.492	0.458
1500	0.484	0.434	0.511	0.476

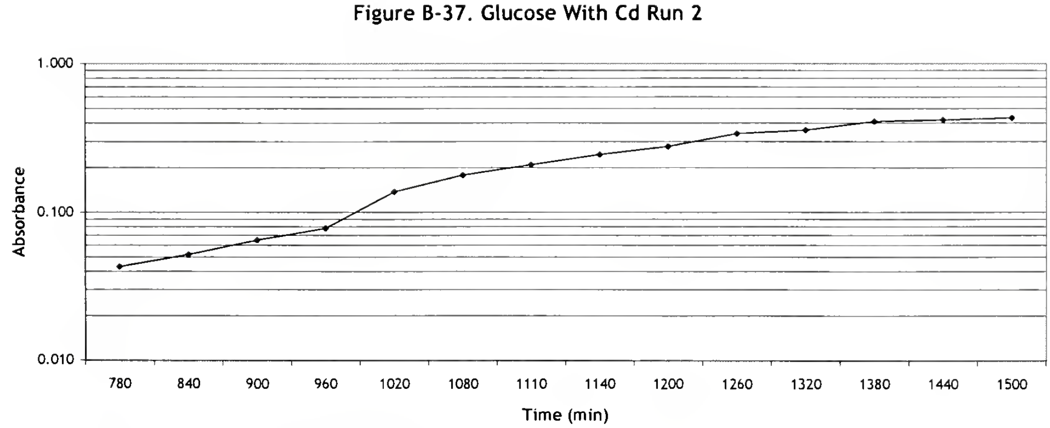
Figure B-36. Glucose With Cd Run 1



Absorbance increases from 0.187 to .0.371

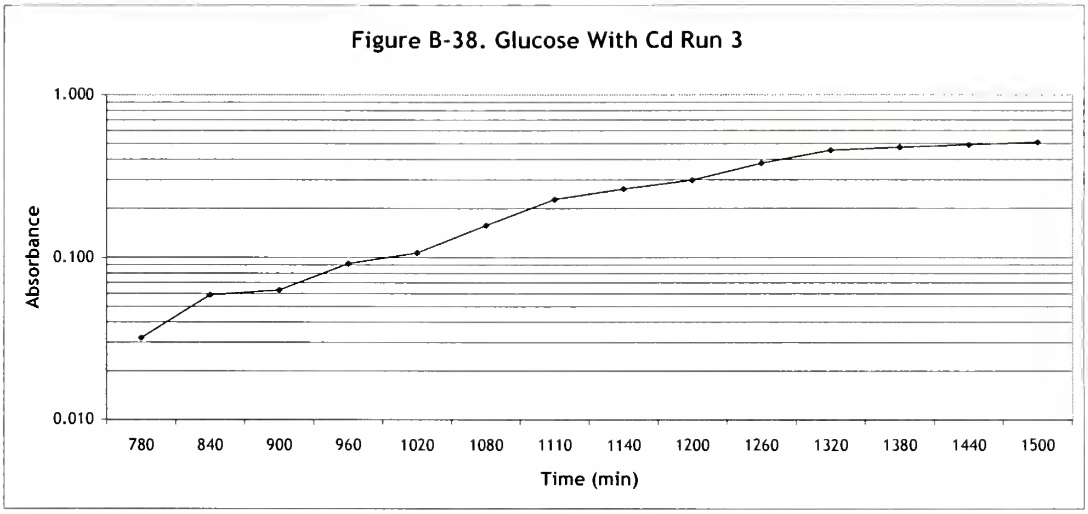
Actual growth rate = (1140 min -960 min) = 180 min

Figure B-37. Glucose With Cd Run 2



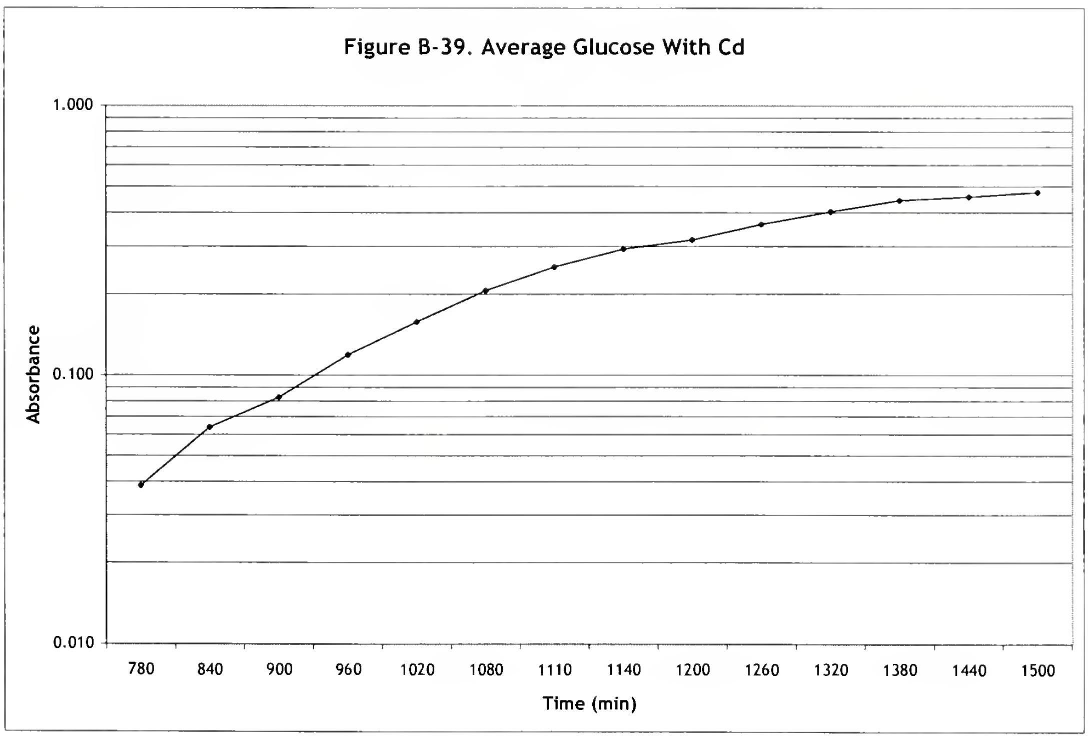
Absorbance increases from 0.138 to .0.278

Actual growth rate = (1200 min -1020 min) = 180 min



Absorbance increases from 0.228 to .0.454

Actual growth rate = **(1320 min -1110 min) = 210 min**



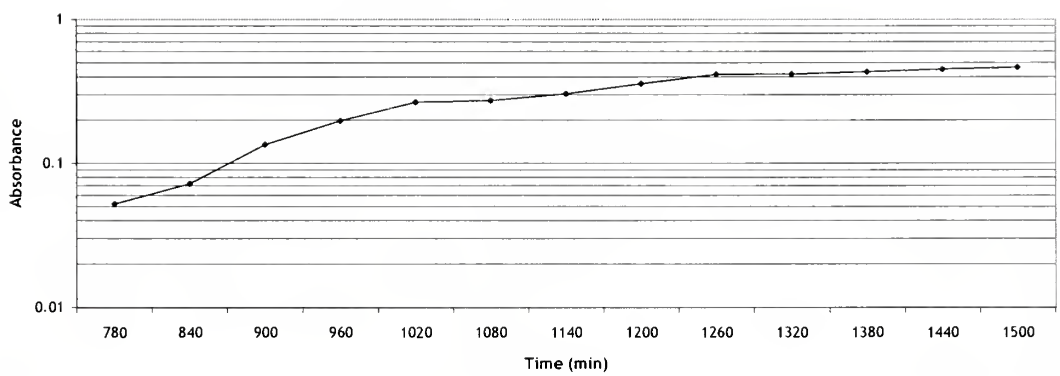
Absorbance increases from 0.158 to .0.317

Actual growth rate = **(1200 min -1020 min) = 180 min**

Data for *M.roseus* from absorbance vs. time in LB Broth- Rich Media

Glucose With $1*10^{-5}$ M Pb				
Time(min)	Run1	Run2	Run3	Average
0				
780	0.052	0.043	0.054	0.050
840	0.072	0.061	0.078	0.070
900	0.135	0.112	0.127	0.125
960	0.198	0.162	0.174	0.178
1020	0.267	0.221	0.235	0.241
1080	0.274	0.232	0.254	0.253
1140	0.305	0.266	0.298	0.290
1200	0.359	0.309	0.342	0.337
1260	0.416	0.337	0.384	0.379
1320	0.418	0.368	0.401	0.396
1380	0.434	0.386	0.422	0.414
1440	0.452	0.395	0.434	0.427
1500	0.466	0.402	0.451	0.440

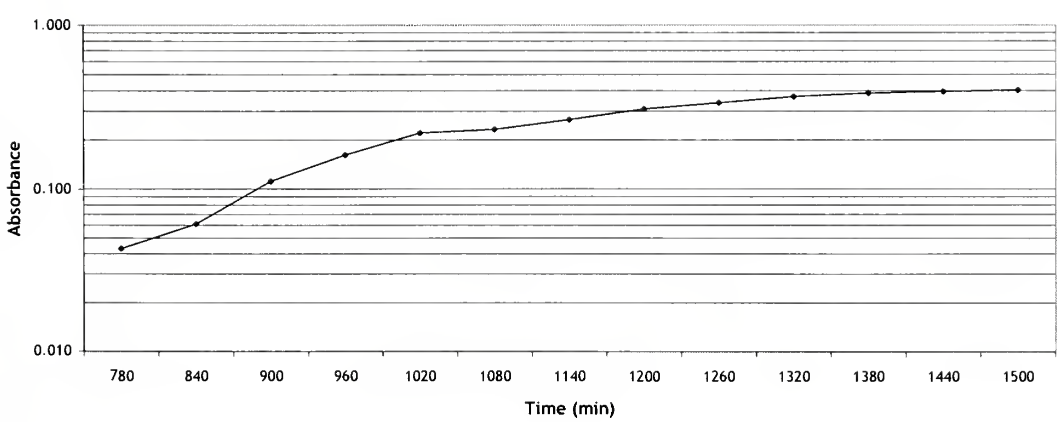
Figure B-40. Glucose With Pb Run 1



Absorbance increases from 0.135 to .267

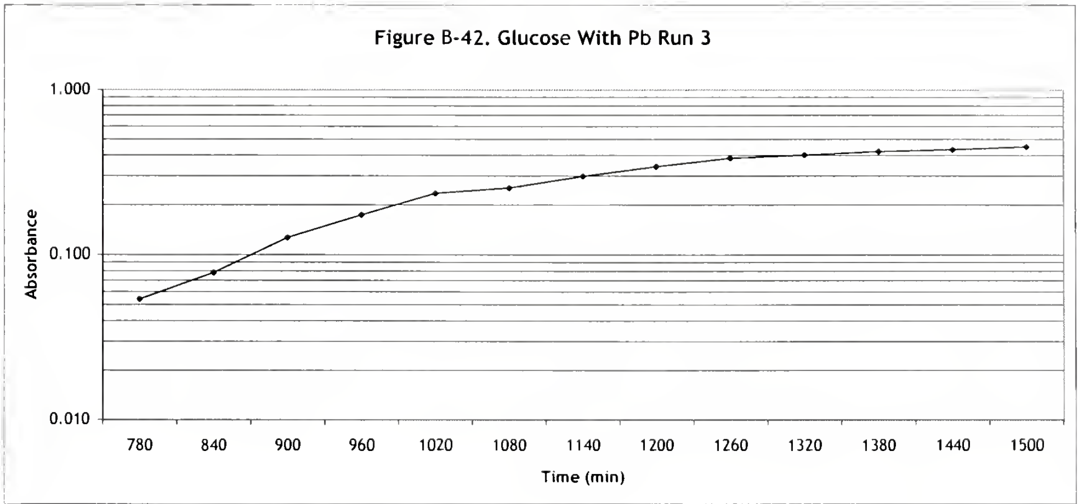
Actual growth rate = (1020 min -900 min) = 120 min

Figure B-41. Glucose With Pb Run 2



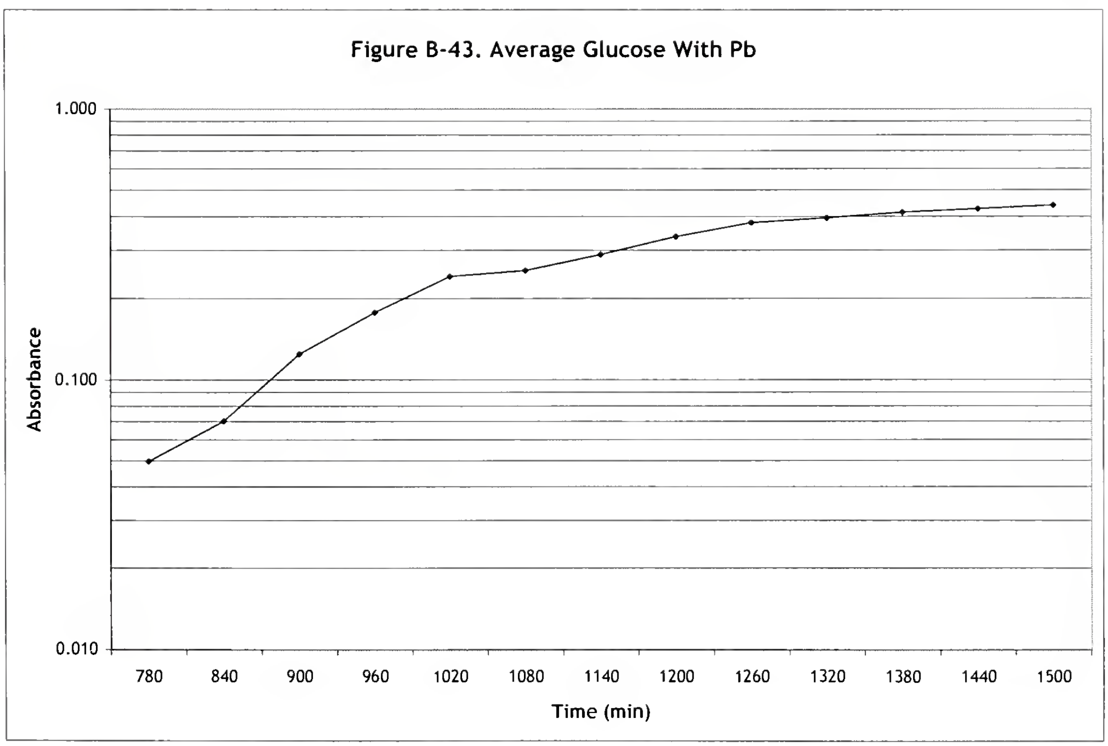
Absorbance increases from 0.112 to .221

Actual growth rate = (1020 min -900 min) = 120 min



Absorbance increases from 0.127 to .254

Actual growth rate = **(1080 min -900 min) = 180 min**



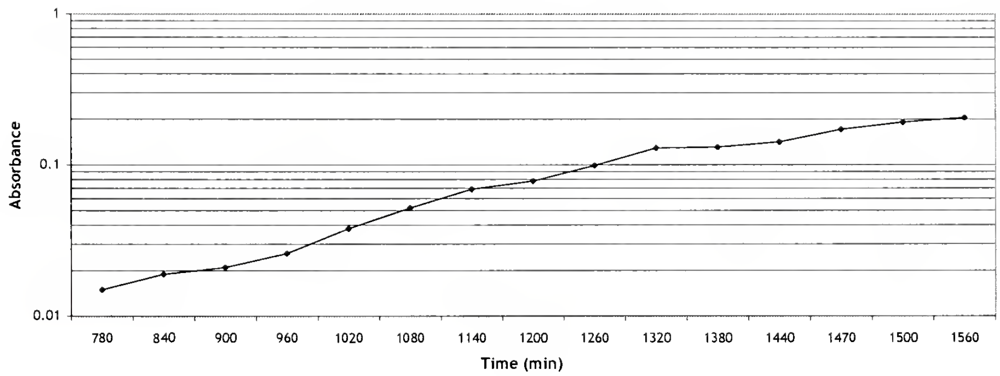
Absorbance increases from 0.125 to .253

Actual growth rate = **(1080 min -900 min) = 180 min**

Data for *M.roseus* from absorbance vs. time in LB Broth- Rich Media

Glucose With $1*10^{-3}$ M Cd				
Time(min)	Run1	Run2	Run3	Average
0				
780	0.015	0.039	0.021	0.025
840	0.019	0.043	0.028	0.030
900	0.021	0.051	0.033	0.035
960	0.026	0.068	0.034	0.043
1020	0.038	0.068	0.047	0.051
1080	0.052	0.071	0.054	0.059
1140	0.069	0.091	0.071	0.077
1200	0.078	0.097	0.082	0.086
1260	0.099	0.102	0.102	0.101
1320	0.129	0.132	0.123	0.128
1380	0.131	0.152	0.133	0.139
1440	0.142	0.192	0.138	0.157
1470	0.172	0.231	0.161	0.188
1500	0.191	0.242	0.177	0.203
1560	0.205	0.261	0.203	0.223

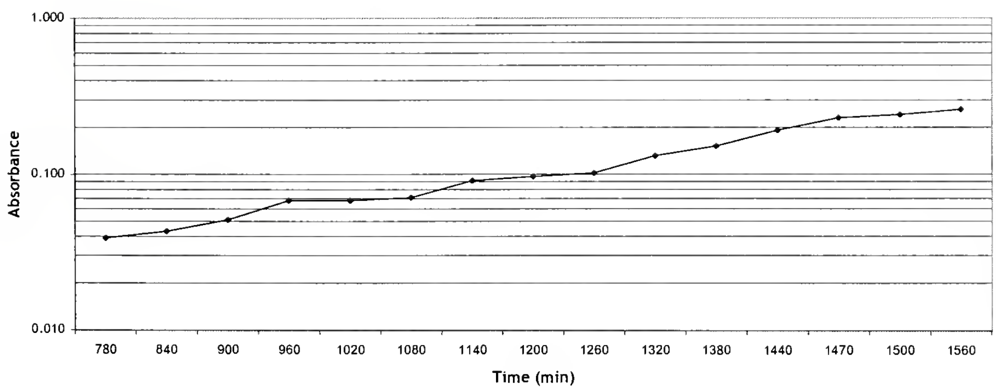
Figure B-44. Glucose With Cd Run 1



Absorbance increases from 0.099 to .0.198

Actual growth rate = **(1500 min - 1260min) = 240 min**

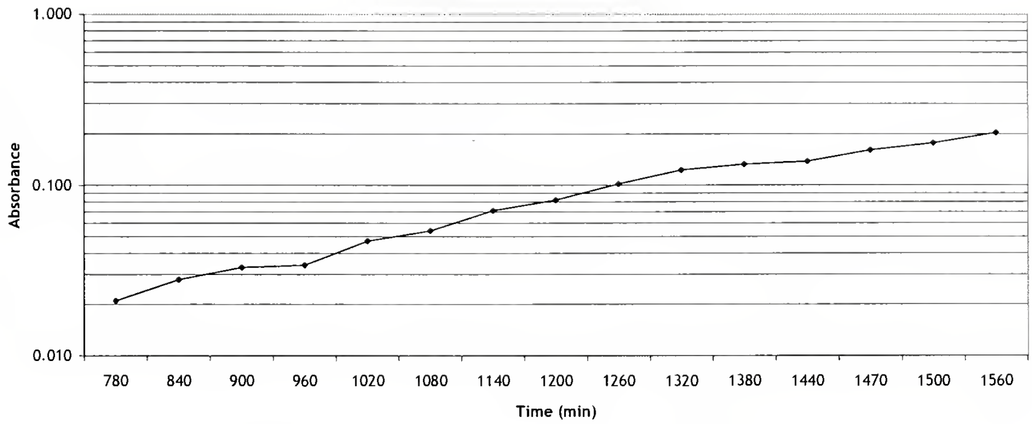
Figure B-45. Glucose With Cd Run 2



Absorbance increases from 0.132 to .0.261

Actual growth rate = **(1560 min - 1320min) = 240 min**

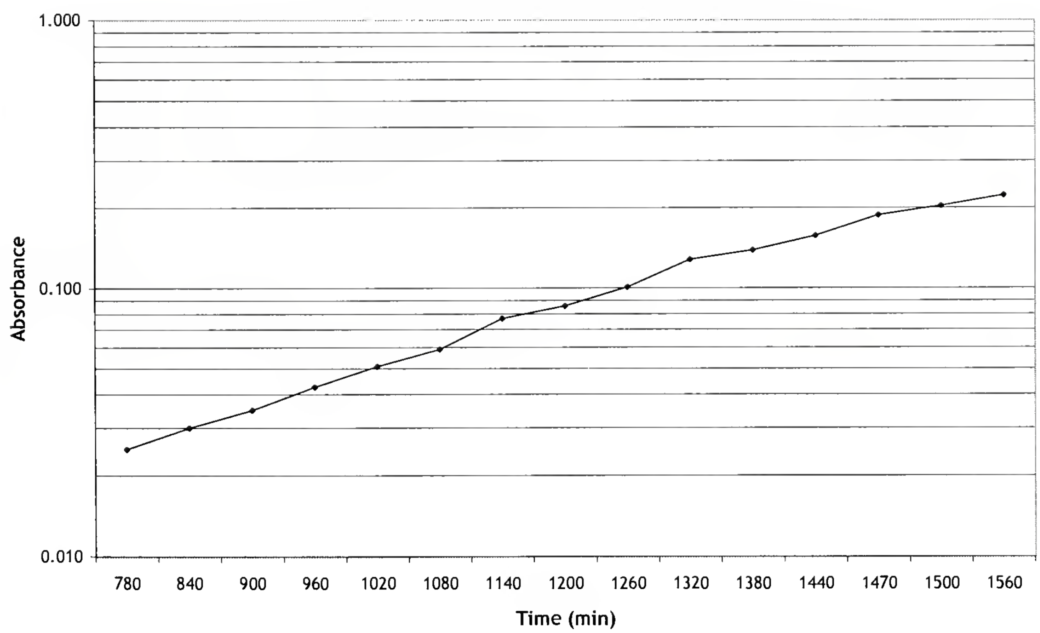
Figure B-46. Glucose With Cd Run 3



Absorbance increases from 0.102 to .0.203

Actual growth rate = (1560 min - 1260min) = 300 min

Figure B-47. Average Glucose With Cd



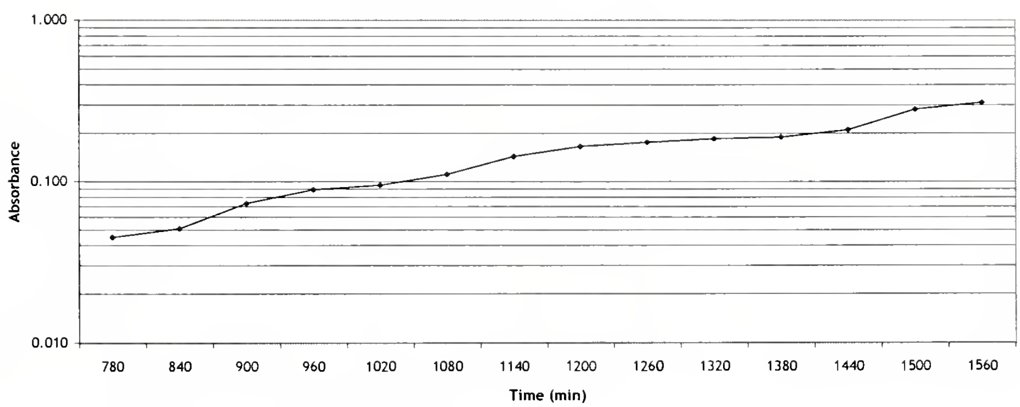
Absorbance increases from 0.101 to .0.203

Actual growth rate = (1500 min - 1260min) = 240 min

Data for *M.roseus* from absorbance vs. time in LB Broth- Rich Media

Glucose With $1*10^{-3}$ M Pb				
Time(min)	Run1	Run2	Run3	Average
0				
780	0.045	0.039	0.041	0.042
840	0.051	0.042	0.062	0.052
900	0.073	0.062	0.081	0.072
960	0.089	0.075	0.086	0.083
1020	0.095	0.085	0.089	0.090
1080	0.111	0.123	0.118	0.117
1140	0.144	0.150	0.138	0.144
1200	0.166	0.204	0.164	0.178
1260	0.176	0.231	0.181	0.196
1320	0.185	0.236	0.188	0.203
1380	0.189	0.25	0.192	0.210
1440	0.211	0.259	0.209	0.226
1500	0.282	0.271	0.273	0.275
1560	0.311	0.298	0.302	0.304

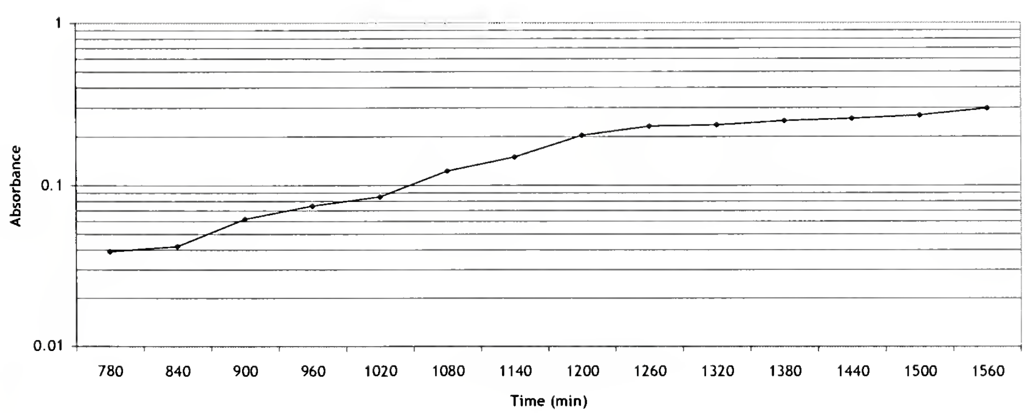
Figure B-48. Glucose With Pb Run 1



Absorbance increases from 0.073 to 0.144

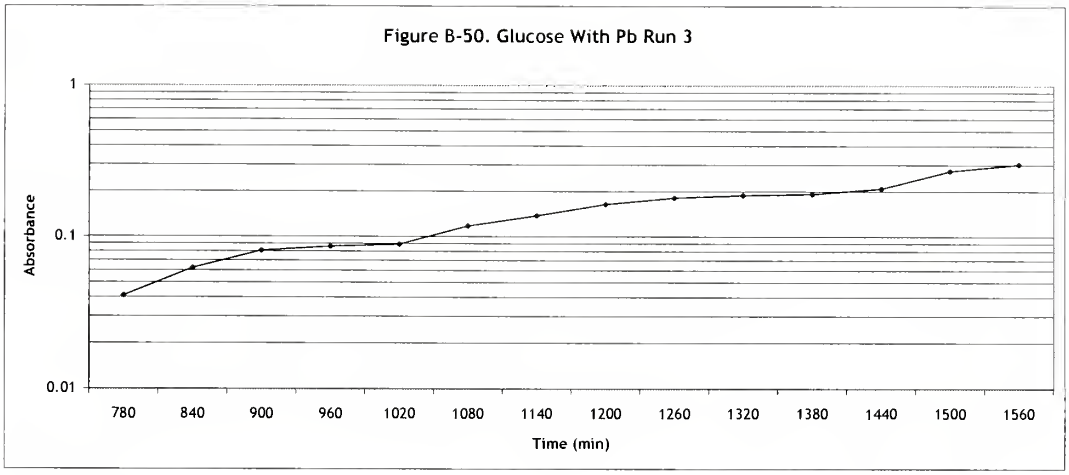
Actual growth rate = (1140 min - 900 min) = 240 min

Figure B-49. Glucose With Pb Run 2



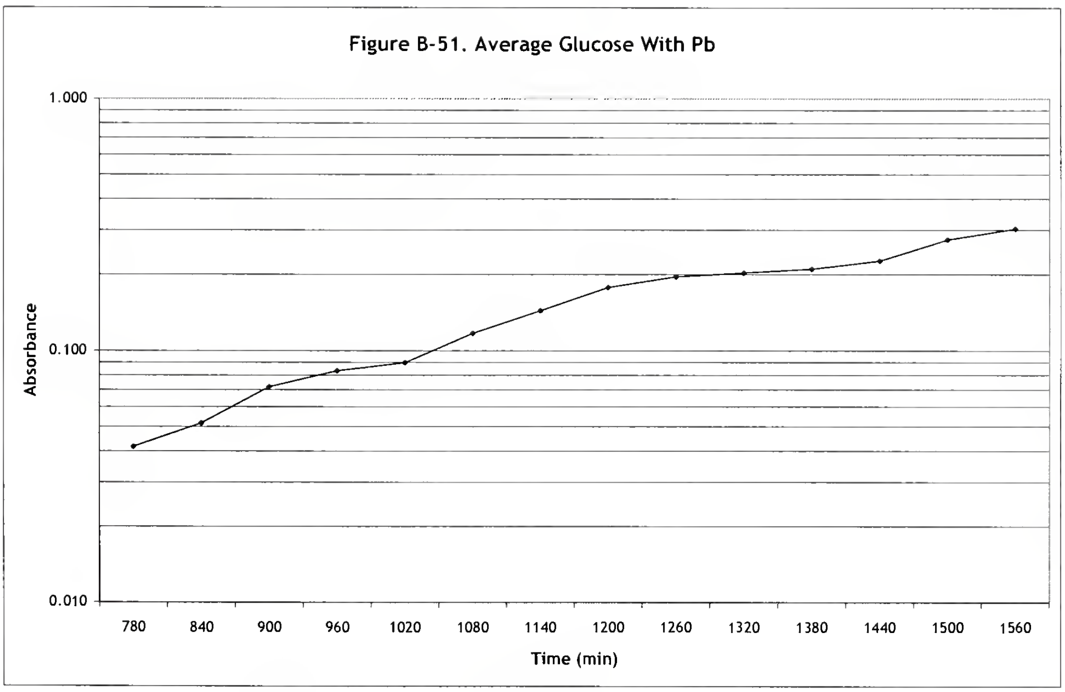
Absorbance increases from 0.075 to 0.150

Actual growth rate = (1140 min - 960min) = 180 min



Absorbance increases from 0.089 to .0.181

Actual growth rate = (1260 min - 1020min) = 240 min



Absorbance increases from 0.090 to 0.178

Actual growth rate = (1200 min - 1020min) = 180 min

M.roseus growth data

Table B-1. M.roseus cell growth over period of time

growth time: 7 hours

	Dilution	
	1:5	
(twice for all media)		
	5	Average
lactose	260	1300
lactose and Cd	110	550
lactose and Pb	150	750
glucose	253	1265
glucose and Cd	88	440
glucose and Pb	232	1160

growth time: 18 hours

	Dilution				
	1:5	1:25	1:50	1:250	
	5	25	50	250	Average
lactose	100 +	100 +	100 +	126	31500
lactose and Cd	100 +	80	41	8	2017
lactose and Pb	100 +	127	61	12	3075
glucose	224	57	29	6	1374
glucose and Cd	116	22	0	0	570
glucose and Pb	260	54	26	5	1300

growth time: 21 hours

	Dilution				
	1:30	1:300	1:900	1:9,000	
	30	300	900	9,000	Average
lactose	100+	100+	54	4	44400
lactose and Cd	154	16	6	0	4940
lactose and Pb	148	15	6	0	4780
glucose	203	21	7	0	6230
glucose and Cd	30	4	1	0	1000
glucose and Pb	90	7	2	0	2200

growth time: 22 hours

	Dilution			
	1:100	1:1,000	1:10,000	
	100	1,000	10,000	Average
(twice la+Cd,glu+Pb)		(twice glu+Cd)	(twice la.)	
lactose	100+	47	5	49000
lactose and Cd	68	5	0	6200
lactose and Pb	95	9	1	9500
glucose	138	14	1	12600
glucose and Cd	20	2	0	2000
glucose and Pb	58	4	0	5200

growth time: 23 hours

	Dilution				
	1:30	1:300	1:3,000	1:30,000	
	30	300	3,000	30,000	Average
	(twice la+Cd,lac+Pb,glu, glu+Pb)				
lactose	100+	143	16	2	50300
lactose and Cd	100+	26	2	0	7200
lactose and Pb	100+	39	3	0	10800
glucose	100+	53	5	0	15600
glucose and Cd	206	19	2	0	5960
glucose and Pb	100+	65	6	0	19000

growth time: 25 hours

	Dilution				
	1:30	1:300	1:3,000	1:30,000	
			(twice glu, glu+Pb)		
	30	300	3,000	30,000	Average
lactose	100+	184	14	2	52400
lactose and Cd	242	28	2	0	7220
lactose and Pb	340	41	4	0	11500
glucose	100+	73	6	0	20600
glucose and Cd	231	20	2	0	6310
glucose and Pb	100+	75	7	0	21500

growth time: 26 hours

	Dilution					
	1:30	1:300	1:3,000	1:30,000	1 :25,000	
		(twice la.,la+Pb,glu+Pb)				
	30	300	3,000	30,000	25,000	Average
lactose	100+	192	18	0	0	58067
lactose and Cd	266	32	2	0	0	7860
lactose and Pb	100+	39	4	0	0	11800
glucose	100+	102	10	1	0	30200
glucose and Cd	249	23	2	0	0	6790
glucose and Pb	100+	83	7	0	0	23600

growth time: 27 hours

	Dilution				
	1:30	1:300	1:3,000	1:30,000	
	(twice la.,la+Pb,glu+Pb)				
	30	300	3,000	30,000	Average
lactose	100+	192	20	0	58400
lactose and Cd	280	26	3	0	8400
lactose and Pb	100+	45	4	0	13000
glucose	100+	147	14	2	48700
glucose and Cd	261	24	2	0	7010
glucose and Pb	100+	83	8	0	24600

growth time: 28 hours

	Dilution					
	1:30	1:300	1:3,000	1:30,000	1 :25,000	
		(twice la.,la+Cd,glu+Pb)	twice(la+Pb)			
	30	300	3,000	30,000	25,000	Average
lactose	100+	196	21	0	0	60200
lactose and Cd	100+	28	3	0	0	8600
lactose and Pb	100+	58	6	0	0	17800
glucose	100+	263	28	3	3	81975
glucose and Cd	263	24	2	0	0	7030
glucose and Pb	100+	90	8	0	0	26000

growth time: 30 hours

	Dilution				
	1:30	1:300	1:3,000	1:30,000	
		twice(glu+Pb)	twice(la.,la+Cd,glu+Cd)		
	30	300	3,000	30,000	Average
lactose	100+	204	23	0	66400
lactose and Cd	100+	48	4	0	12800
lactose and Pb	100+	72	8	1	25200
glucose	100+	285	32	3	90500
glucose and Cd	240	30	3	0	8400
glucose and Pb	100+	93	9	0	27600

growth time: 31 hours

	Dilution						
	1:30	1:300	1:3,000	1:30,000	1 :25,000	:125,000	
		twice(la+Cd, la+Pb)	twice (glu +Pb,lac+Pb)				
	30	300	3,000	30,000	25,000	125,000	Average
lactose	100+	100+	32	3	5	1	109000
lactose and Cd	100+	121	10	0	0	0	34200
lactose and Pb	100+	117	12	1	0	0	34275
glucose	100+	294	36	3	4	0	96550
glucose and Cd	262	29	3	0	0	0	8520
glucose and Pb	100+	98	9	0	0	0	27800

growth time: 32 hours

	Dilution				
	1:30	1:300	1:3,000	1:30,000	
			twice(la+Cd, glu+Cd)	(twice la.,la+Pb,glu)	
	30	300	3,000	30,000	Average
lactose	100+	100+	63	6	183000
lactose and Cd	100+	144	13	0	40400
lactose and Pb	100+	100+	39	3	99000
glucose	100+	100+	40	5	140000
glucose and Cd	100+	26	3	0	8600
glucose and Pb	100+	106	10	1	30600

growth time: 33 hours

	Dilution						
	1:30	1:300	1:3,000	1:30,000	1:25,000	:125,000	
		twice(glu+Cd)					
	30	300	3,000	30,000	25,000	125,000	Average
lactose	100+	100+	74	7	8	2	220500
lactose and Cd	100+	153	14	2	2	0	49475
lactose and Pb	100+	100+	74	6	8	1	181750
glucose	100+	100+	44	5	7	1	145500
glucose and Cd	100+	31	3	0	0	0	9200
glucose and Pb	100+	121	11	1	2	0	37325

growth time: 34 hours

	Dilution						
	1:30	1:300	1:3,000	1:30,000	1:25,000	:125,000	
			(twice glu+Cd)				
	30	300	3,000	30,000	25,000	125,000	Average
lactose	100+	100+	89	10	11	2	273000
lactose and Cd	100+	177	16	2	2	0	52775
lactose and Pb	100+	100+	78	6	8	2	216000
glucose	100+	100+	74	7	8	2	220500
glucose and Cd	100+	33	4	0	0	0	11300
glucose and Pb	100+	127	12	1	2	0	38525

growth t 35 hours

	Dilution						
	1:30	1:300	1:3,000	1:30,000	1:25,000	1:125,000	
		(twice glu+Cd)					
	30	300	3,000	30,000	25,000	125,000	Average
lactose	100+	100+	128	11	13	3	353500
lactose and Cd	100+	192	18	2	2	0	55400
lactose and Pb	100+	100+	79	6	8	2	216750
glucose	100+	100+	87	7	9	2	236500
glucose and Cd	100+	35	4	0	0	0	11250
glucose and Pb	100+	161	15	1	2	0	43325

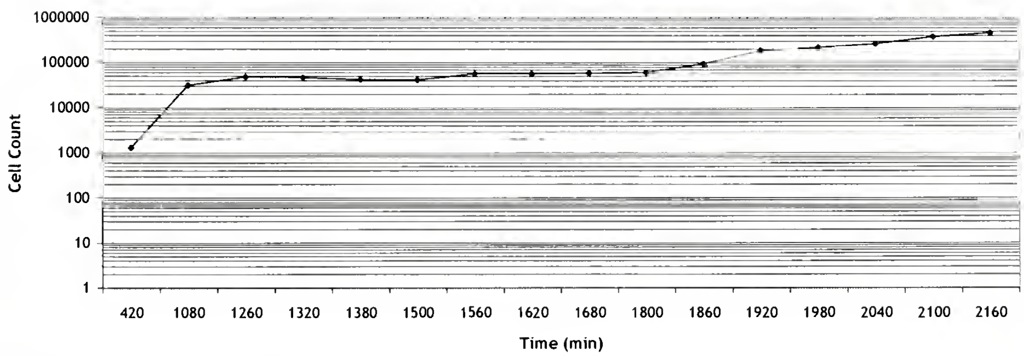
growth time: 36 hours

	Dilution						
	1:30	1:300	1:3,000	1:30,000	1:25,000	:125,000	
		(twice glu+Cd)					
	30	300	3,000	30,000	25,000	125,000	Average
lactose	100+	100+	157	14	16	3	416500
lactose and Cd	100+	176	17	2	2	0	53450
lactose and Pb	100+	100+	86	7	8	2	229500
glucose	100+	100+	133	10	11	2	306000
glucose and Cd	100+	41	4	0	0	0	12200
glucose and Pb	100+	165	16	1	2	0	44375

Cell Growth vs. Time
LACTOSE ONLY CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Cell Count 4	Average Cell Count
420	1300	1300	1300		1300
1080	31500	31500	31500		31500
1260	48600	48600	36000		44400
1320	47000	50000	50000		49000
1380	42900	48000	60000		50300
1500	42000	60000	55200		52400
1560	57600	57600	59000		58067
1620	57600	57600	60000		58400
1680	58800	58800	63000		60200
1800	61200	69000	69000		66400
1860	96000	90000	125000	125000	109000
1920	189000	180000	180000		183000
1980	222000	210000	250000	200000	220500
2040	267000	300000	275000	250000	273000
2100	384000	330000	325000	375000	353500
2160	471000	420000	400000	375000	416500

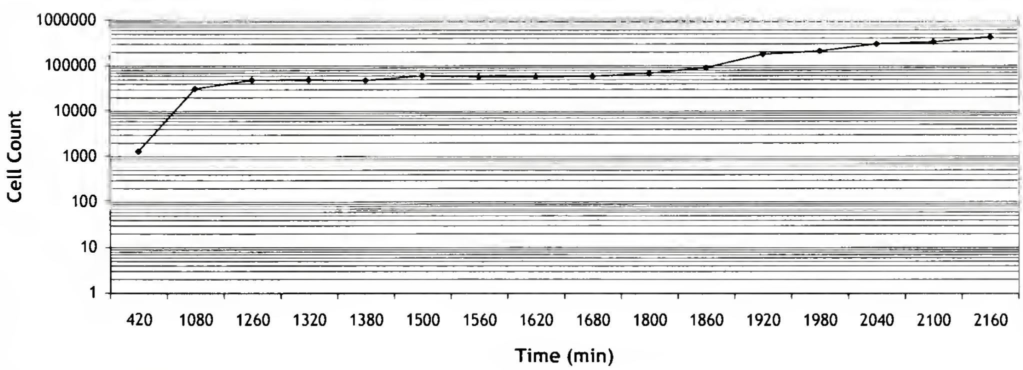
Figure B-52. Lactose Control Cell Count Growth Curve 1



Cell Count increases from 96000 to 189000

Actual growth rate = (1920min -1860min) = 60 min

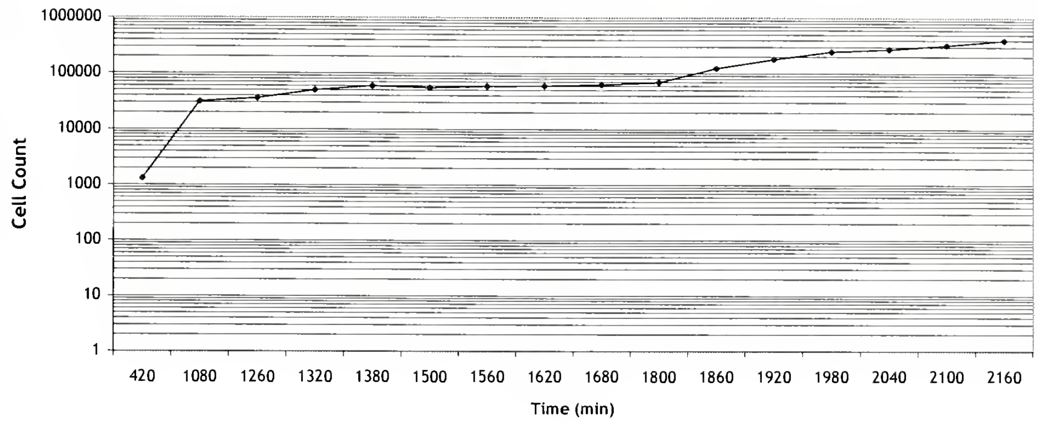
Figure B-53. Lactose Control Cell Count Growth Curve 2



Cell Count increases from 90000 to 180000

Actual growth rate = (1920min -1860min) = 60 min

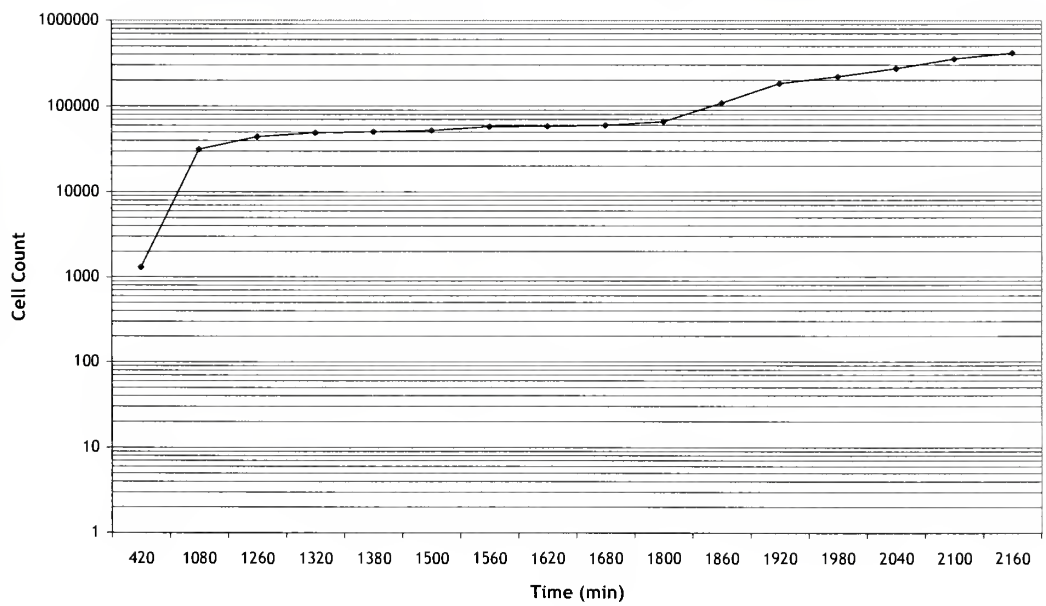
Figure B-54. Lactose Control Cell Count Growth Curve 3



Cell Count increases from 125000 to 250000

Actual growth rate = **(1980min -1860min) = 120 min**

Figure B-55. Average Lactose Control Cell Count Growth Curve



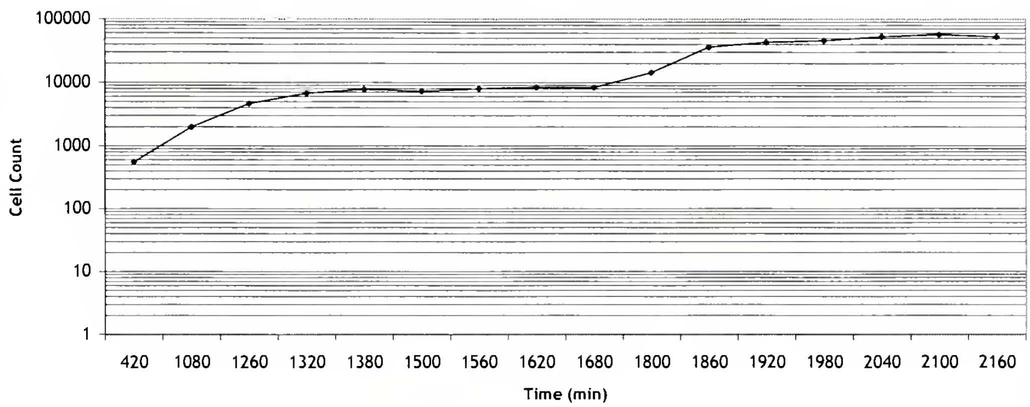
Cell Count increases from 109000 to 220500

Actual growth rate = **(1980min -1860min) = 120 min**

LACTOSE WITH Cd CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Cell Count 4	Average Cell Count
420	550	550	550		550
1080	2000	2050	2000		2017
1260	4620	4800	5400		4940
1320	6800	6800	5000		6200
1380	7800	7800	6000		7200
1500	7260	8400	6000		7220
1560	7980	9600	6000		7860
1620	8400	7800	9000		8400
1680	8400	8400	9000		8600
1800	14400	12000	12000		12800
1860	36300	36300	30000		34200
1920	43200	39000	39000		40400
1980	45900	42000	50000	60000	49475
2040	53100	48000	60000	50000	52775
2100	57600	54000	60000	50000	55400
2160	52800	51000	60000	50000	53450

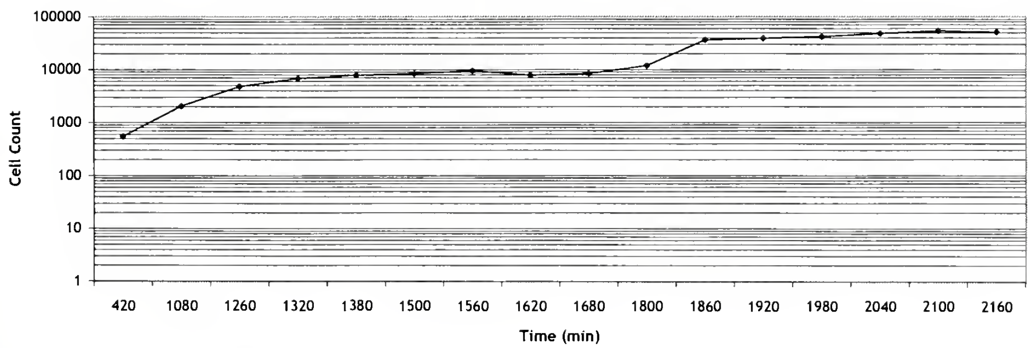
Figure B-56. Lactose With Cd Cell Count Growth Curve 1



Cell Count increases from 7260 to 14400

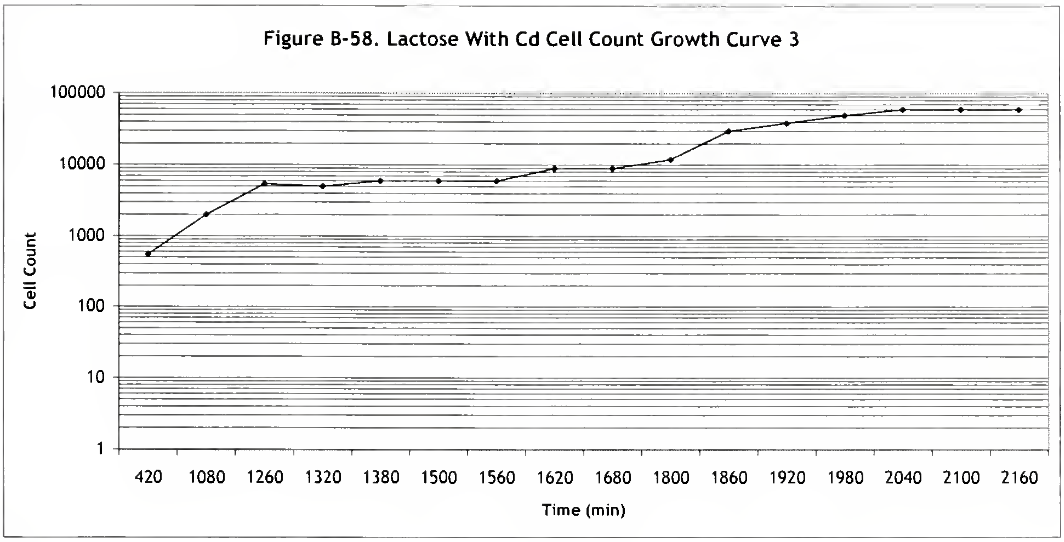
Actual growth rate = (1800min -1500min) = 300 min

Figure B-57. Lactose With Cd Cell Count Growth Curve 2



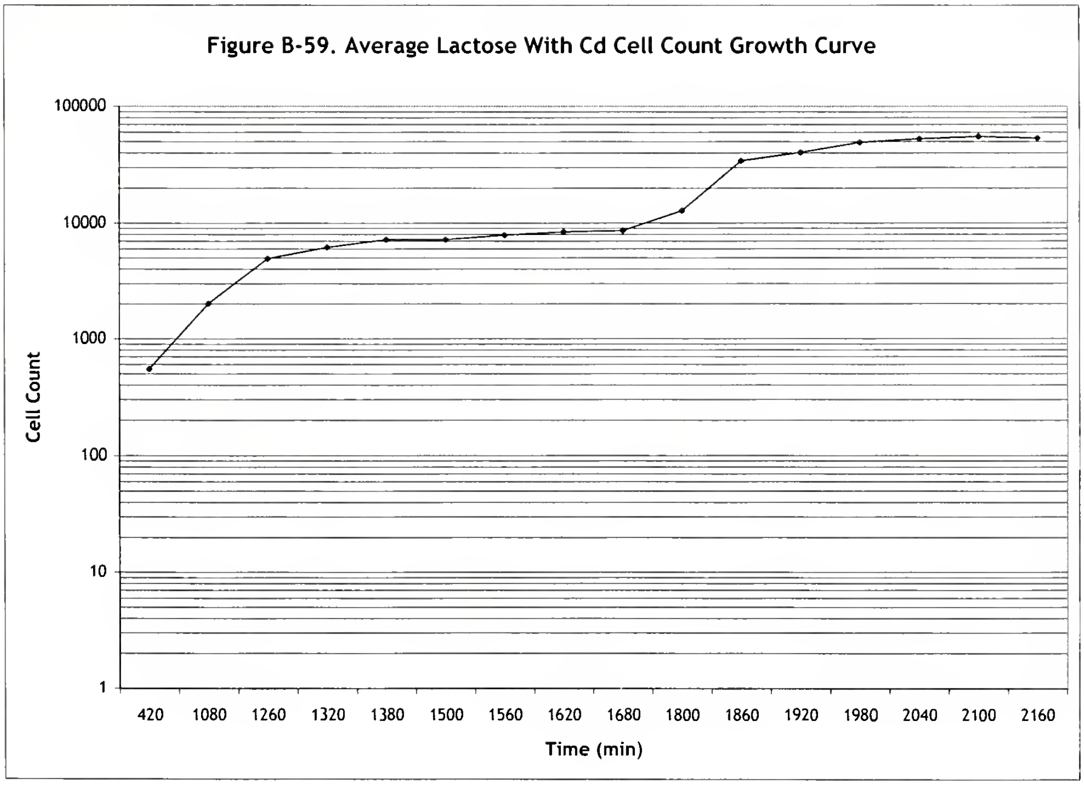
Cell Count increases from 4800 to 9600

Actual growth rate = (1560min -1260min) = 300 min



Cell Count increases from 30000to 60000

Actual growth rate = (2040min -1860min) = 180 min



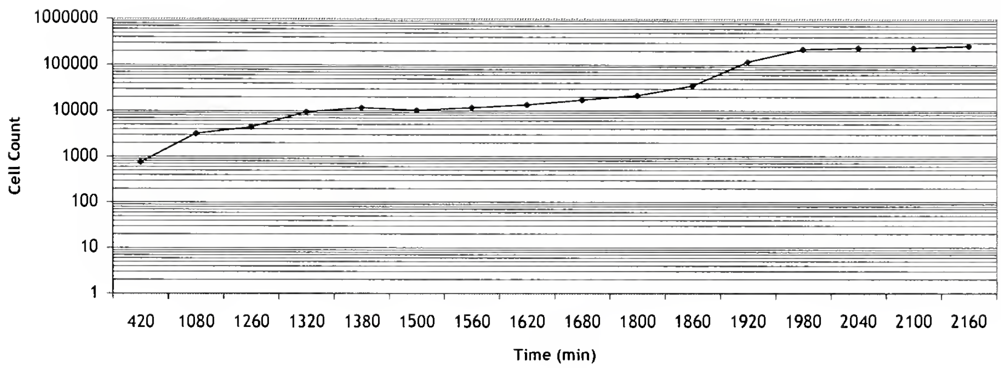
Cell Count increases from 6200 to 12800

Actual growth rate = (1800min -1380min) = 420 min

LACTOSE WITH Pb CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Cell Count 4	Average Cell Count
420	750	750	750		750
1080	3175	3050	3000		3075
1260	4440	4500	5400		4780
1320	9500	9000	10000		9500
1380	11700	9000	11700		10800
1500	10200	12300	12000		11500
1560	11700	11700	12000		11800
1620	13500	13500	12000		13000
1680	17400	18000	18000		17800
1800	21600	24000	30000		25200
1860	35100	36000	36000	30000	34275
1920	117000	90000	90000		99000
1980	222000	180000	200000	125000	181750
2040	234000	180000	200000	250000	216000
2100	237000	180000	200000	250000	216750
2160	258000	210000	200000	250000	229500

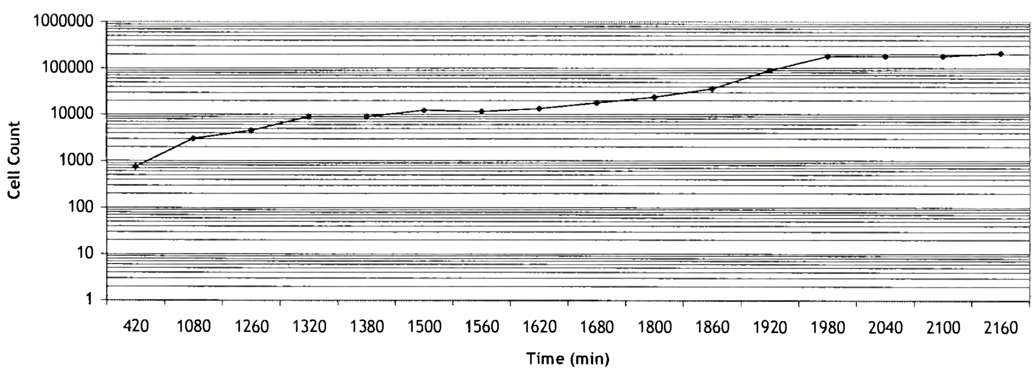
Figure B-60. Lactose With Pb Cell Growth Curve 1



Cell Count increases from 17400 to 35100

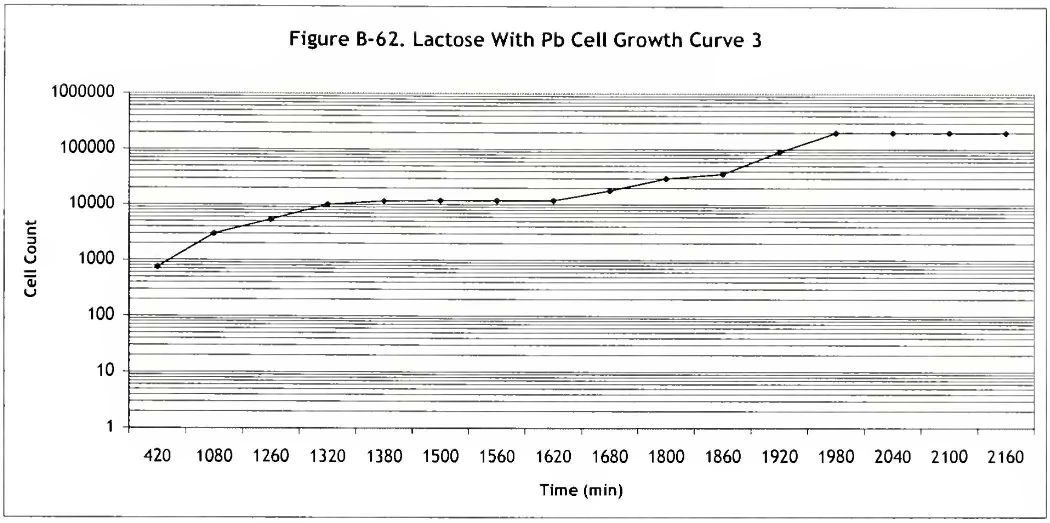
Actual growth rate = (1860min -1680min) = 180 min

Figure B-61. Lactose With Pb Cell Growth Curve 2



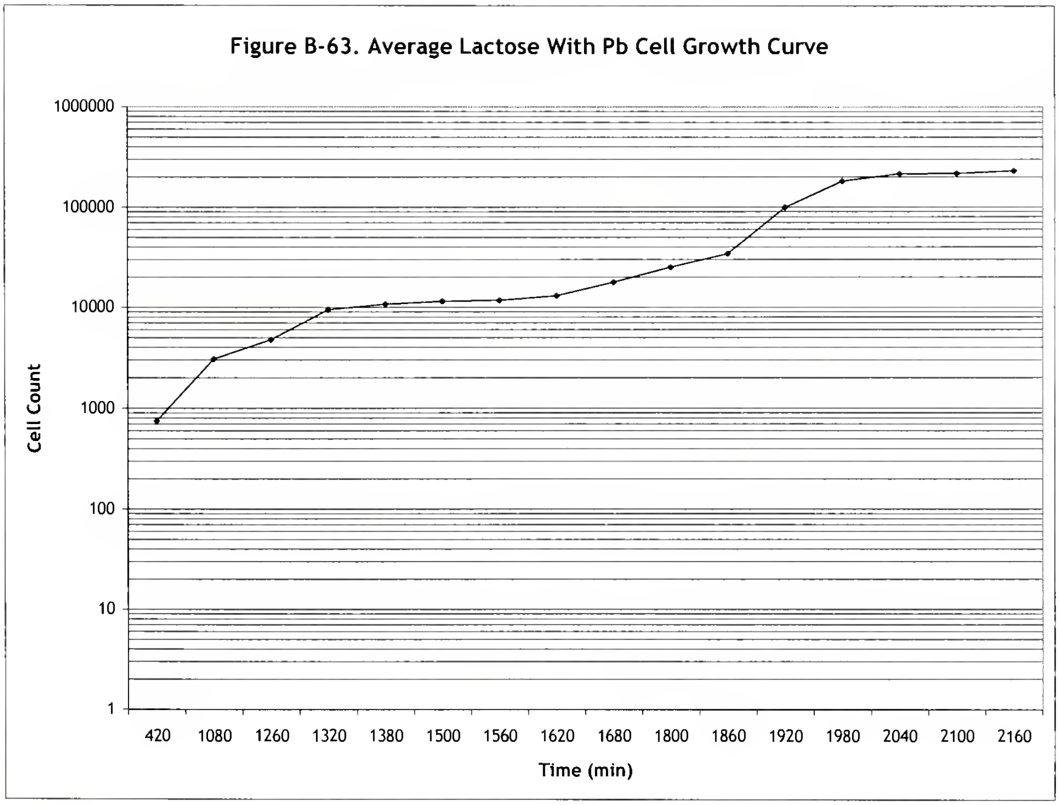
Cell Count increases from 18000 to 36000

Actual growth rate = (1860min -1680min) = 180 min



Cell Count increases from 18000 to 36000

Actual growth rate = (1860min -1680min) = 180 min



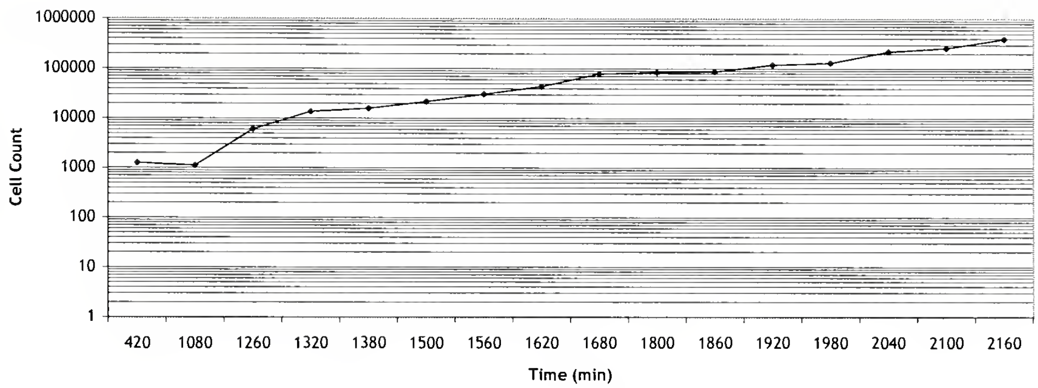
Cell Count increases from 13000 to 25200

Actual growth rate = (1800min -1620min) = 180 min

GLUCOSE ONLY CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Cell Count 4	Average Cell Count
420	1265	1265	1265		1265
1080	1120	1425	1450	1500	1374
1260	6090	6300	6300		6230
1320	13800	14000	10000		12600
1380	15900	15900	15000		15600
1500	21900	21900	18000		20600
1560	30600	30000	30000		30200
1620	44100	42000	60000		48700
1680	78900	84000	90000	75000	81975
1800	85500	96000	90000		90500
1860	88200	108000	100000	90000	96550
1920	120000	150000	150000		140000
1980	132000	150000	175000	125000	145500
2040	222000	210000	200000	250000	220500
2100	261000	210000	225000	250000	236500
2160	399000	300000	275000	250000	306000

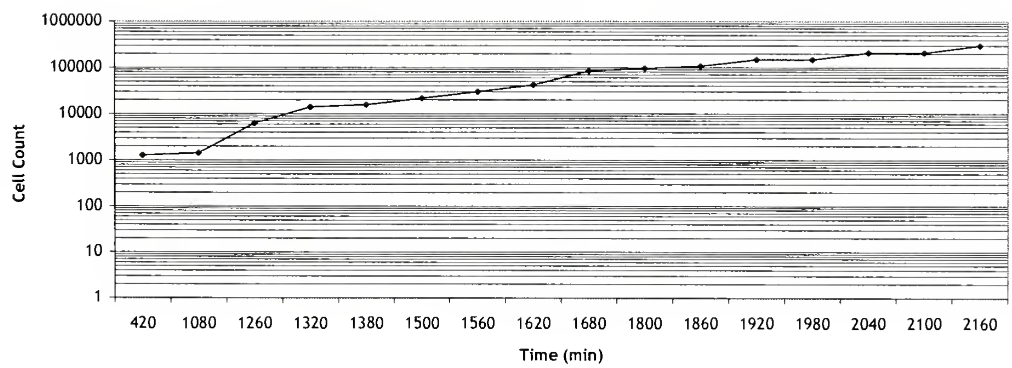
Figure B-64. Glucose Control Cell Growth Curve 1



Cell Count increases from 21900 to 44100

Actual growth rate = (1620min -1500min) = 120 min

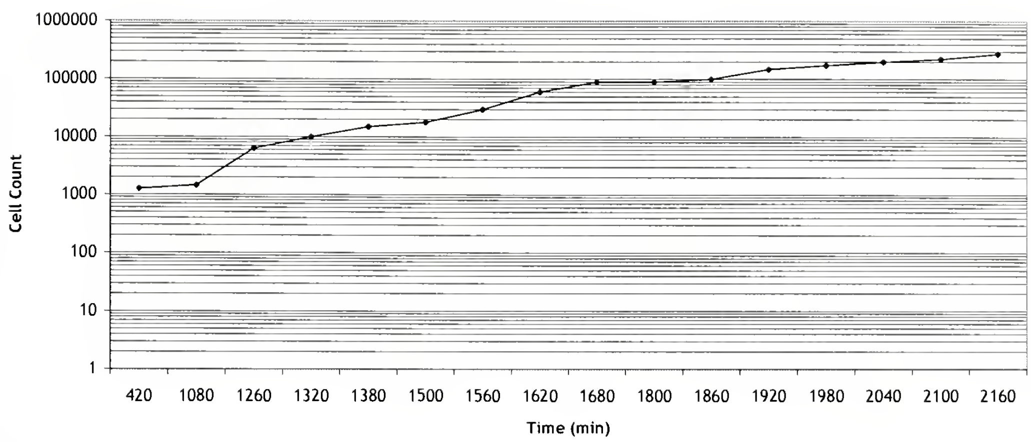
Figure B-65. Glucose Control Cell Growth Curve 2



Cell Count increases from 42000 to 84000

Actual growth rate = (1680min -1620min) = 60 min

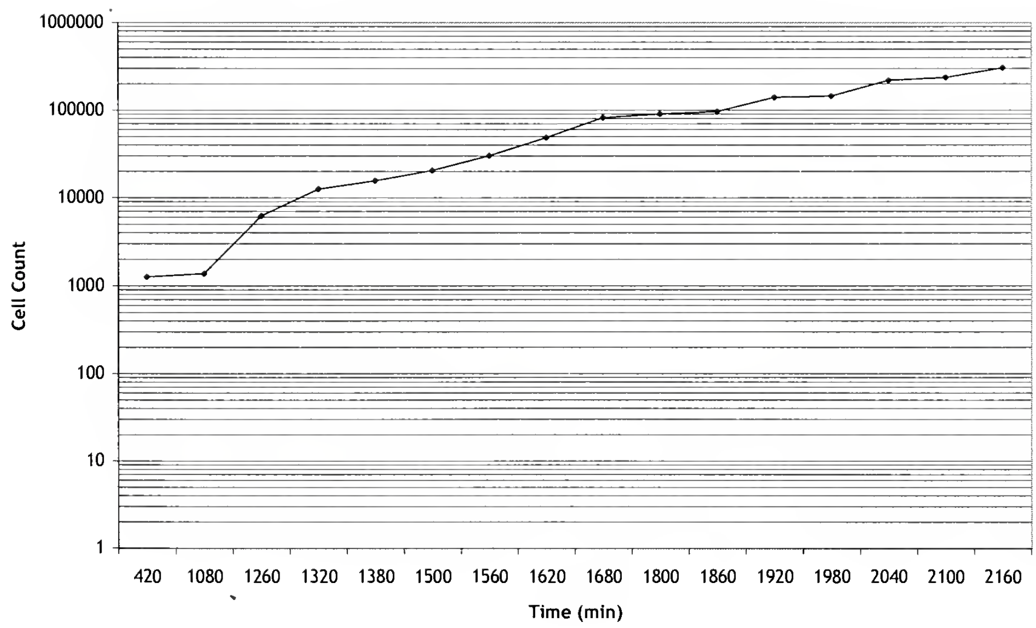
Figure B-66. Glucose Control Cell Growth Curve 3



Cell Count increases from 30000 to 60000

Actual growth rate = **(1620min -1560min) = 60 min**

Figure B-67. Average Glucose Control Cell Growth Curve



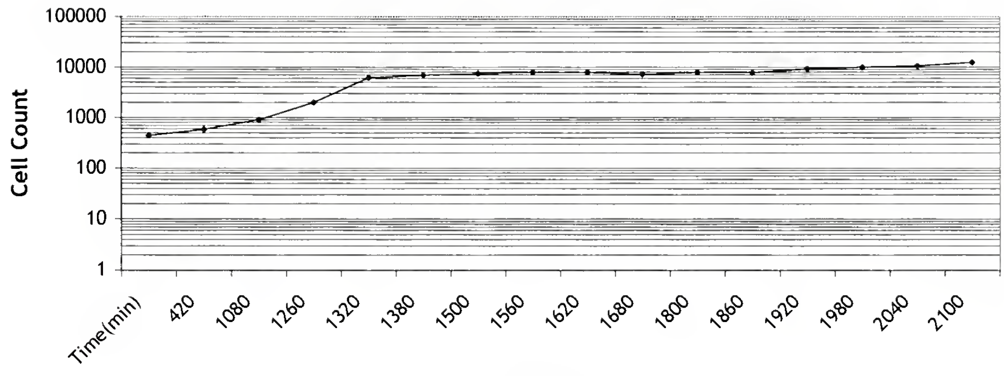
Cell Count increases from 15600 to 30200

Actual growth rate = **(1560min -1380min) = 180 min**

GLOSE WITH Cd CELL COUNT

Time(min)	Cell Count 1	Cell Count 2	Cell Count 3	Average Cell Count
420	440	440	440	440
1080	580	550	580	570
1260	900	1200	900	1000
1320	2000	2000	2000	2000
1380	6180	5700	6000	5960
1500	6930	6000	6000	6310
1560	7470	6900	6000	6790
1620	7830	7200	6000	7010
1680	7890	7200	6000	7030
1800	7200	9000	9000	8400
1860	7860	8700	9000	8520
1920	7800	9000	9000	8600
1980	9300	9300	9000	9200
2040	9900	12000	12000	11300
2100	10500	12000	12000	11500
2160	12300	12300	12000	12200

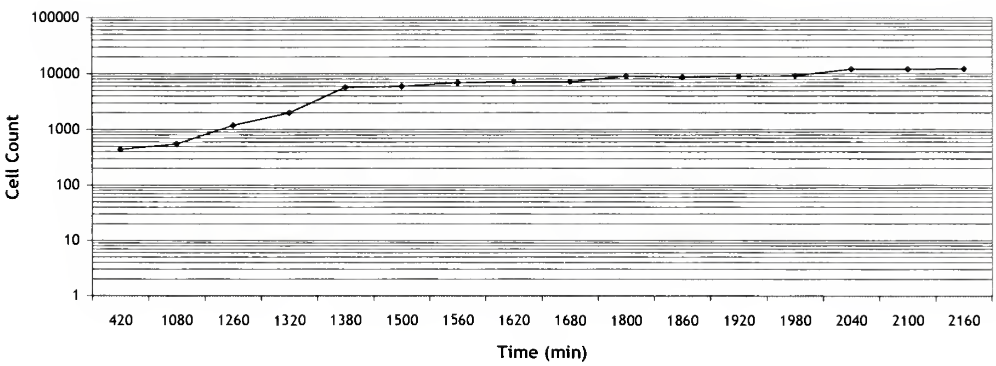
Figure B-68. Glucose With Cd Cell Growth Curve 1



Cell Count increases from 6180 to 12300

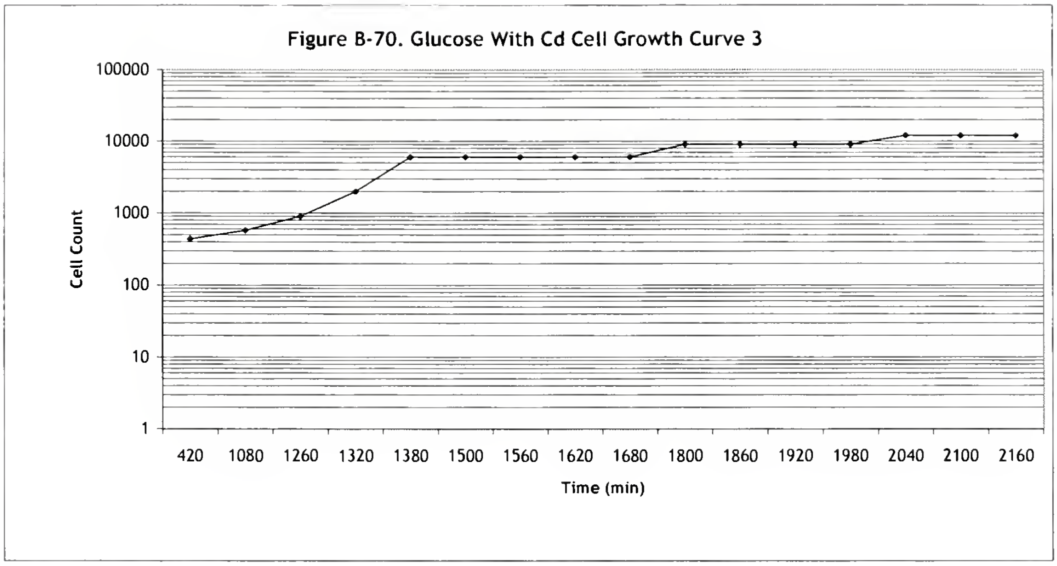
Actual growth rate = (2160min -1500min) = 660 min

Figure B-69. Glucose With Cd Cell Growth Curve 2



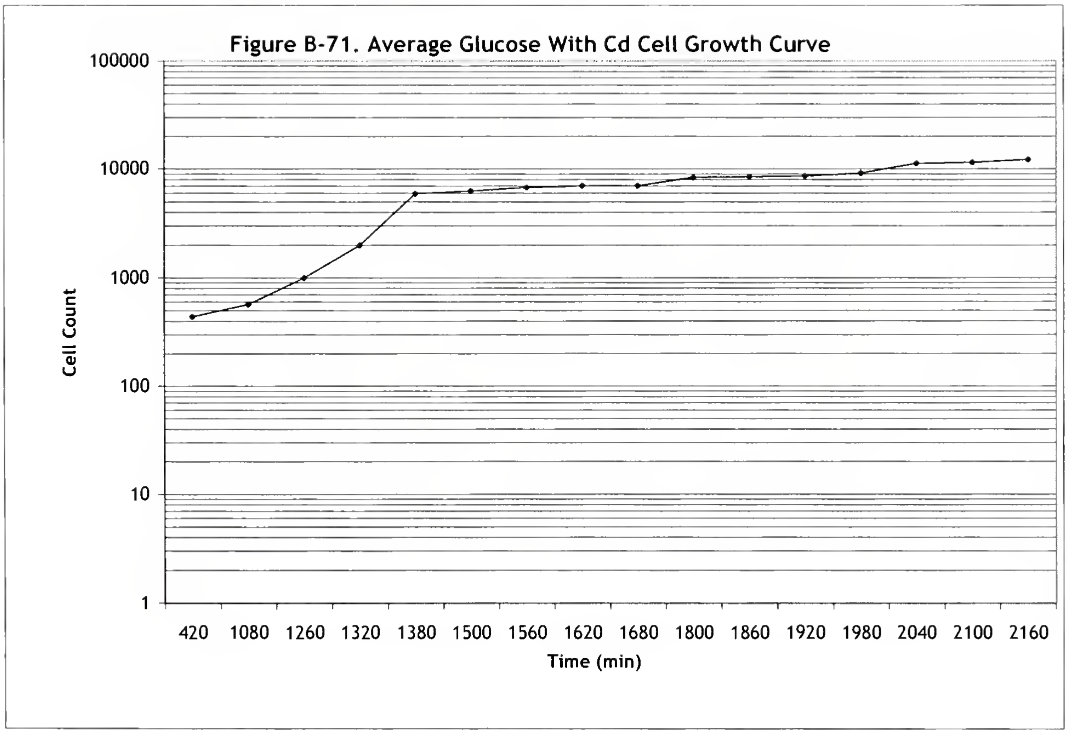
Cell Count increases from 6000 to 12000

Actual growth rate = (2040min -1500min) = 540 min



Cell Count increases from 6000 to 12000

Actual growth rate = **(2040min -1680min) = 360 min**



Cell Count increases from 5960 to 11500

Actual growth rate = **(2100min -1380min) = 720 min**

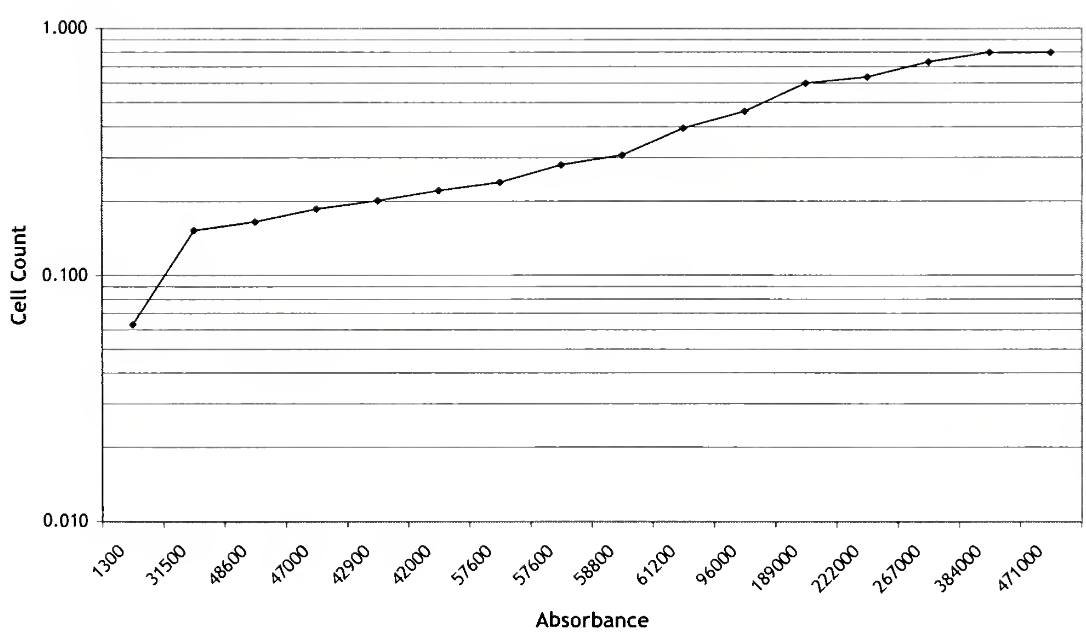
M.roseus Cell growth vs. Absorbance

LACTOSE ONLY CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
420	1300	0.063	1.16	0.865
1080	31500	0.152	1.42	0.705
1260	48600	0.165	1.46	0.684
1320	47000	0.186	1.53	0.652
1380	42900	0.201	1.59	0.630
1500	42000	0.221	1.66	0.601
1560	57600	0.239	1.73	0.577
1620	57600	0.281	1.91	0.524
1680	58800	0.308	2.03	0.492
1800	61200	0.395	2.48	0.403
1860	96000	0.461	2.89	0.346
1920	189000	0.599	3.97	0.252
1980	222000	0.635	4.32	0.232
2040	267000	0.732	5.40	0.185
2100	384000	0.798	6.28	0.159
2160	471000	0.798	6.28	0.159

Cell Count	Absorbance
1300	0.063
31500	0.152
48600	0.165
47000	0.186
42900	0.201
42000	0.221
57600	0.239
57600	0.281
58800	0.308
61200	0.395
96000	0.461
189000	0.599
222000	0.635
267000	0.732
384000	0.798
471000	0.798

Figure B-76. Lactose Control Absorbance vs. Cell Count 1



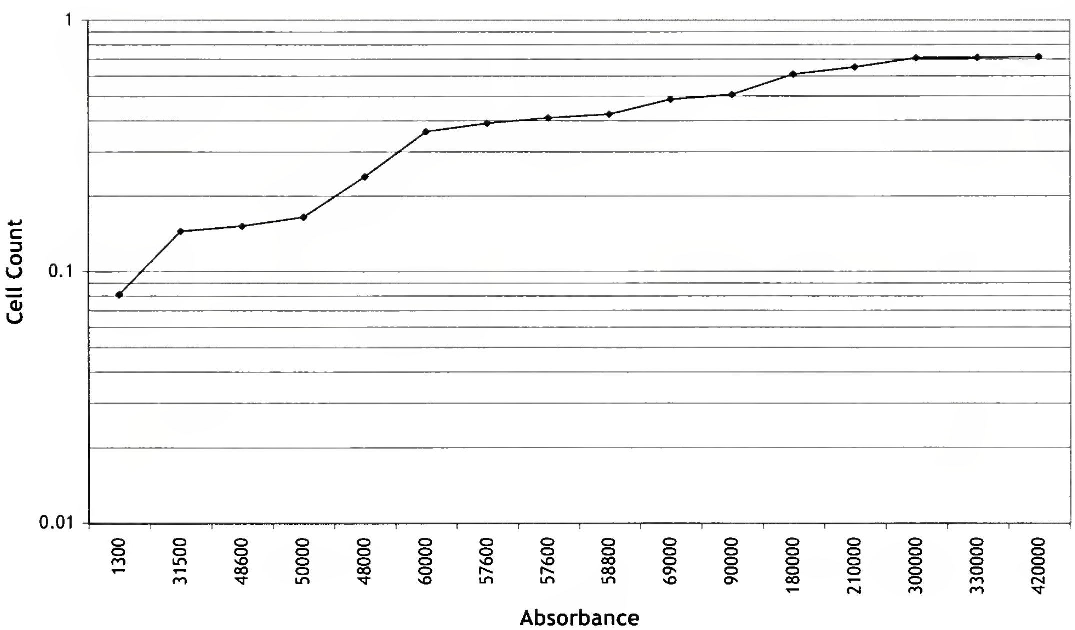
Cell Count increases from 96000 to 189000

Difference in absorbance = (0.599 -0.461) = 0.138

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
420	1300	0.081	1.21	0.830
1080	31500	0.145	1.40	0.716
1260	48600	0.152	1.42	0.705
1320	50000	0.165	1.46	0.684
1380	48000	0.239	1.73	0.577
1500	60000	0.362	2.30	0.435
1560	57600	0.391	2.46	0.406
1620	57600	0.411	2.58	0.388
1680	58800	0.424	2.65	0.377
1800	69000	0.485	3.05	0.327
1860	90000	0.508	3.22	0.310
1920	180000	0.612	4.09	0.244
1980	210000	0.653	4.50	0.222
2040	300000	0.708	5.11	0.196
2100	330000	0.711	5.14	0.195
2160	420000	0.715	5.19	0.193

Cell Count	Absorbance
1300	0.081
31500	0.145
48600	0.152
50000	0.165
48000	0.239
60000	0.362
57600	0.391
57600	0.411
58800	0.424
69000	0.485
90000	0.508
180000	0.612
210000	0.653
300000	0.708
330000	0.711
420000	0.715

Figure B.77. Lactose Control Absorbance vs. Cell Count 2



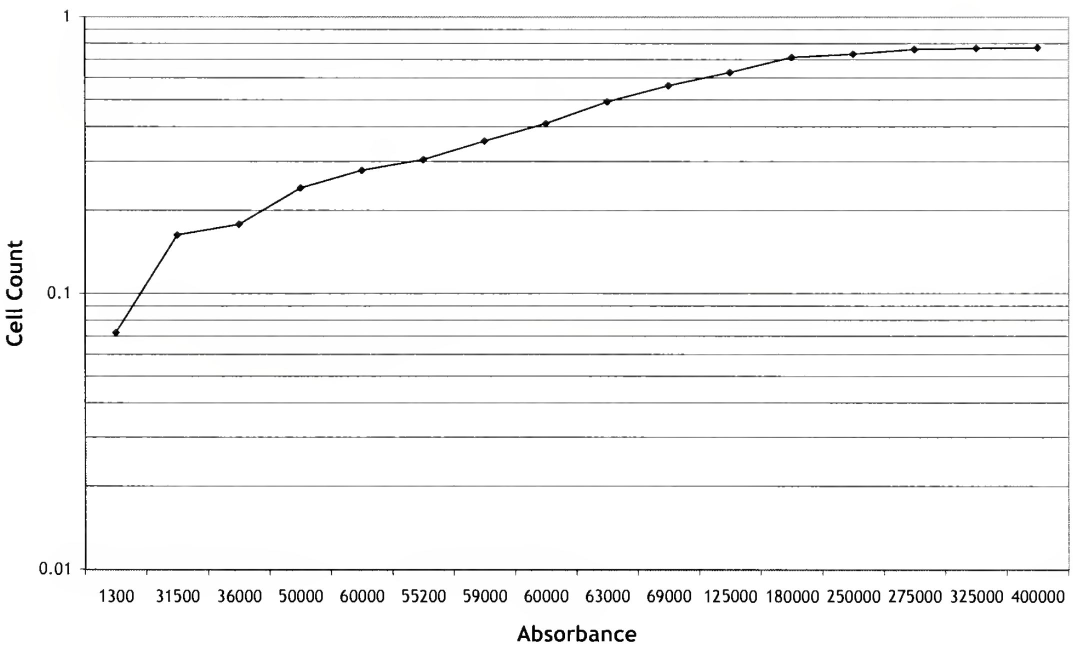
Cell Count increases from 90000 to 180000

Difference in absorbance = (0.612 -0.508) = 0.104

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
420	1300	0.072	1.18	0.847
1080	31500	0.163	1.46	0.687
1260	36000	0.178	1.51	0.664
1320	50000	0.241	1.74	0.574
1380	60000	0.279	1.90	0.526
1500	55200	0.305	2.02	0.495
1560	59000	0.355	2.26	0.442
1620	60000	0.411	2.58	0.388
1680	63000	0.492	3.10	0.322
1800	69000	0.563	3.66	0.274
1860	125000	0.628	4.25	0.236
1920	180000	0.713	5.16	0.194
1980	250000	0.732	5.40	0.185
2040	275000	0.763	5.79	0.173
2100	325000	0.768	5.86	0.171
2160	400000	0.773	5.93	0.169

Cell Count	Absorbance
1300	0.072
31500	0.163
36000	0.178
50000	0.241
60000	0.279
55200	0.305
59000	0.355
60000	0.411
63000	0.492
69000	0.563
125000	0.628
180000	0.713
250000	0.732
275000	0.763
325000	0.768
400000	0.773

Figure B-78. Lactose Control Absorbance vs. Cell Count 3



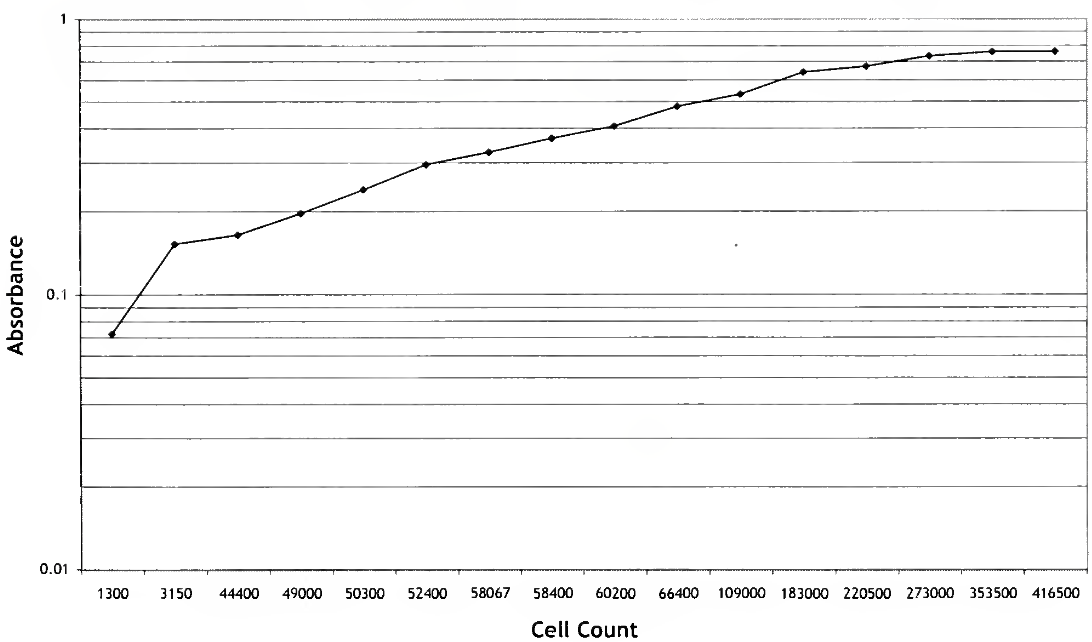
Cell Count increases from 125000 to 250000

Difference in absorbance = (0.732 -0.628) = 0.104

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
420	1300	0.072	1.18	0.847
1080	3150	0.153	1.42	0.703
1260	44400	0.165	1.46	0.684
1320	49000	0.197	1.58	0.635
1380	50300	0.240	1.74	0.576
1500	52400	0.296	1.98	0.506
1560	58067	0.328	2.13	0.470
1620	58400	0.368	2.33	0.429
1680	60200	0.408	2.56	0.391
1800	66400	0.481	3.03	0.330
1860	109000	0.532	3.41	0.294
1920	183000	0.641	4.38	0.228
1980	220500	0.673	4.71	0.212
2040	273000	0.734	5.42	0.184
2100	353500	0.759	5.74	0.174
2160	416500	0.762	5.78	0.173

Cell Count	Absorbance
1300	0.072
3150	0.153
44400	0.165
49000	0.197
50300	0.24
52400	0.296
58067	0.328
58400	0.368
60200	0.408
66400	0.481
109000	0.532
183000	0.641
220500	0.673
273000	0.734
353500	0.759
416500	0.762

Figure B-79. Average Lactose Control Absorbance vs. Cell Count



Cell Count increases from 109000 to 220500

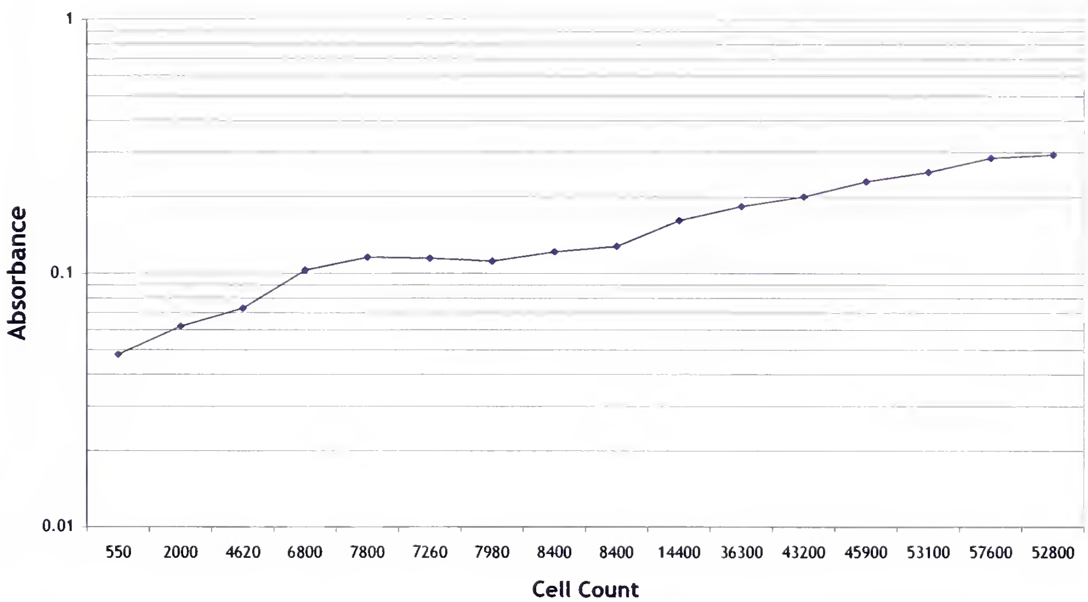
Difference in absorbance = (0.673 -0.532) = 0.141

LACTOSE WITH Cd CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
420	550	0.048	1.12	0.895
1080	2000	0.062	1.15	0.867
1260	4620	0.073	1.18	0.845
1320	6800	0.103	1.27	0.789
1380	7800	0.116	1.31	0.766
1500	7260	0.115	1.30	0.767
1560	7980	0.112	1.29	0.773
1620	8400	0.122	1.32	0.755
1680	8400	0.128	1.34	0.745
1800	14400	0.162	1.45	0.689
1860	36300	0.184	1.53	0.655
1920	43200	0.201	1.59	0.630
1980	45900	0.231	1.70	0.587
2040	53100	0.251	1.78	0.561
2100	57600	0.286	1.93	0.518
2160	52800	0.294	1.97	0.508

Cell Count	Absorbance
550	0.048
2000	0.062
4620	0.073
6800	0.103
7800	0.116
7260	0.115
7980	0.112
8400	0.122
8400	0.128
14400	0.162
36300	0.184
43200	0.201
45900	0.231
53100	0.251
57600	0.286
52800	0.294

Figure B-80. Lactose with Cd Absorbance vs. Cell Count 1



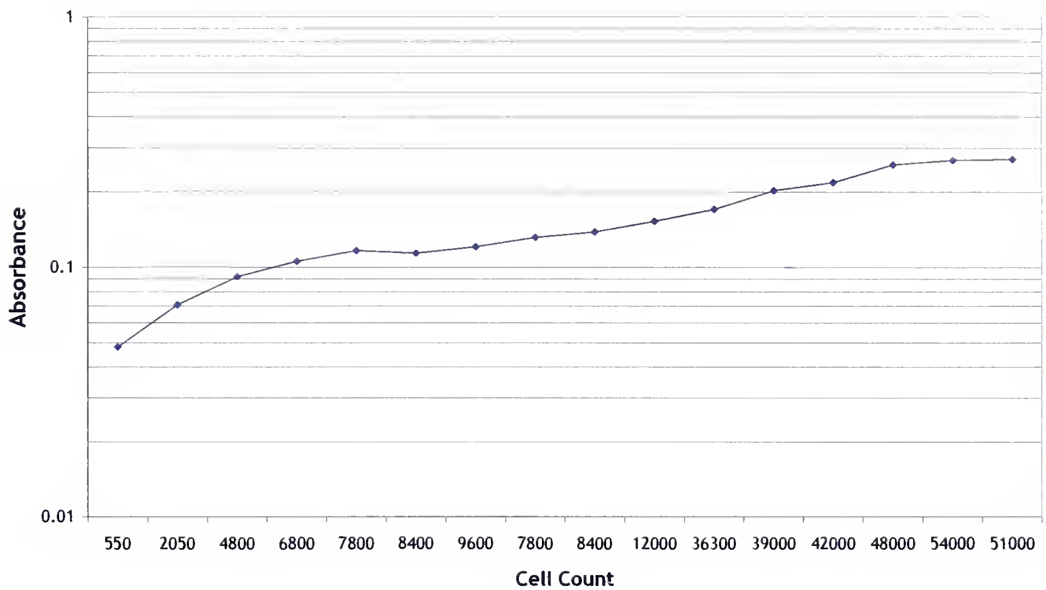
Cell Count increases from 7260 to 14400

Difference in absorbance = (0.162 -0.115) = 0.047

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
420	550	0.048	1.12	0.895
1080	2050	0.071	1.18	0.849
1260	4800	0.092	1.24	0.809
1320	6800	0.106	1.28	0.783
1380	7800	0.117	1.31	0.764
1500	8400	0.114	1.30	0.769
1560	9600	0.121	1.32	0.757
1620	7800	0.132	1.36	0.738
1680	8400	0.139	1.38	0.726
1800	12000	0.153	1.42	0.703
1860	36300	0.171	1.48	0.675
1920	39000	0.203	1.60	0.627
1980	42000	0.219	1.66	0.604
2040	48000	0.258	1.81	0.552
2100	54000	0.269	1.86	0.538
2160	51000	0.271	1.87	0.536

Cell Count	Absorbance
550	0.048
2050	0.071
4800	0.092
6800	0.106
7800	0.117
8400	0.114
9600	0.121
7800	0.132
8400	0.139
12000	0.153
36300	0.171
39000	0.203
42000	0.219
48000	0.258
54000	0.269
51000	0.271

Figure B-81. Lactose with Cd Absorbance vs. Cell Count 2



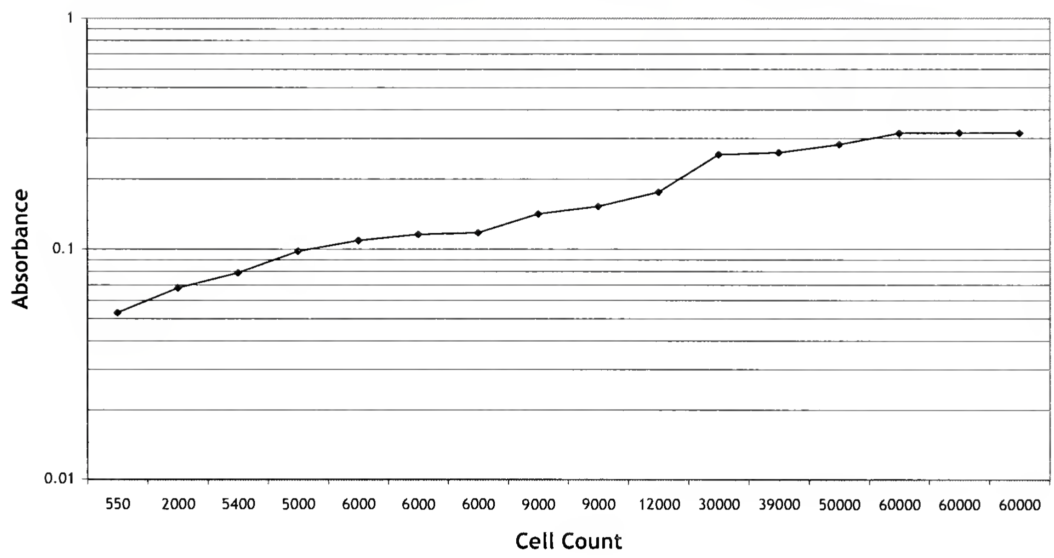
Cell Count increases from 4800 to 9600

Difference in absorbance = (0.121 -0.071) = 0.05

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
420	550	0.053	1.13	0.885
1080	2000	0.068	1.17	0.855
1260	5400	0.079	1.20	0.834
1320	5000	0.098	1.25	0.798
1380	6000	0.109	1.29	0.778
1500	6000	0.116	1.31	0.766
1560	6000	0.118	1.31	0.762
1620	9000	0.142	1.39	0.721
1680	9000	0.153	1.42	0.703
1800	12000	0.176	1.50	0.667
1860	30000	0.256	1.80	0.555
1920	39000	0.261	1.82	0.548
1980	50000	0.283	1.92	0.521
2040	60000	0.316	2.07	0.483
2100	60000	0.317	2.07	0.482
2160	60000	0.316	2.07	0.483

Cell Count	Absorbance
550	0.053
2000	0.068
5400	0.079
5000	0.098
6000	0.109
6000	0.116
6000	0.118
9000	0.142
9000	0.153
12000	0.176
30000	0.256
39000	0.261
50000	0.283
60000	0.316
60000	0.317
60000	0.316

Figure B-82. Lactose with Cd Absorbance vs. Cell Count 3



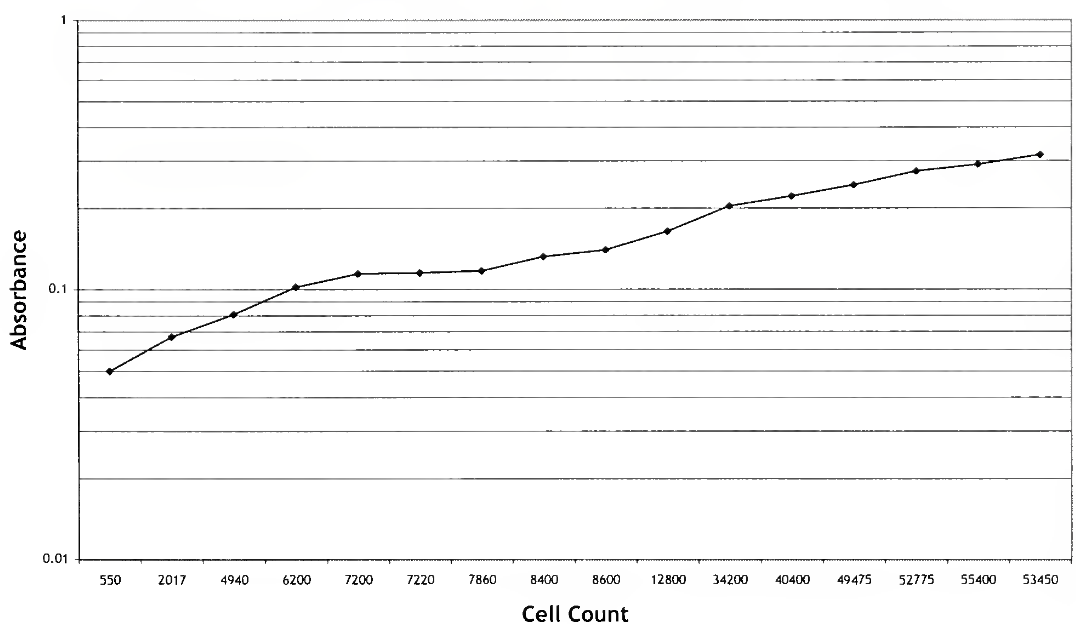
Cell Count increases from 30000 to 60000

Difference in absorbance = (0.316 -0.256) = 0.06

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
420	550	0.050	1.12	0.892
1080	2017	0.067	1.17	0.857
1260	4940	0.081	1.21	0.829
1320	6200	0.102	1.27	0.790
1380	7200	0.114	1.30	0.769
1500	7220	0.115	1.30	0.767
1560	7860	0.117	1.31	0.764
1620	8400	0.132	1.36	0.738
1680	8600	0.140	1.38	0.724
1800	12800	0.164	1.46	0.686
1860	34200	0.204	1.60	0.626
1920	40400	0.222	1.67	0.600
1980	49475	0.244	1.76	0.570
2040	52775	0.275	1.88	0.531
2100	55400	0.291	1.95	0.512
2160	53450	0.294	1.97	0.509

Cell Count	Absorbance
550	0.05
2017	0.067
4940	0.081
6200	0.102
7200	0.114
7220	0.115
7860	0.117
8400	0.132
8600	0.14
12800	0.164
34200	0.204
40400	0.222
49475	0.244
52775	0.275
55400	0.291
53450	0.316

Figure B-83. Average Lactose with Cd Absorbance vs. Cell Count



Cell Count increases from 6200 to 12800

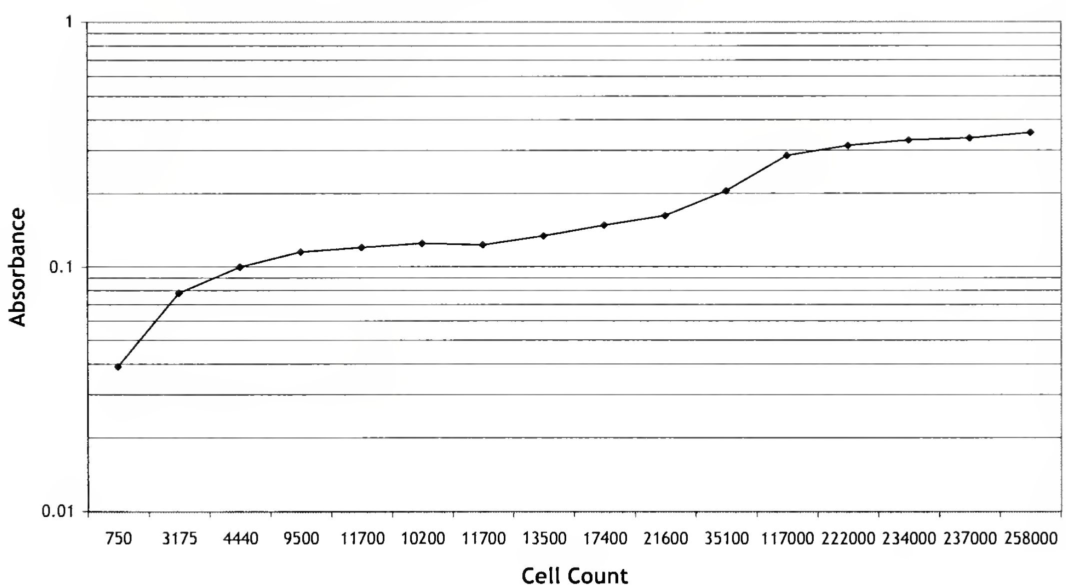
Difference in absorbance = (0.164 -0.102) = 0.062

LACTOSE WITH Pb CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
420	750	0.039	1.09	0.914
1080	3175	0.078	1.20	0.836
1260	4440	0.1	1.26	0.794
1320	9500	0.115	1.30	0.767
1380	11700	0.12	1.32	0.759
1500	10200	0.125	1.33	0.750
1560	11700	0.123	1.33	0.753
1620	13500	0.134	1.36	0.735
1680	17400	0.148	1.41	0.711
1800	21600	0.162	1.45	0.689
1860	35100	0.205	1.60	0.624
1920	117000	0.286	1.93	0.518
1980	222000	0.314	2.06	0.485
2040	234000	0.331	2.14	0.467
2100	237000	0.337	2.17	0.460
2160	258000	0.355	2.26	0.442

Cell Count	Absorbance
750	0.039
3175	0.078
4440	0.1
9500	0.115
11700	0.12
10200	0.125
11700	0.123
13500	0.134
17400	0.148
21600	0.162
35100	0.205
117000	0.286
222000	0.314
234000	0.331
237000	0.337
258000	0.355

Figure B-84. Lactose With Pb Absorbance vs. Cell Count 1



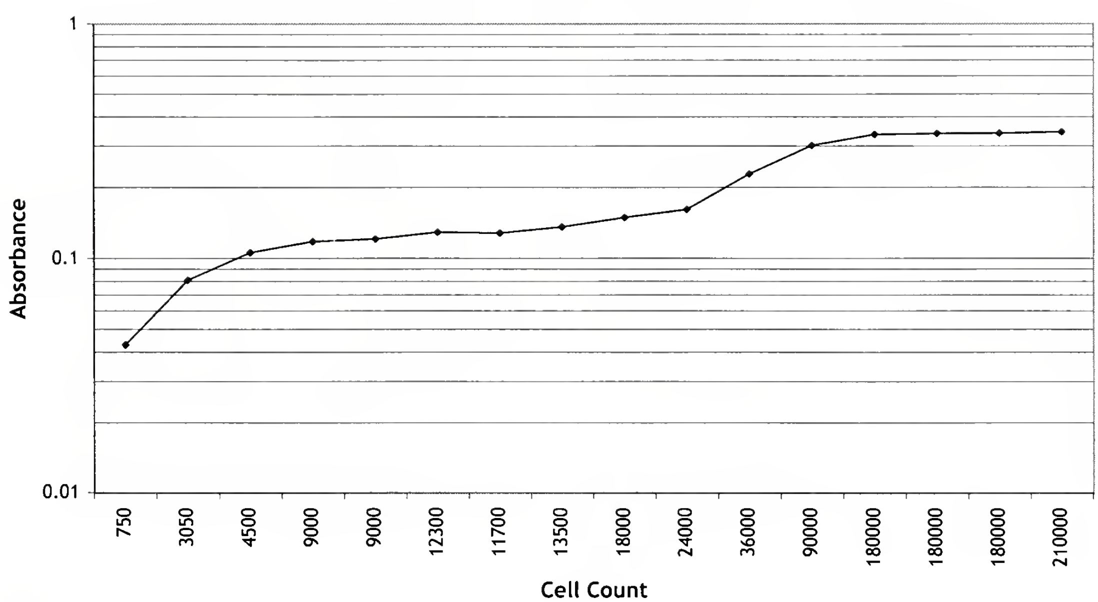
Cell Count increases from 17400 to 35100

Difference in absorbance = (0.205 -0.148) = 0.057

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
420	750	0.043	1.10	0.906
1080	3050	0.081	1.21	0.830
1260	4500	0.106	1.28	0.783
1320	9000	0.118	1.31	0.762
1380	9000	0.121	1.32	0.757
1500	12300	0.129	1.35	0.743
1560	11700	0.128	1.34	0.745
1620	13500	0.136	1.37	0.731
1680	18000	0.149	1.41	0.710
1800	24000	0.161	1.45	0.690
1860	36000	0.228	1.69	0.592
1920	90000	0.302	2.00	0.499
1980	180000	0.336	2.17	0.461
2040	180000	0.339	2.18	0.458
2100	180000	0.341	2.19	0.456
2160	210000	0.345	2.21	0.452

Cell Count	Absorbance
750	0.043
3050	0.081
4500	0.106
9000	0.118
9000	0.121
12300	0.129
11700	0.128
13500	0.136
18000	0.149
24000	0.161
36000	0.228
90000	0.302
180000	0.336
180000	0.339
180000	0.341
210000	0.345

Figure B-85. Lactose With Pb Absorbance vs. Cell Count 2



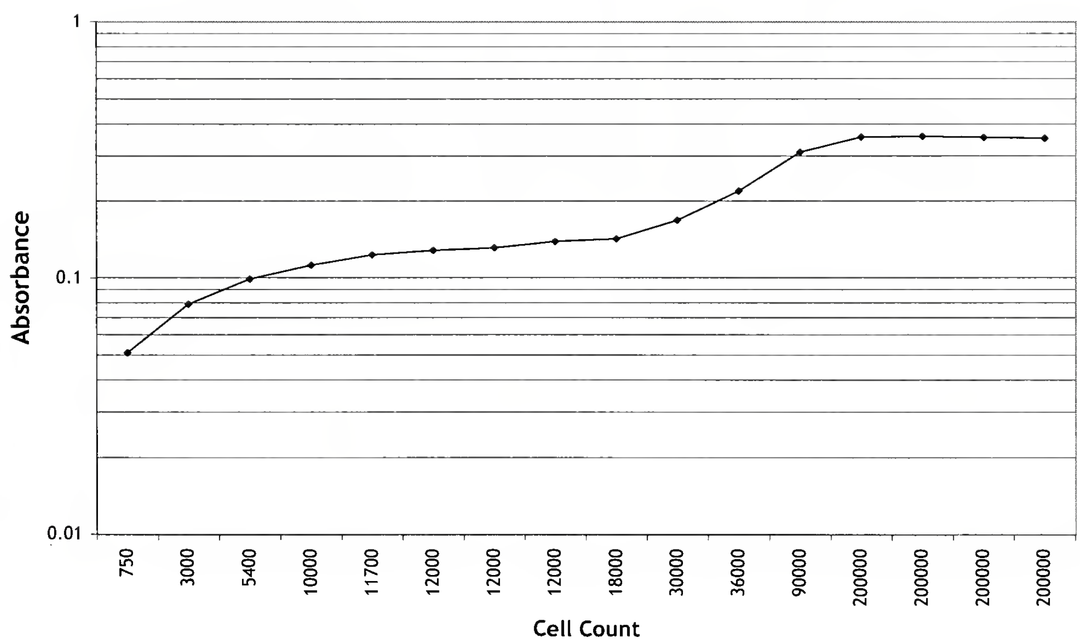
Cell Count increases from 18000 to 36000

Difference in absorbance = (0.228 -0.149) = 0.079

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
420	750	0.051	1.12	0.889
1080	3000	0.079	1.20	0.834
1260	5400	0.099	1.26	0.796
1320	10000	0.112	1.29	0.773
1380	11700	0.123	1.33	0.753
1500	12000	0.128	1.34	0.745
1560	12000	0.131	1.35	0.740
1620	12000	0.139	1.38	0.726
1680	18000	0.142	1.39	0.721
1800	30000	0.168	1.47	0.679
1860	36000	0.219	1.66	0.604
1920	90000	0.311	2.05	0.489
1980	200000	0.356	2.27	0.441
2040	200000	0.358	2.28	0.439
2100	200000	0.355	2.26	0.442
2160	200000	0.352	2.25	0.445

Cell Count	Absorbance
750	0.051
3000	0.079
5400	0.099
10000	0.112
11700	0.123
12000	0.128
12000	0.131
12000	0.139
18000	0.142
30000	0.168
36000	0.219
90000	0.311
200000	0.356
200000	0.358
200000	0.355
200000	0.352

Figure B-86. Lactose With Pb Absorbance vs. Cell Count 3



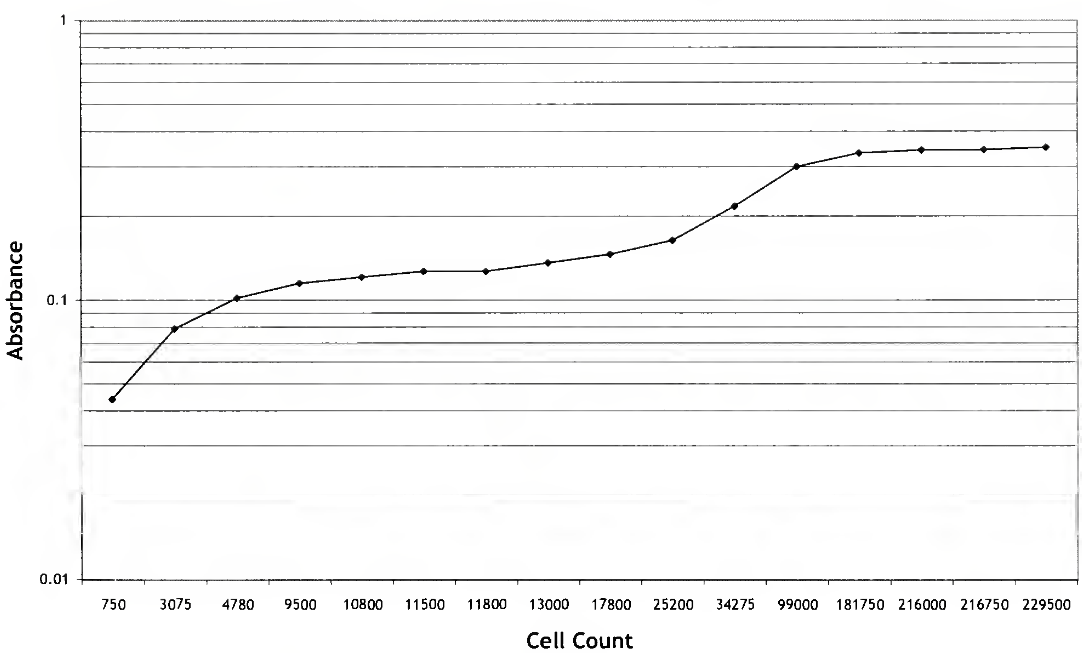
Cell Count increases from 18000 to 36000

Difference in absorbance = (0.219 -0.142) = 0.077

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
420	750	0.044	1.11	0.903
1080	3075	0.079	1.20	0.833
1260	4780	0.102	1.26	0.791
1320	9500	0.115	1.30	0.767
1380	10800	0.121	1.32	0.756
1500	11500	0.127	1.34	0.746
1560	11800	0.127	1.34	0.746
1620	13000	0.136	1.37	0.731
1680	17800	0.146	1.40	0.714
1800	25200	0.164	1.46	0.686
1860	34275	0.217	1.65	0.606
1920	99000	0.300	1.99	0.502
1980	181750	0.335	2.16	0.462
2040	216000	0.343	2.20	0.454
2100	216750	0.344	2.21	0.453
2160	229500	0.351	2.24	0.446

Cell Count	Absorbance
750	0.044
3075	0.079
4780	0.102
9500	0.115
10800	0.121
11500	0.127
11800	0.127
13000	0.136
17800	0.146
25200	0.164
34275	0.217
99000	0.3
181750	0.335
216000	0.343
216750	0.344
229500	0.351

Figure B-87. Average Lactose With Pb Absorbance vs. Cell Count



Cell Count increases from 13000 to 25200

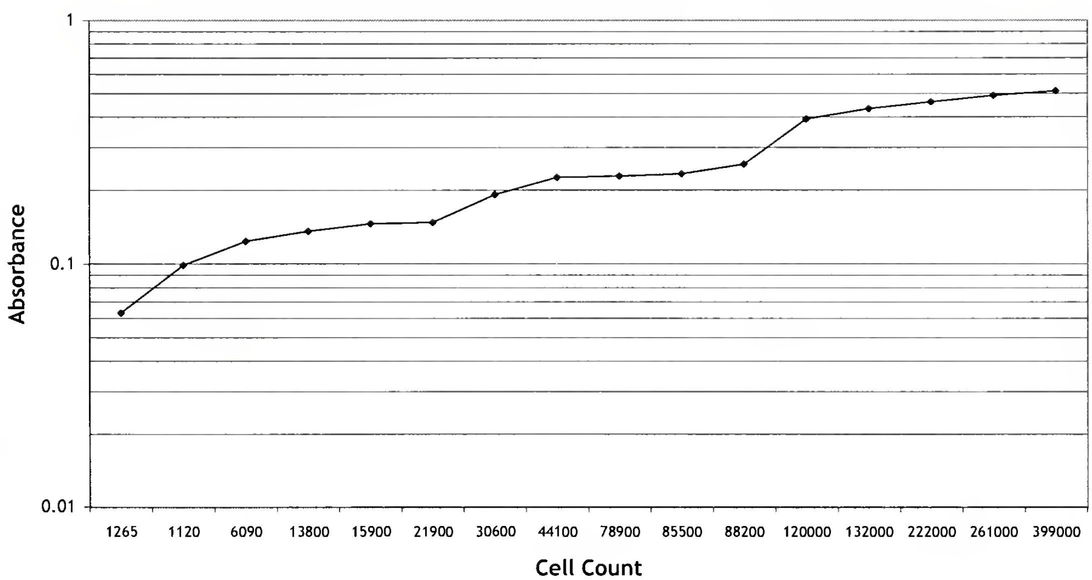
Difference in absorbance = (0.164 -0.136) = 0.028

GLUCOSE ONLY CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
420	1265	0.063	1.16	0.865
1080	1120	0.099	1.26	0.796
1260	6090	0.124	1.33	0.752
1320	13800	0.136	1.37	0.731
1380	15900	0.146	1.40	0.714
1500	21900	0.148	1.41	0.711
1560	30600	0.192	1.56	0.643
1620	44100	0.226	1.68	0.594
1680	78900	0.229	1.69	0.590
1800	85500	0.234	1.71	0.583
1860	88200	0.256	1.80	0.555
1920	120000	0.393	2.47	0.405
1980	132000	0.434	2.72	0.368
2040	222000	0.462	2.90	0.345
2100	261000	0.491	3.10	0.323
2160	399000	0.513	3.26	0.307

Cell Count	Absorbance
1265	0.063
1120	0.099
6090	0.124
13800	0.136
15900	0.146
21900	0.148
30600	0.192
44100	0.226
78900	0.229
85500	0.234
88200	0.256
120000	0.393
132000	0.434
222000	0.462
261000	0.491
399000	0.513

Figure B-88. Glucose Control Absorbance vs. Cell Count 1



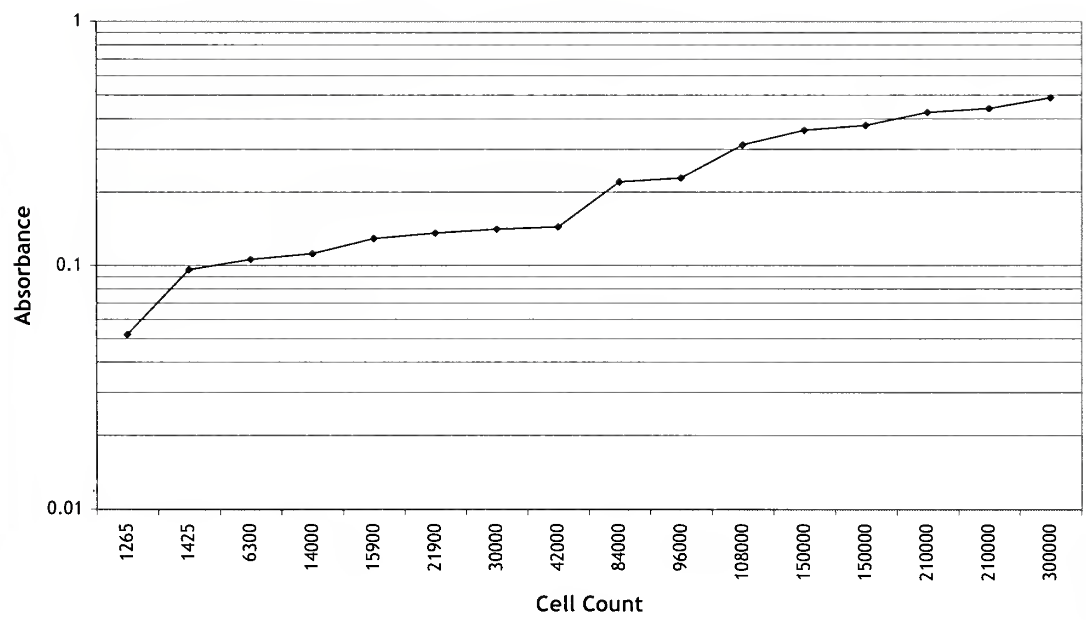
Cell Count increases from 21900 to 44100

Difference in absorbance = (0.226 -0.148) = 0.078

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
420	1265	0.052	1.13	0.887
1080	1425	0.096	1.25	0.802
1260	6300	0.106	1.28	0.783
1320	14000	0.112	1.29	0.773
1380	15900	0.129	1.35	0.743
1500	21900	0.136	1.37	0.731
1560	30000	0.141	1.38	0.723
1620	42000	0.144	1.39	0.718
1680	84000	0.221	1.66	0.601
1800	96000	0.229	1.69	0.590
1860	108000	0.313	2.06	0.486
1920	150000	0.359	2.29	0.438
1980	150000	0.376	2.38	0.421
2040	210000	0.425	2.66	0.376
2100	210000	0.441	2.76	0.362
2160	300000	0.488	3.08	0.325

Cell Count	Absorbance
1265	0.052
1425	0.096
6300	0.106
14000	0.112
15900	0.129
21900	0.136
30000	0.141
42000	0.144
84000	0.221
96000	0.229
108000	0.313
150000	0.359
150000	0.376
210000	0.425
210000	0.441
300000	0.488

Figure B-89. Glucose Control Absorbance vs. Cell Count 2



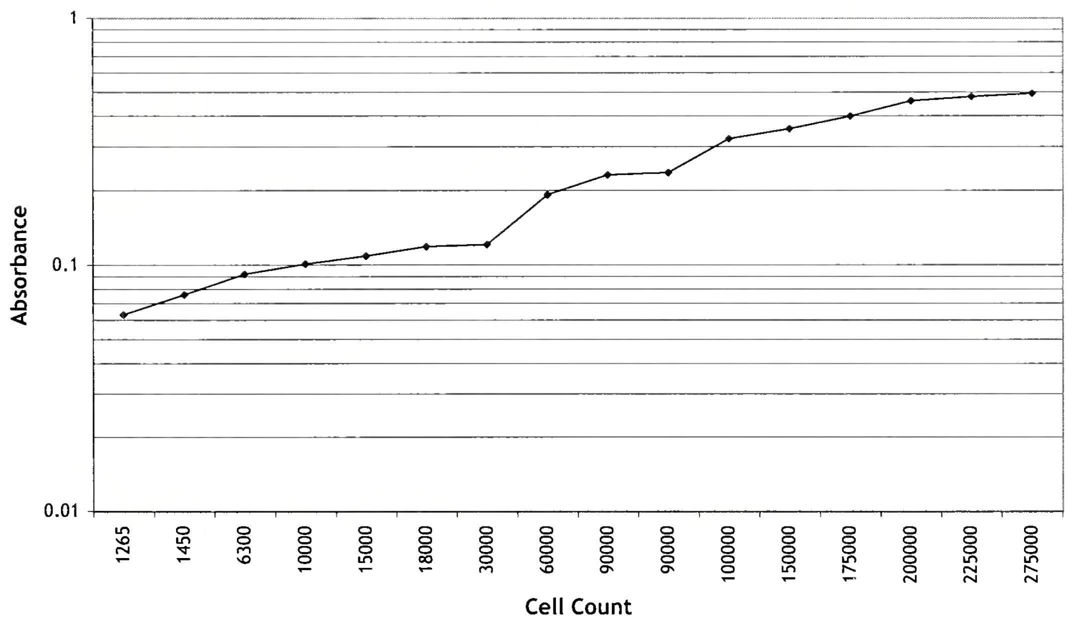
Cell Count increases from 42000 to 84000

Difference in absorbance = (0.221 -0.144) = 0.077

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
420	1265	0.063	1.16	0.865
1080	1450	0.076	1.19	0.839
1260	6300	0.092	1.24	0.809
1320	10000	0.101	1.26	0.793
1380	15000	0.109	1.29	0.778
1500	18000	0.119	1.32	0.760
1560	30000	0.121	1.32	0.757
1620	60000	0.192	1.56	0.643
1680	90000	0.231	1.70	0.587
1800	90000	0.236	1.72	0.581
1860	100000	0.323	2.10	0.475
1920	150000	0.355	2.26	0.442
1980	175000	0.399	2.51	0.399
2040	200000	0.461	2.89	0.346
2100	225000	0.479	3.01	0.332
2160	275000	0.493	3.11	0.321

Cell Count	Absorbance
1265	0.063
1450	0.076
6300	0.092
10000	0.101
15000	0.109
18000	0.119
30000	0.121
60000	0.192
90000	0.231
90000	0.236
100000	0.323
150000	0.355
175000	0.399
200000	0.461
225000	0.479
275000	0.493

Figure B-90. Glucose Control Absorbance vs. Cell Count 3



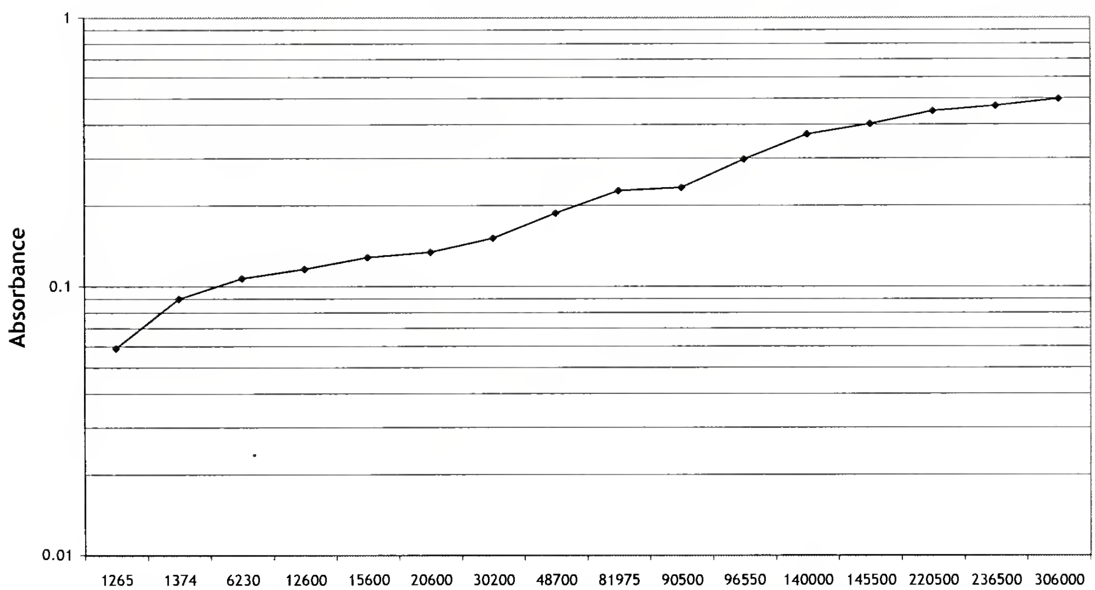
Cell Count increases from 30000 to 60000

Difference in absorbance = (0.192 -0.121) = 0.071

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
420	1265	0.059	1.15	0.872
1080	1374	0.090	1.23	0.812
1260	6230	0.107	1.28	0.781
1320	12600	0.116	1.31	0.765
1380	15600	0.128	1.34	0.745
1500	20600	0.134	1.36	0.734
1560	30200	0.151	1.42	0.706
1620	48700	0.187	1.54	0.650
1680	81975	0.227	1.69	0.593
1800	90500	0.233	1.71	0.585
1860	96550	0.297	1.98	0.504
1920	140000	0.369	2.34	0.428
1980	145500	0.403	2.53	0.395
2040	220500	0.449	2.81	0.355
2100	236500	0.470	2.95	0.339
2160	306000	0.498	3.15	0.318

Cell Count	Absorbance
1265	0.059
1374	0.09
6230	0.107
12600	0.116
15600	0.128
20600	0.134
30200	0.151
48700	0.187
81975	0.227
90500	0.233
96550	0.297
140000	0.369
145500	0.403
220500	0.449
236500	0.47
306000	0.498

Figure B-91. Average Glucose Control Absorbance vs. Cell Count



Cell Count increases from 15600 to 30200

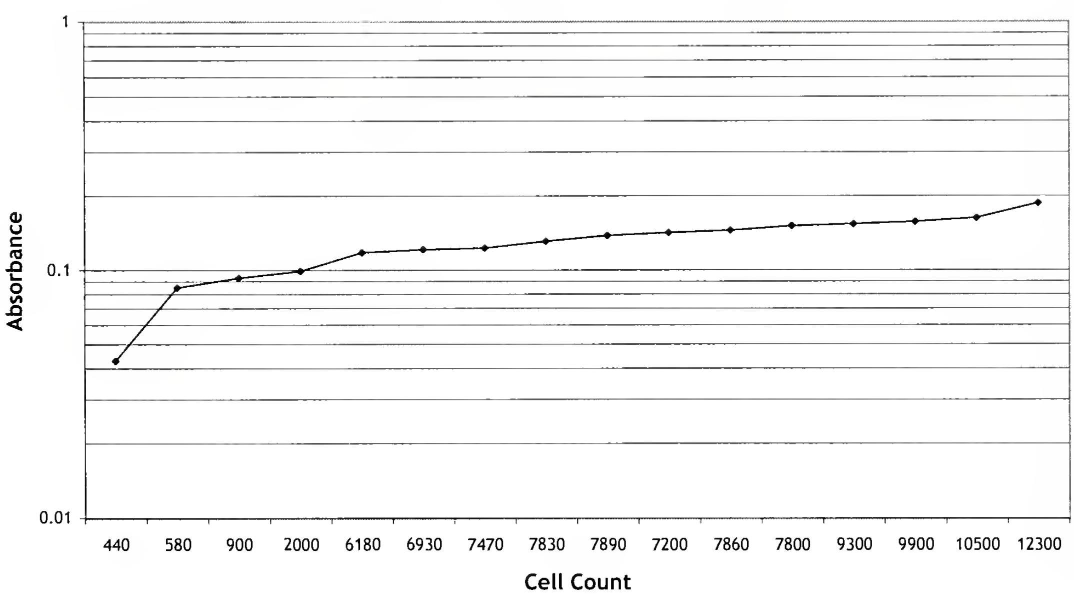
Difference in absorbance = (0.151 -0.128) = 0.023

GLUCOSE WITH Cd CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
420	440	0.043	1.10	0.906
1080	580	0.085	1.22	0.822
1260	900	0.093	1.24	0.807
1320	2000	0.099	1.26	0.796
1380	6180	0.118	1.31	0.762
1500	6930	0.121	1.32	0.757
1560	7470	0.123	1.33	0.753
1620	7830	0.131	1.35	0.740
1680	7890	0.138	1.37	0.728
1800	7200	0.142	1.39	0.721
1860	7860	0.145	1.40	0.716
1920	7800	0.151	1.42	0.706
1980	9300	0.154	1.43	0.701
2040	9900	0.157	1.44	0.697
2100	10500	0.163	1.46	0.687
2160	12300	0.187	1.54	0.650

Cell Count	Absorbance
440	0.043
580	0.085
900	0.093
2000	0.099
6180	0.118
6930	0.121
7470	0.123
7830	0.131
7890	0.138
7200	0.142
7860	0.145
7800	0.151
9300	0.154
9900	0.157
10500	0.163
12300	0.187

Figure B-92. Glucose With Cd Absorbance vs. Cell Count 1



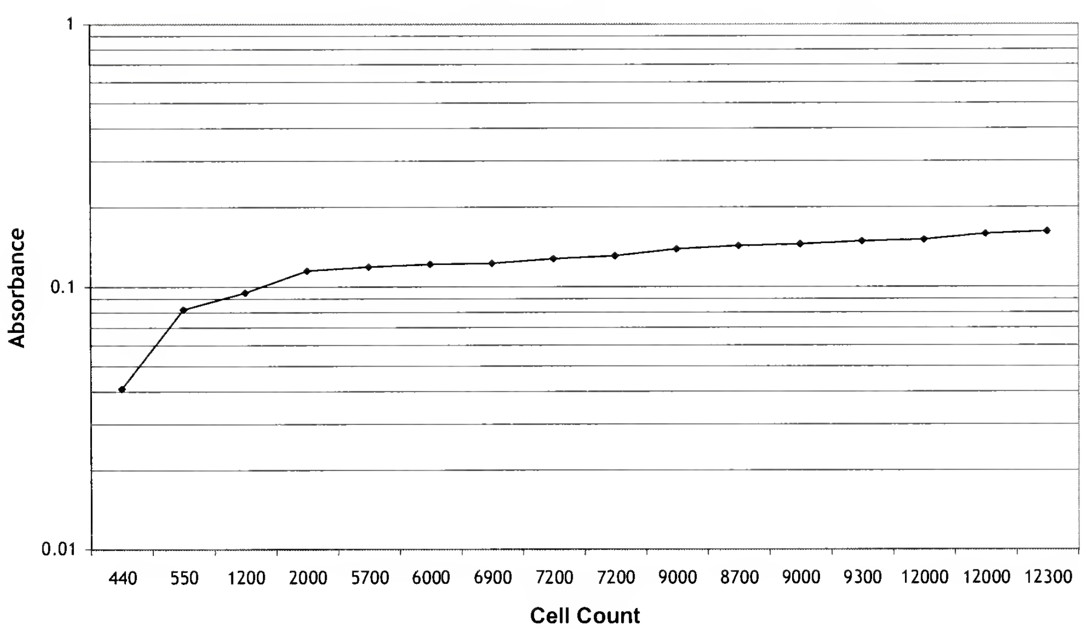
Cell Count increases from 6180 to 12300

Difference in absorbance = (0.187 -0.118) = 0.069

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
420	440	0.041	1.10	0.910
1080	550	0.082	1.21	0.828
1260	1200	0.095	1.24	0.804
1320	2000	0.115	1.30	0.767
1380	5700	0.119	1.32	0.760
1500	6000	0.122	1.32	0.755
1560	6900	0.123	1.33	0.753
1620	7200	0.128	1.34	0.745
1680	7200	0.131	1.35	0.740
1800	9000	0.139	1.38	0.726
1860	8700	0.143	1.39	0.719
1920	9000	0.145	1.40	0.716
1980	9300	0.149	1.41	0.710
2040	12000	0.162	1.45	0.689
2100	12000	0.168	1.47	0.679
2160	12300	0.171	1.48	0.675

Cell Count	Absorbance
440	0.041
550	0.082
1200	0.095
2000	0.115
5700	0.119
6000	0.122
6900	0.123
7200	0.128
7200	0.131
9000	0.139
8700	0.143
9000	0.145
9300	0.149
12000	0.151
12000	0.159
12300	0.162

Figure B-93. Glucose With Cd Absorbance vs. Cell Count 2



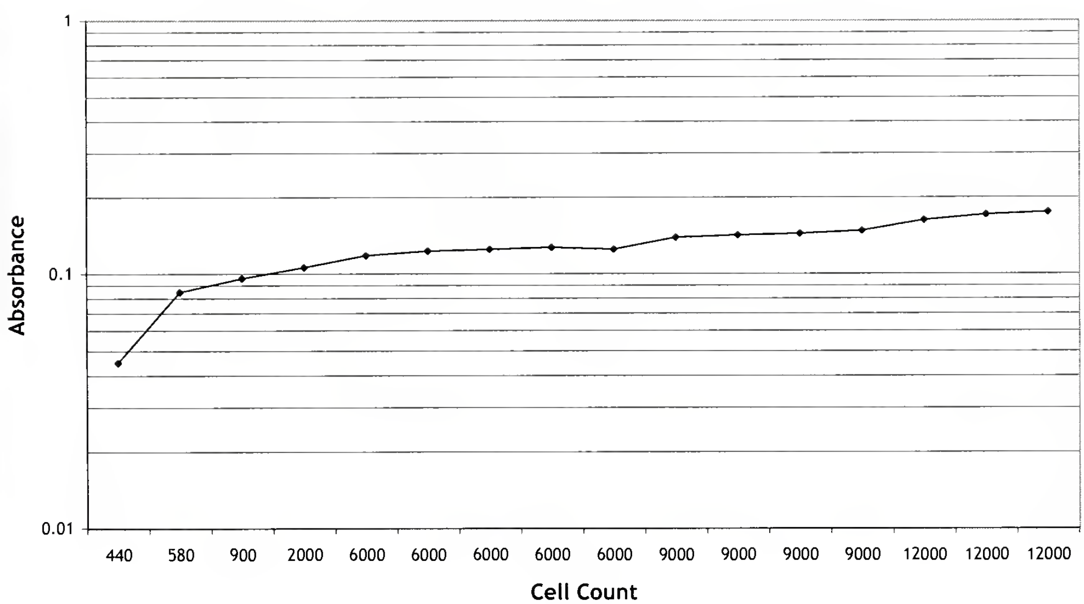
Cell Count increases from 6000 to 12000

Difference in absorbance = (0.162 -0.122) = 0.04

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
420	440	0.045	1.11	0.902
1080	580	0.085	1.22	0.822
1260	900	0.096	1.25	0.802
1320	2000	0.106	1.28	0.783
1380	6000	0.118	1.31	0.762
1500	6000	0.123	1.33	0.753
1560	6000	0.125	1.33	0.750
1620	6000	0.127	1.34	0.746
1680	6000	0.125	1.33	0.750
1800	9000	0.139	1.38	0.726
1860	9000	0.142	1.39	0.721
1920	9000	0.144	1.39	0.718
1980	9000	0.148	1.41	0.711
2040	12000	0.163	1.46	0.687
2100	12000	0.171	1.48	0.675
2160	12000	0.175	1.50	0.668

Cell Count	Absorbance
440	0.045
580	0.085
900	0.096
2000	0.106
6000	0.118
6000	0.123
6000	0.125
6000	0.127
6000	0.125
9000	0.139
9000	0.142
9000	0.144
9000	0.148
12000	0.163
12000	0.171
12000	0.175

Figure B-94. Glucose With Cd Absorbance vs. Cell Count 3



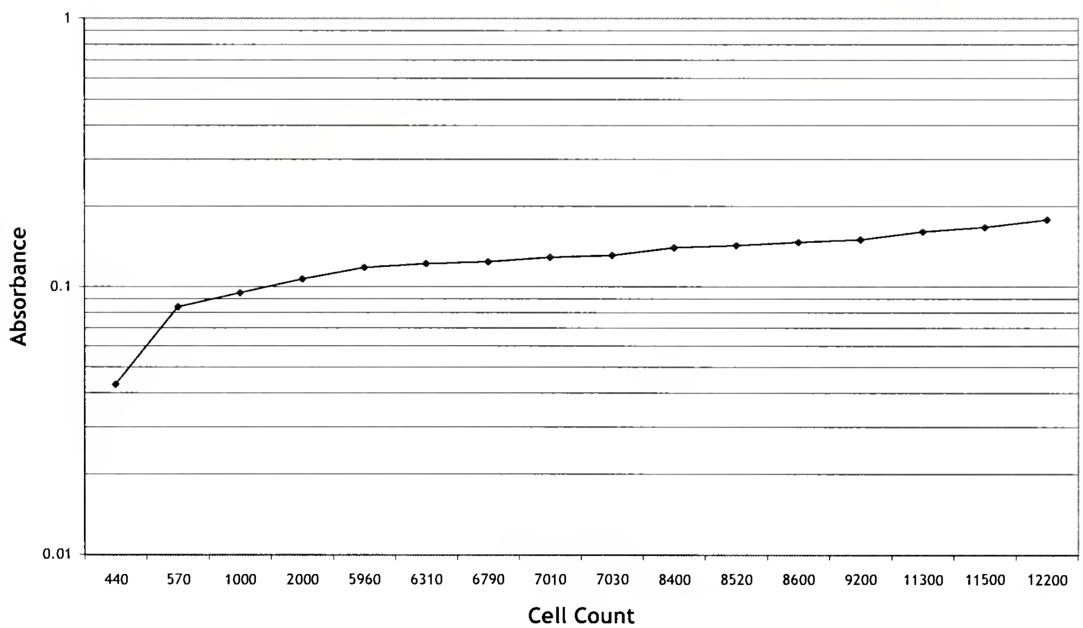
Cell Count increases from 6000 to 12000

Difference in absorbance = (0.163 -0.127) = 0.038

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
420	440	0.043	1.10	0.906
1080	570	0.084	1.21	0.824
1260	1000	0.095	1.24	0.804
1320	2000	0.107	1.28	0.782
1380	5960	0.118	1.31	0.761
1500	6310	0.122	1.32	0.755
1560	6790	0.124	1.33	0.752
1620	7010	0.129	1.34	0.744
1680	7030	0.131	1.35	0.739
1800	8400	0.140	1.38	0.724
1860	8520	0.143	1.39	0.719
1920	8600	0.147	1.40	0.713
1980	9200	0.150	1.41	0.707
2040	11300	0.161	1.45	0.691
2100	11500	0.167	1.47	0.680
2160	12200	0.178	1.51	0.664

Cell Count	Absorbance
440	0.043
570	0.084
1000	0.095
2000	0.107
5960	0.118
6310	0.122
6790	0.124
7010	0.129
7030	0.131
8400	0.14
8520	0.143
8600	0.147
9200	0.15
11300	0.161
11500	0.167
12200	0.178

Figure B-95. Average Glucose With Cd Absorbance vs. Cell Count



Cell Count increases from 5960 to 11500

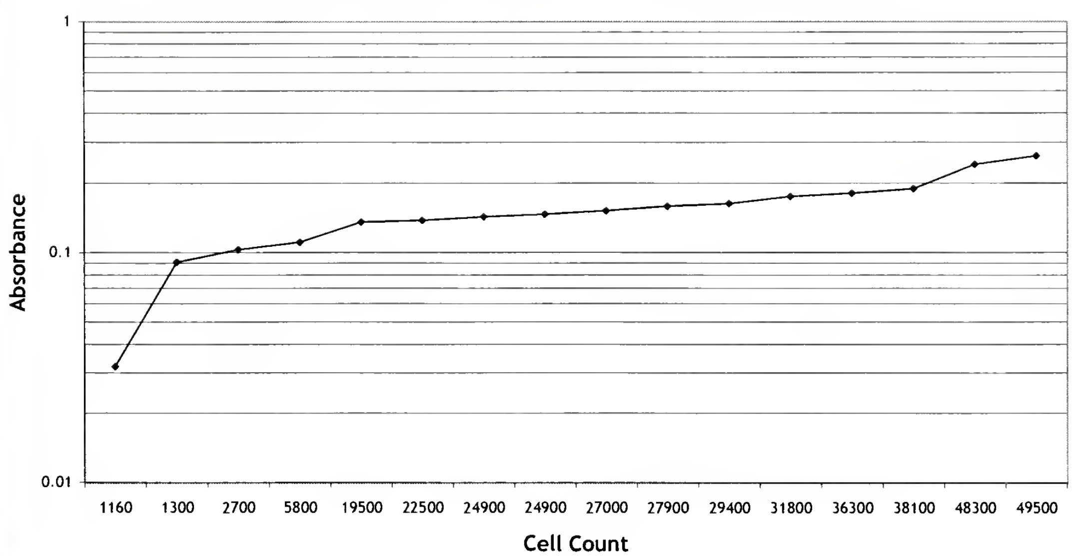
Difference in absorbance = (0.161 -0.118) = 0.043

GLUCOSE WITH Pb CELL COUNT

Time(min)	Cell Count 1	Absorbance	Antilog	Transmitance
420	1160	0.032	1.08	0.929
1080	1300	0.091	1.23	0.811
1260	2700	0.103	1.27	0.789
1320	5800	0.111	1.29	0.774
1380	19500	0.136	1.37	0.731
1500	22500	0.138	1.37	0.728
1560	24900	0.143	1.39	0.719
1620	24900	0.147	1.40	0.713
1680	27000	0.152	1.42	0.705
1800	27900	0.159	1.44	0.693
1860	29400	0.163	1.46	0.687
1920	31800	0.175	1.50	0.668
1980	36300	0.181	1.52	0.659
2040	38100	0.189	1.55	0.647
2100	48300	0.241	1.74	0.574
2160	49500	0.262	1.83	0.547

Cell Count	Absorbance
1160	0.032
1300	0.091
2700	0.103
5800	0.111
19500	0.136
22500	0.138
24900	0.143
24900	0.147
27000	0.152
27900	0.159
29400	0.163
31800	0.175
36300	0.181
38100	0.189
48300	0.241
49500	0.262

Figure B-96. Glucose With Pb Absorbance vs. Cell Count 1



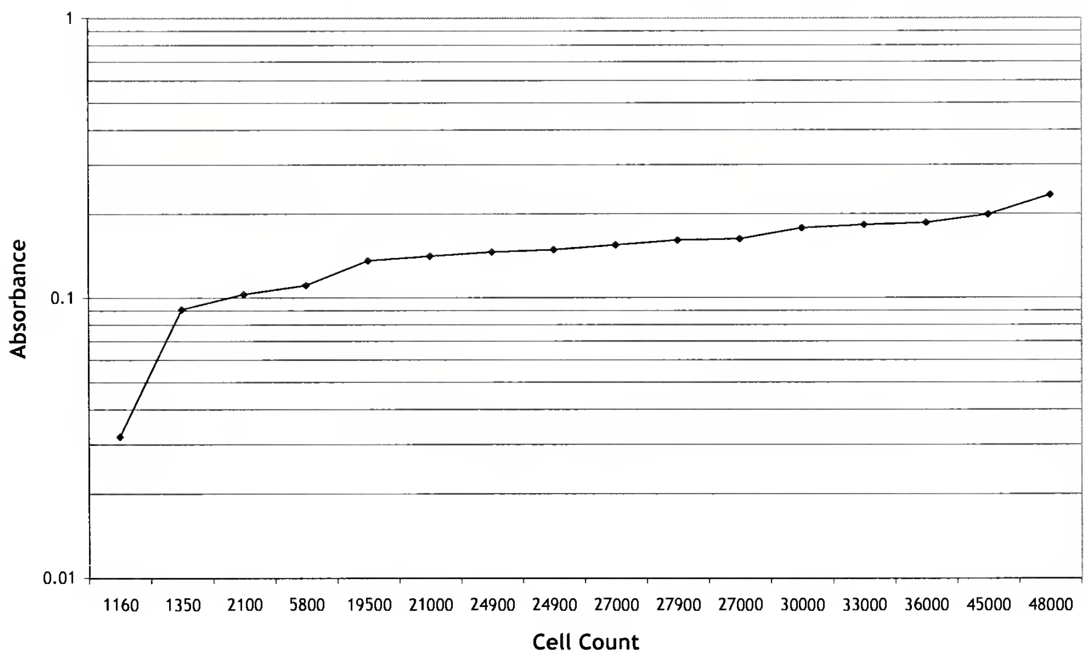
Cell Count increases from 19500 to 38100

Difference in absorbance = (0.169 -0.136) = 0.053

Time(min)	Cell Count 2	Absorbance	Antilog	Transmitance
420	1160	0.032	1.08	0.929
1080	1350	0.091	1.23	0.811
1260	2100	0.103	1.27	0.789
1320	5800	0.111	1.29	0.774
1380	19500	0.136	1.37	0.731
1500	21000	0.141	1.38	0.723
1560	24900	0.146	1.40	0.714
1620	24900	0.149	1.41	0.710
1680	27000	0.155	1.43	0.700
1800	27900	0.161	1.45	0.690
1860	27000	0.163	1.46	0.687
1920	30000	0.178	1.51	0.664
1980	33000	0.183	1.52	0.656
2040	36000	0.186	1.53	0.652
2100	45000	0.199	1.58	0.632
2160	48000	0.234	1.71	0.583

Cell Count	Absorbance
1160	0.032
1350	0.091
2100	0.103
5800	0.111
19500	0.136
21000	0.141
24900	0.146
24900	0.149
27000	0.155
27900	0.161
27000	0.163
30000	0.178
33000	0.183
36000	0.186
45000	0.199
48000	0.234

Figure B-97. Glucose With Pb Absorbance vs. Cell Count 2



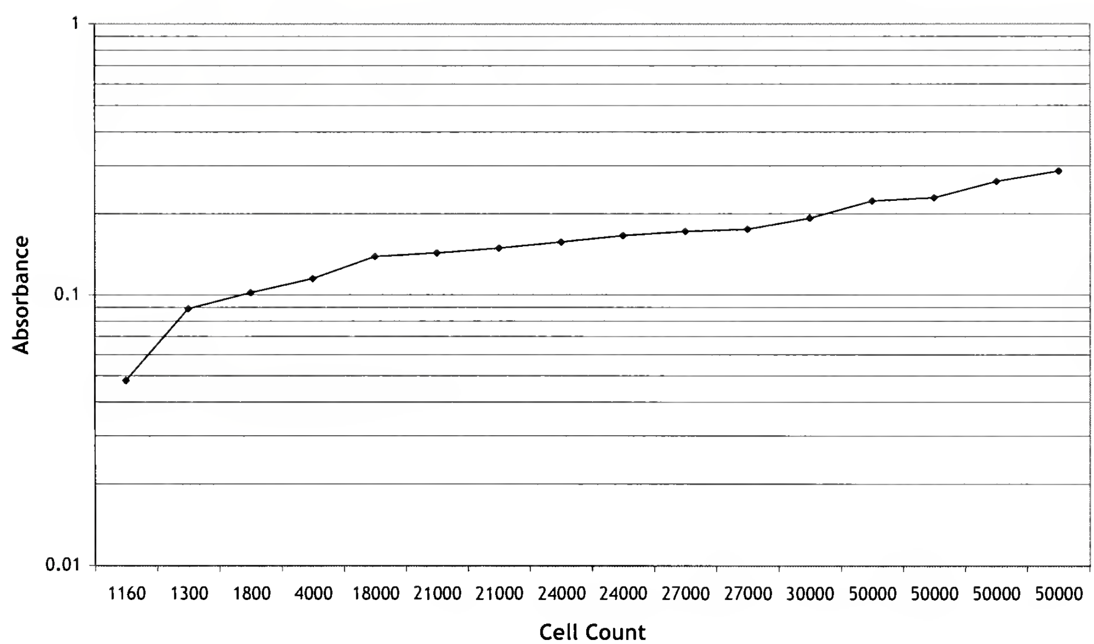
Cell Count increases from 21000 to 45000

Difference in absorbance = (0.199 -0.141) = 0.058

Time(min)	Cell Count 3	Absorbance	Antilog	Transmitance
420	1160	0.048	1.12	0.895
1080	1300	0.089	1.23	0.815
1260	1800	0.102	1.26	0.791
1320	4000	0.115	1.30	0.767
1380	18000	0.139	1.38	0.726
1500	21000	0.143	1.39	0.719
1560	21000	0.149	1.41	0.710
1620	24000	0.157	1.44	0.697
1680	24000	0.166	1.47	0.682
1800	27000	0.172	1.49	0.673
1860	27000	0.175	1.50	0.668
1920	30000	0.192	1.56	0.643
1980	50000	0.223	1.67	0.598
2040	50000	0.229	1.69	0.590
2100	50000	0.263	1.83	0.546
2160	50000	0.287	1.94	0.516

Cell Count	Absorbance
1160	0.048
1300	0.089
1800	0.102
4000	0.115
18000	0.139
21000	0.143
21000	0.149
24000	0.157
24000	0.166
27000	0.172
27000	0.175
30000	0.192
50000	0.223
50000	0.229
50000	0.263
50000	0.287

Figure B-98. Glucose With Pb Absorbance vs. Cell Count 3



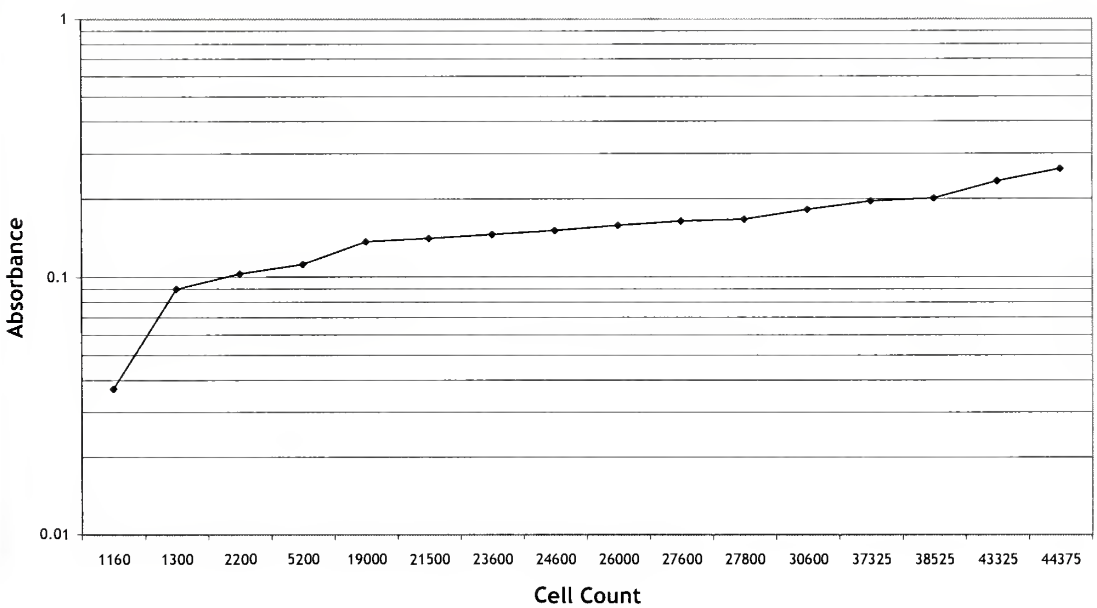
Cell Count increases from 24000 to 50000

Difference in absorbance = (0.223 -0.166) = 0.057

Time(min)	Average Cell Count	Average Absorbance	Antilog	Transmitance
420	1160	0.037	1.09	0.918
1080	1300	0.090	1.23	0.812
1260	2200	0.103	1.27	0.789
1320	5200	0.112	1.30	0.772
1380	19000	0.137	1.37	0.729
1500	21500	0.141	1.38	0.723
1560	23600	0.146	1.40	0.714
1620	24600	0.151	1.42	0.706
1680	26000	0.158	1.44	0.696
1800	27600	0.164	1.46	0.685
1860	27800	0.167	1.47	0.681
1920	30600	0.182	1.52	0.658
1980	37325	0.196	1.57	0.637
2040	38525	0.201	1.59	0.629
2100	43325	0.234	1.72	0.583
2160	44375	0.261	1.82	0.548

Cell Count	Absorbance
1160	0.037
1300	0.09
2200	0.103
5200	0.112
19000	0.137
21500	0.141
23600	0.146
24600	0.151
26000	0.158
27600	0.164
27800	0.167
30600	0.182
37325	0.196
38525	0.201
43325	0.234
44375	0.261

Figure B-99. Average Glucose With Pb Absorbance vs. Cell Count



Cell Count increases from 19000 to 38525

Difference in absorbance = (0.201 -0.137) = 0.064

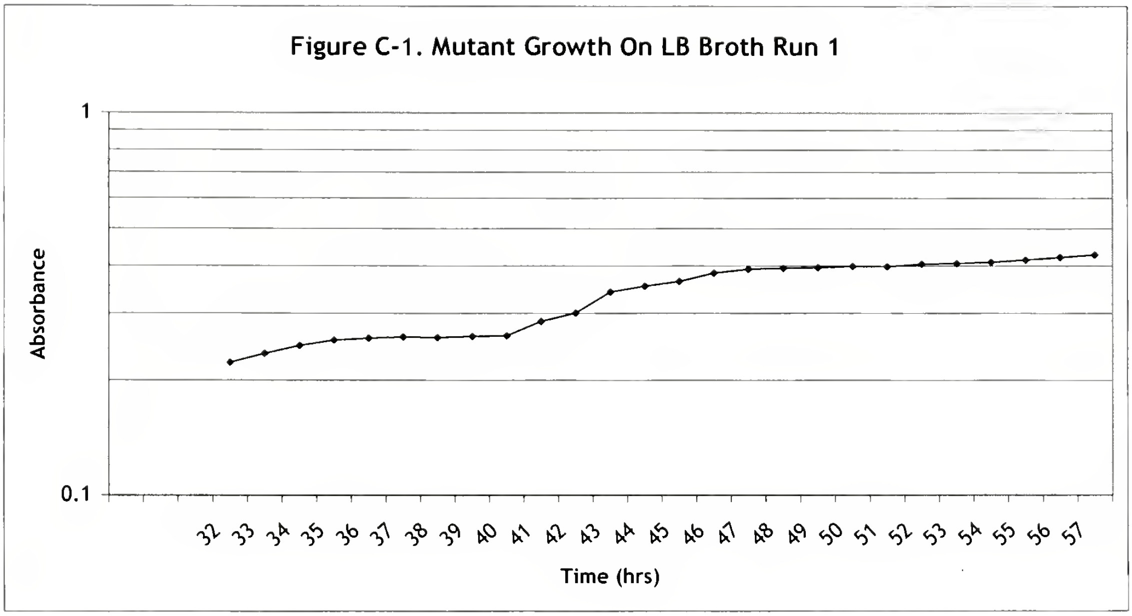
APPENDIX C

All of the doubling times for suspected mutant, donor and recepiet strains are presented here

MUTANT growth rates on LB broth

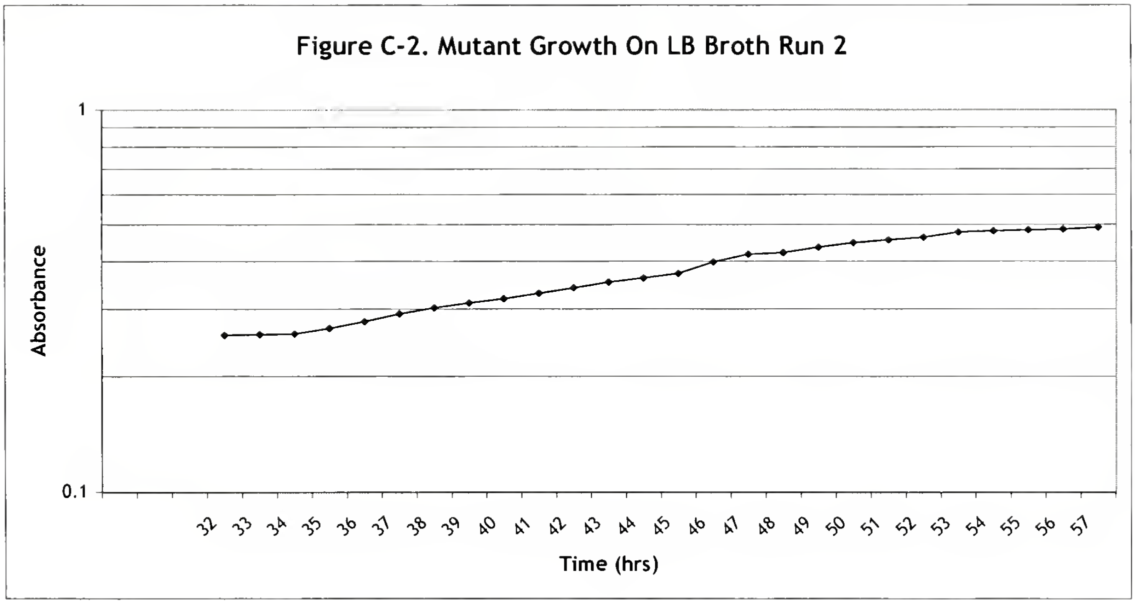
Time(mi	Time(hrs)	Run1	Run2	Run3	Average
0					
30					
60	1	0.021	0.019	0.018	0.019
120	2	0.032	0.024	0.029	0.028
180	3	0.041	0.038	0.037	0.039
240	4	0.047	0.052	0.045	0.048
300	5	0.054	0.059	0.051	0.055
360	6	0.056	0.063	0.062	0.060
420	7	0.064	0.071	0.068	0.068
480	8	0.075	0.076	0.079	0.077
540	9	0.085	0.079	0.086	0.083
600	10	0.090	0.084	0.097	0.090
660	11	0.091	0.089	0.098	0.093
720	12	0.096	0.102	0.101	0.100
780	13	0.101	0.104	0.105	0.103
840	14	0.107	0.107	0.111	0.108
900	15	0.113	0.109	0.123	0.115
960	16	0.122	0.114	0.132	0.123
1020	17	0.134	0.118	0.139	0.130
1080	18	0.139	0.129	0.147	0.138
1140	19	0.142	0.135	0.153	0.143
1200	20	0.149	0.148	0.159	0.152
1260	21	0.154	0.159	0.163	0.159
1320	22	0.171	0.163	0.168	0.167
1380	23	0.173	0.171	0.185	0.176
1440	24	0.178	0.183	0.189	0.183
1500	25	0.181	0.194	0.193	0.189
1560	26	0.185	0.216	0.212	0.204
1620	27	0.192	0.228	0.228	0.216
1680	28	0.196	0.244	0.231	0.224
1740	29	0.215	0.251	0.236	0.234
1800	30	0.217	0.255	0.244	0.239
1860	31	0.221	0.257	0.253	0.244

1920	32	0.223	0.256	0.255	0.245
1980	33	0.235	0.257	0.259	0.250
2040	34	0.247	0.258	0.261	0.255
2100	35	0.255	0.267	0.265	0.262
2160	36	0.258	0.278	0.269	0.268
2220	37	0.26	0.291	0.272	0.274
2280	38	0.259	0.302	0.278	0.280
2340	39	0.261	0.311	0.283	0.285
2400	40	0.262	0.319	0.299	0.293
2460	41	0.286	0.33	0.312	0.309
2520	42	0.301	0.341	0.314	0.319
2580	43	0.342	0.353	0.321	0.339
2640	44	0.354	0.362	0.331	0.349
2700	45	0.364	0.372	0.338	0.358
2760	46	0.383	0.399	0.34	0.374
2820	47	0.392	0.418	0.341	0.384
2880	48	0.394	0.422	0.352	0.389
2940	49	0.396	0.436	0.361	0.398
3000	50	0.399	0.448	0.375	0.407
3060	51	0.398	0.456	0.389	0.414
3120	52	0.404	0.463	0.396	0.421
3180	53	0.406	0.478	0.404	0.429
3240	54	0.409	0.481	0.412	0.434
3300	55	0.415	0.484	0.423	0.441
3360	56	0.421	0.486	0.436	0.448
3420	57	0.428	0.492	0.448	0.456



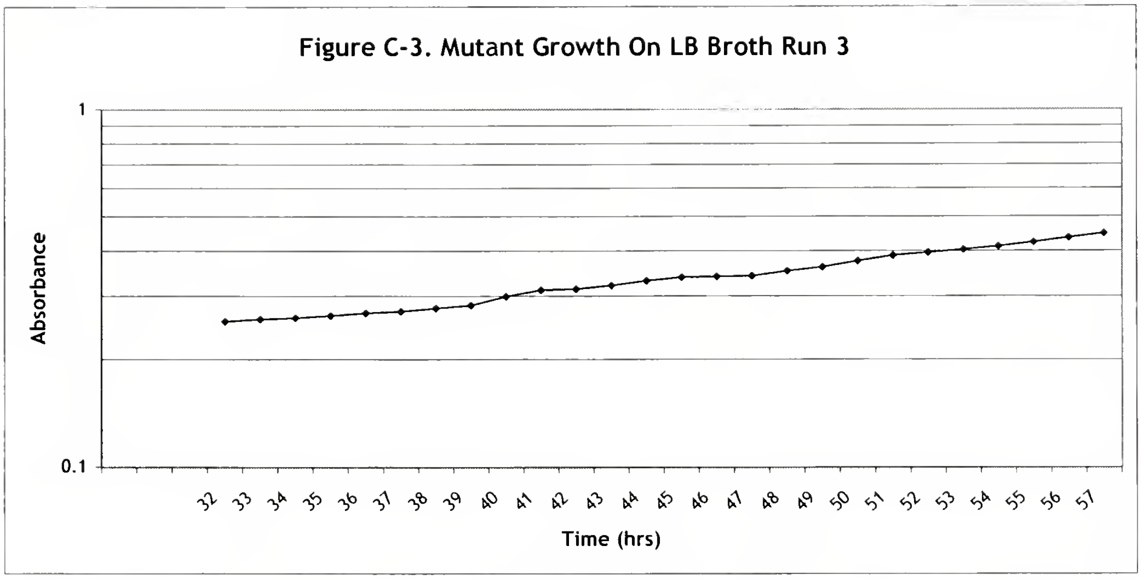
Absorbance increases from 0.107 to 0.215

Actual growth rate = **(29 hrs -14 hrs) = 15 hrs**



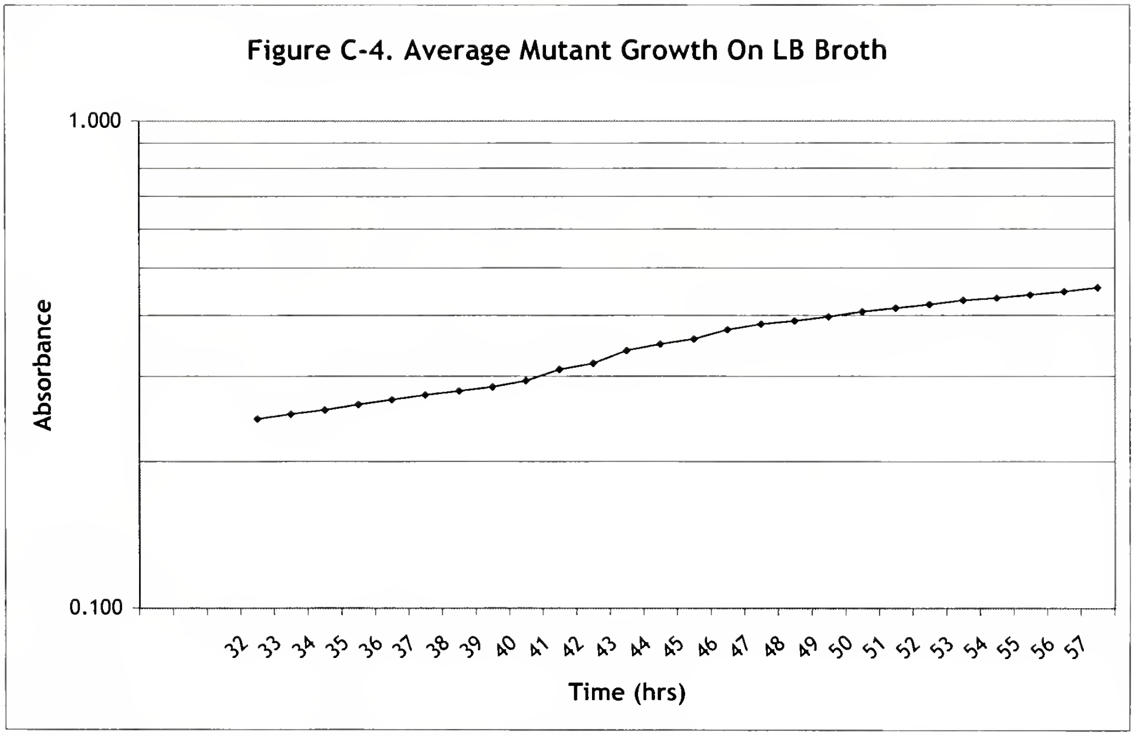
Absorbance increases from 0.109 to 0.216

Actual growth rate = **(26 hrs -15 hrs) = 11 hrs**



Absorbance increases from 0.105 to 0.212

Actual growth rate = (26 hrs -13 hrs) = 13 hrs



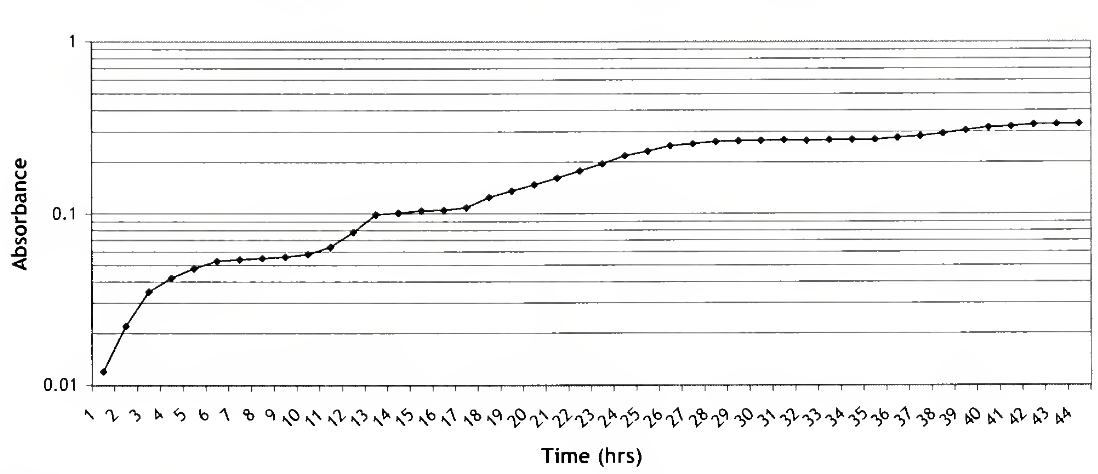
Absorbance increases from 0.108 to 0.216

Actual growth rate = (27 hrs -14 hrs) = 13 hrs

MUTANT growth rates on LB broth with Rif and Tc

Time(min)	Time(hrs)	Run1	Run2	Run3	Average
0					
30					
60	1	0.012	0.011	0.009	0.011
120	2	0.022	0.024	0.018	0.021
180	3	0.035	0.032	0.028	0.032
240	4	0.042	0.048	0.056	0.049
300	5	0.048	0.054	0.063	0.055
360	6	0.053	0.059	0.072	0.061
420	7	0.054	0.063	0.084	0.067
480	8	0.055	0.069	0.090	0.071
540	9	0.056	0.073	0.092	0.074
600	10	0.058	0.084	0.097	0.080
660	11	0.064	0.095	0.102	0.087
720	12	0.078	0.098	0.112	0.096
780	13	0.099	0.099	0.120	0.106
840	14	0.101	0.103	0.129	0.111
900	15	0.104	0.104	0.138	0.115
960	16	0.105	0.106	0.143	0.118
1020	17	0.109	0.108	0.152	0.123
1080	18	0.125	0.128	0.167	0.140
1140	19	0.136	0.136	0.171	0.148
1200	20	0.148	0.143	0.179	0.157
1260	21	0.162	0.151	0.186	0.166
1320	22	0.178	0.158	0.198	0.178
1380	23	0.196	0.167	0.243	0.202
1440	24	0.218	0.175	0.279	0.224
1500	25	0.232	0.189	0.282	0.234
1560	26	0.249	0.201	0.288	0.246
1620	27	0.256	0.215	0.293	0.255
1680	28	0.264	0.245	0.297	0.269
1740	29	0.266	0.264	0.301	0.277
1800	30	0.267	0.297	0.307	0.290
1860	31	0.269	0.303	0.309	0.294
1920	32	0.267	0.308	0.313	0.296
1980	33	0.269	0.311	0.311	0.297
2040	34	0.27	0.313	0.315	0.299
2100	35	0.271	0.318	0.317	0.302
2160	36	0.278	0.321	0.318	0.306
2220	37	0.284	0.326	0.331	0.314
2280	38	0.293	0.328	0.346	0.322
2340	39	0.306	0.336	0.358	0.333
2400	40	0.318	0.342	0.372	0.344
2460	41	0.323	0.349	0.382	0.351
2520	42	0.331	0.351	0.387	0.356
2580	43	0.332	0.353	0.398	0.361
2640	44	0.334	0.354	0.401	0.363

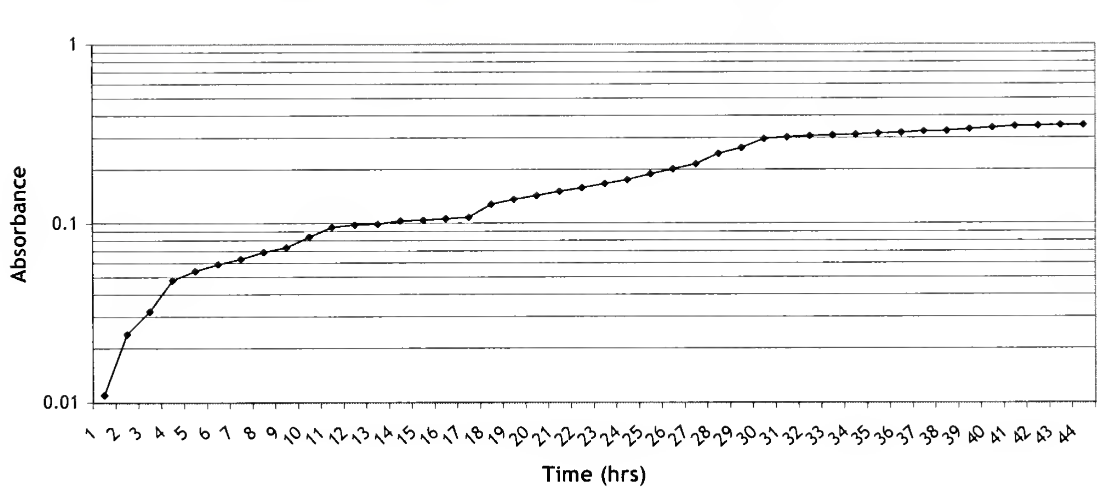
Figure C-5. Mutant Growth with Rf and Tc Run 1



Absorbance increases from 0.109 to 0.218

Actual growth rate = **(24 hrs -17 hrs) = 7 hrs**

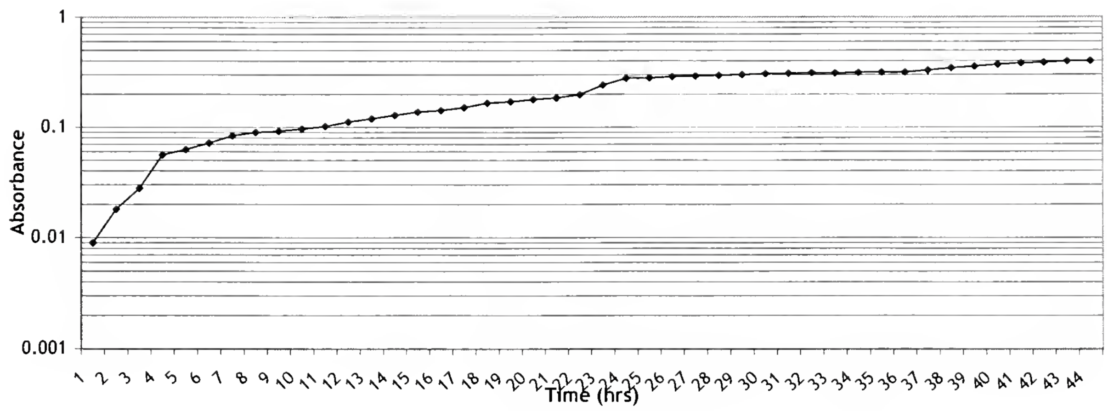
Figure C-6. Mutant Growth with Rf and Tc Run 2



Absorbance increases from 0.108 to 0.215

Actual growth rate = **(27 hrs -17 hrs) = 10 hrs**

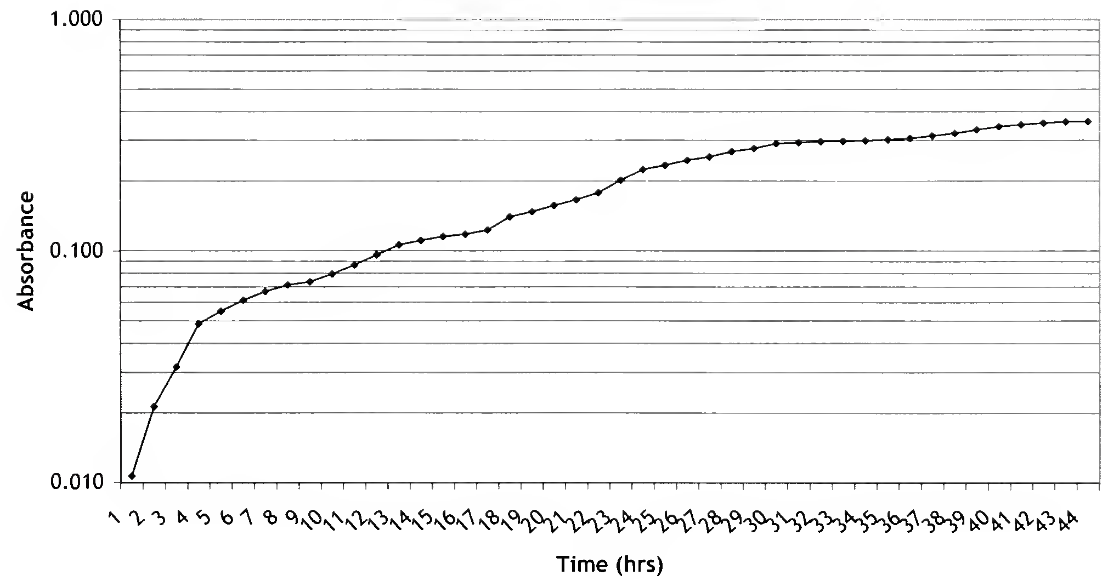
Figure C-7. Mutant Growth with Rf and Tc Run 3



Absorbance increases from 0.120 to 0.243

Actual growth rate = (23 hrs -13 hrs) = 10 hrs

Figure C-8. Average Mutant Growth with Rf and Tc

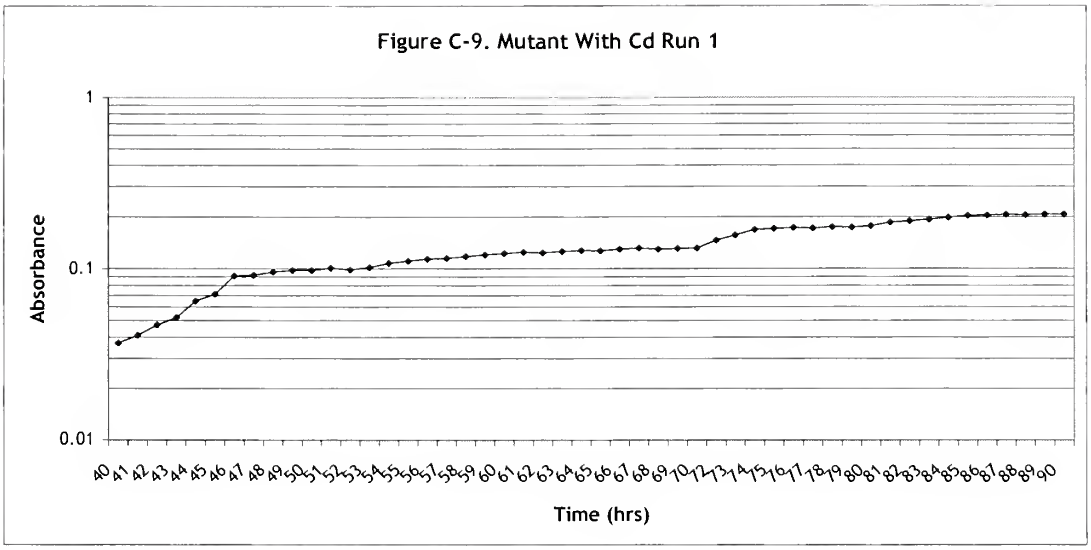


Absorbance increases from 0.111 to 0.224

Actual growth rate = (24 hrs -14 hrs) = 10 hrs

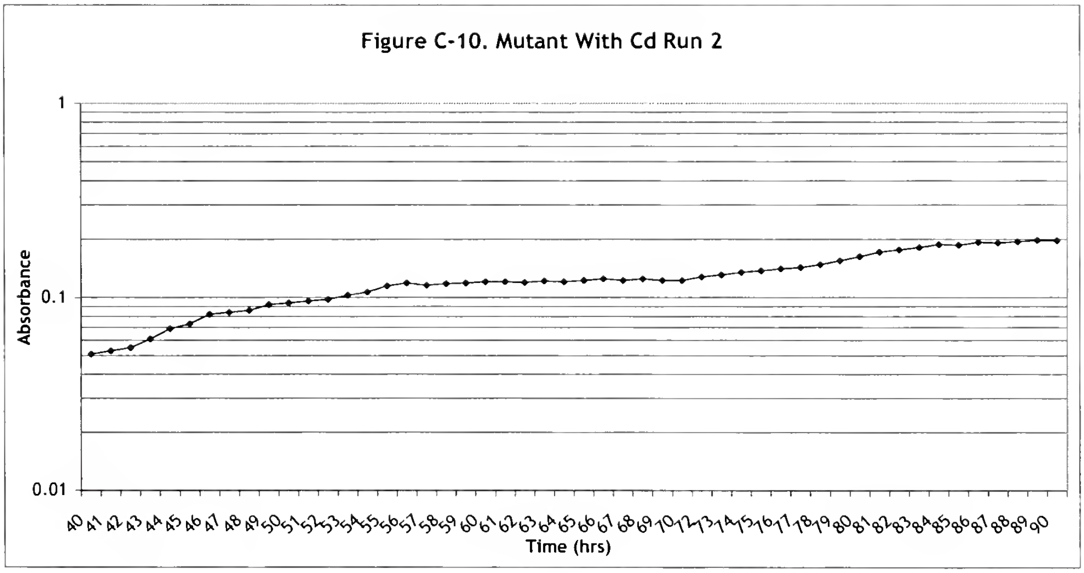
MUTANT growth rates with Cd

$50*10^{-6}$ M $CdCl_2$				
Time(hrs)	Run1	Run2	Run3	Average
40	0.037	0.051	0.052	0.047
41	0.041	0.053	0.06	0.051
42	0.047	0.055	0.062	0.055
43	0.052	0.061	0.069	0.061
44	0.065	0.069	0.075	0.070
45	0.071	0.073	0.077	0.074
46	0.091	0.082	0.079	0.084
47	0.092	0.084	0.083	0.086
48	0.096	0.086	0.088	0.090
49	0.098	0.092	0.086	0.092
50	0.098	0.094	0.091	0.094
51	0.101	0.096	0.095	0.097
52	0.099	0.098	0.096	0.098
53	0.102	0.103	0.098	0.101
54	0.108	0.107	0.101	0.105
55	0.111	0.115	0.104	0.110
56	0.114	0.119	0.106	0.113
57	0.115	0.116	0.11	0.114
58	0.118	0.118	0.115	0.117
59	0.121	0.119	0.117	0.119
60	0.123	0.121	0.123	0.122
61	0.125	0.121	0.124	0.123
62	0.124	0.12	0.126	0.123
63	0.126	0.122	0.129	0.126
64	0.128	0.121	0.128	0.126
65	0.127	0.123	0.134	0.128
66	0.13	0.125	0.136	0.130
67	0.132	0.123	0.139	0.131
68	0.13	0.125	0.141	0.132
69	0.131	0.123	0.143	0.132
70	0.132	0.123	0.144	0.133
72	0.146	0.128	0.152	0.142
73	0.157	0.131	0.158	0.149
74	0.169	0.135	0.163	0.156
75	0.171	0.138	0.165	0.158
76	0.173	0.141	0.168	0.161
77	0.172	0.143	0.173	0.163
78	0.175	0.148	0.177	0.167
79	0.174	0.155	0.179	0.169
80	0.177	0.163	0.183	0.174
81	0.186	0.172	0.186	0.181
82	0.189	0.177	0.189	0.185
83	0.193	0.182	0.188	0.188
84	0.198	0.188	0.194	0.193
85	0.203	0.187	0.198	0.196
86	0.204	0.193	0.206	0.201
87	0.206	0.192	0.209	0.202
88	0.205	0.195	0.211	0.204
89	0.207	0.198	0.213	0.206
90	0.207	0.197	0.215	0.206



Absorbance increases from 0.102 to 0.204

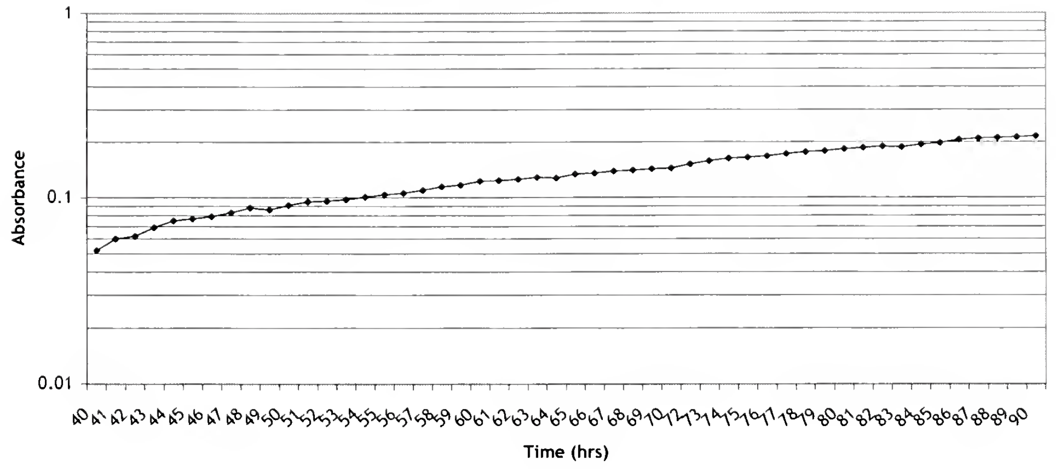
Actual growth rate = **(86 hrs -53 hrs) = 33 hrs**



Absorbance increases from 0.096 to 0.192

Actual growth rate = **(87 hrs -51 hrs) = 36 hrs**

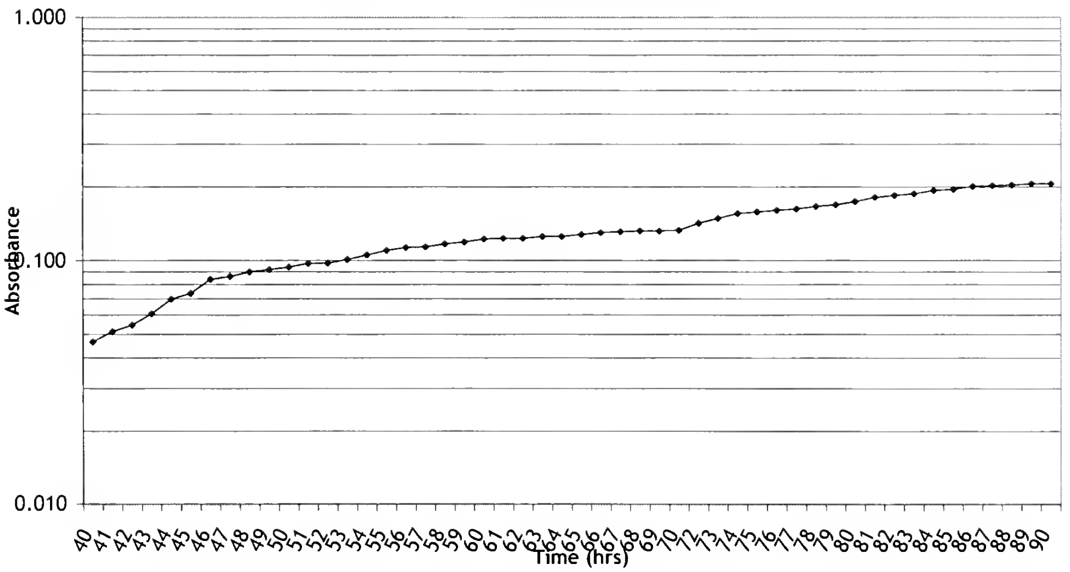
Figure C-11. Mutant With Cd Run 3



Absorbance increases from 0.104 to 0.209

Actual growth rate = **(87 hrs -55 hrs) = 32 hrs**

Figure C-12. Average Mutant With Cd



Absorbance increases from 0.101 to 0.202

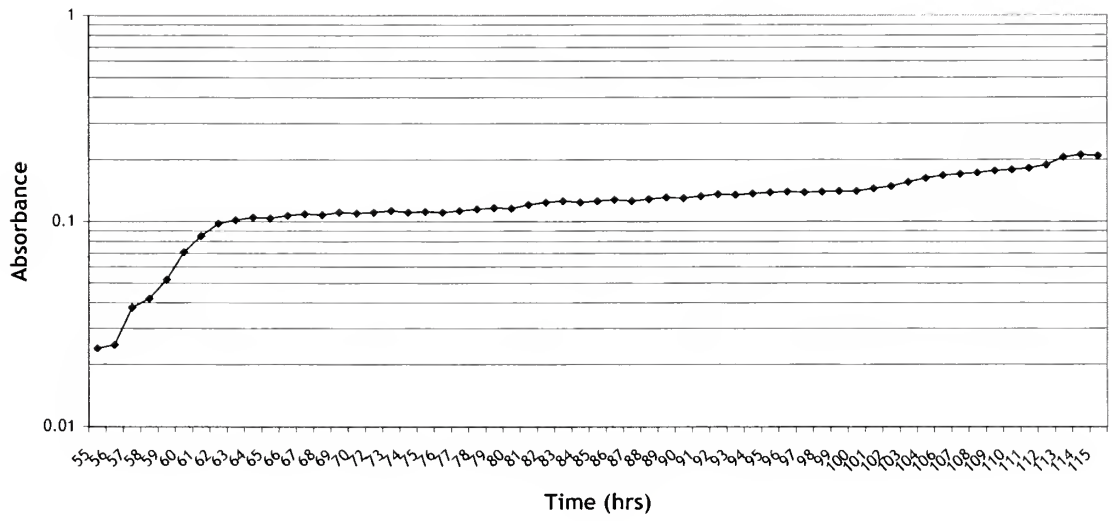
Actual growth rate = **(87 hrs -53 hrs) = 34 hrs**

MUTANT growth rates with Cd

$100*10^{-6}$ M $CdCl_2$				
Time(hrs)	Run1	Run2	Run3	Average
55	0.024	0.026	0.021	0.024
56	0.025	0.028	0.028	0.027
57	0.038	0.032	0.035	0.035
58	0.042	0.039	0.039	0.040
59	0.052	0.042	0.042	0.045
60	0.071	0.056	0.063	0.063
61	0.085	0.075	0.075	0.078
62	0.098	0.085	0.085	0.089
63	0.102	0.092	0.093	0.096
64	0.105	0.095	0.102	0.101
65	0.104	0.103	0.105	0.104
66	0.107	0.11	0.109	0.109
67	0.109	0.113	0.108	0.110
68	0.108	0.116	0.108	0.111
69	0.111	0.117	0.11	0.113
70	0.11	0.118	0.112	0.113
72	0.111	0.119	0.111	0.114
73	0.113	0.121	0.113	0.116
74	0.111	0.124	0.114	0.116
75	0.112	0.129	0.115	0.119
76	0.111	0.135	0.114	0.120
77	0.113	0.136	0.115	0.121
78	0.115	0.139	0.116	0.123
79	0.117	0.138	0.118	0.124
80	0.116	0.144	0.119	0.126
81	0.121	0.149	0.117	0.129
82	0.124	0.15	0.12	0.131
83	0.126	0.152	0.122	0.133
84	0.124	0.151	0.123	0.133
85	0.126	0.153	0.124	0.134
86	0.128	0.155	0.128	0.137
87	0.126	0.154	0.13	0.137
88	0.129	0.156	0.129	0.138
89	0.131	0.159	0.131	0.140
90	0.13	0.158	0.135	0.141
91	0.133	0.157	0.137	0.142
92	0.136	0.159	0.139	0.145
93	0.135	0.161	0.141	0.146

94	0.137	0.161	0.143	0.147
95	0.139	0.16	0.147	0.149
96	0.14	0.162	0.151	0.151
97	0.139	0.163	0.154	0.152
98	0.14	0.162	0.156	0.153
99	0.141	0.162	0.155	0.153
100	0.141	0.165	0.158	0.155
101	0.145	0.164	0.163	0.157
102	0.149	0.168	0.168	0.162
103	0.156	0.169	0.167	0.164
104	0.163	0.176	0.174	0.171
106	0.168	0.181	0.178	0.176
107	0.171	0.186	0.181	0.179
108	0.173	0.189	0.184	0.182
109	0.177	0.192	0.188	0.186
110	0.179	0.195	0.193	0.189
111	0.182	0.198	0.195	0.192
112	0.189	0.213	0.198	0.200
113	0.206	0.217	0.211	0.211
114	0.211	0.22	0.218	0.216
115	0.209	0.221	0.216	0.215

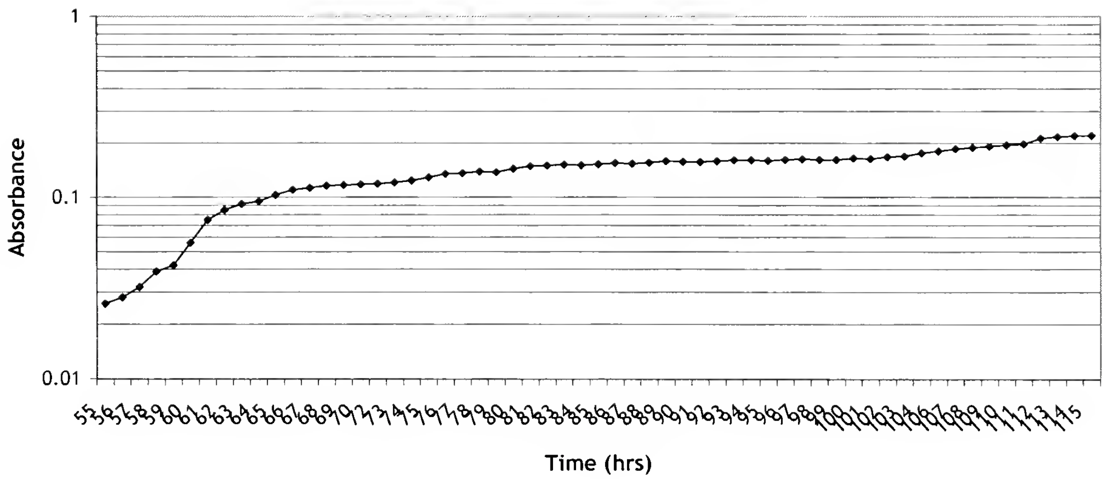
Figure C-13. Mutant With Cd Run 1



Absorbance increases from 0.105 to 0.211

Actual growth rate = (114 hrs -64 hrs) = 50 hrs

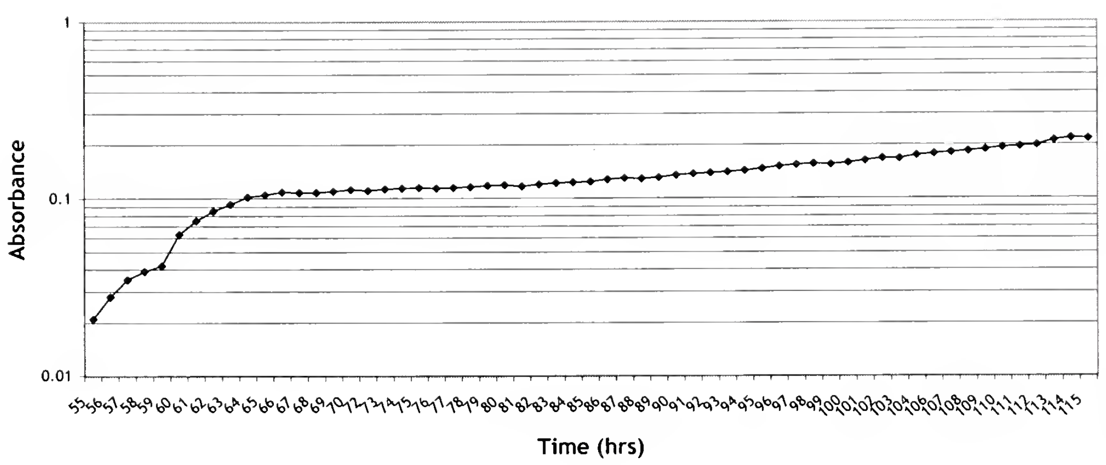
Figure C-14. Mutant With Cd Run 2



Absorbance increases from 0.111 to 0.221

Actual growth rate = (115 hrs -66 hrs) = 49 hrs

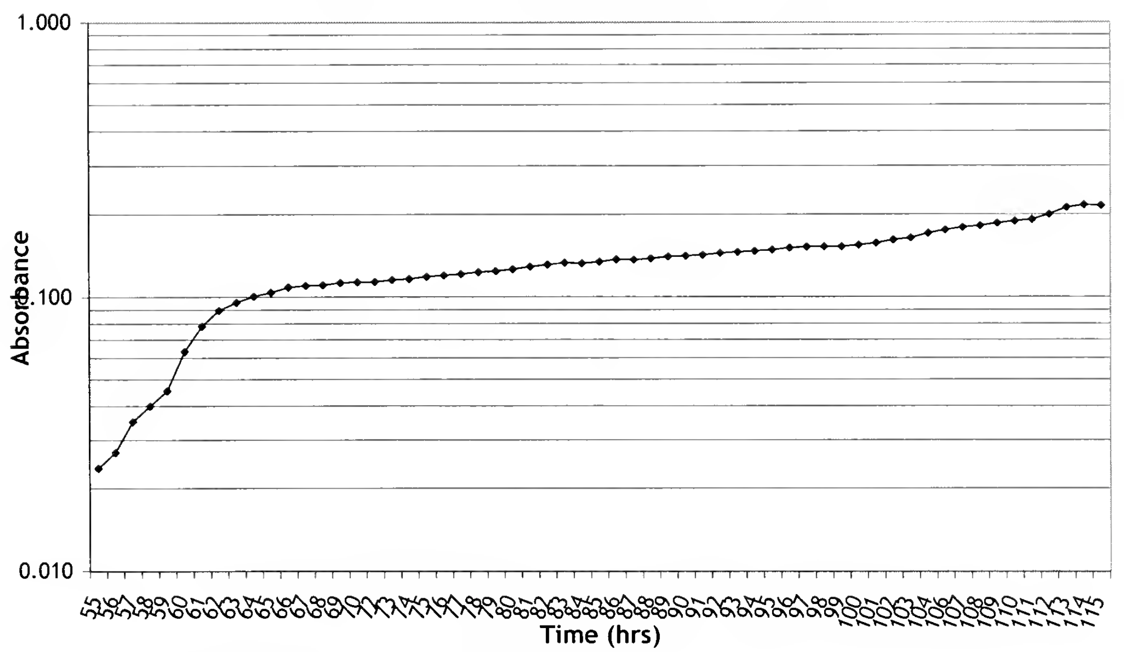
Figure C-15. Mutant With Cd Run 3



Absorbance increases from 0.105 to 0.211

Actual growth rate = **(113 hrs -65 hrs) = 48 hrs**

Figure C-16. Average Mutant With Cd

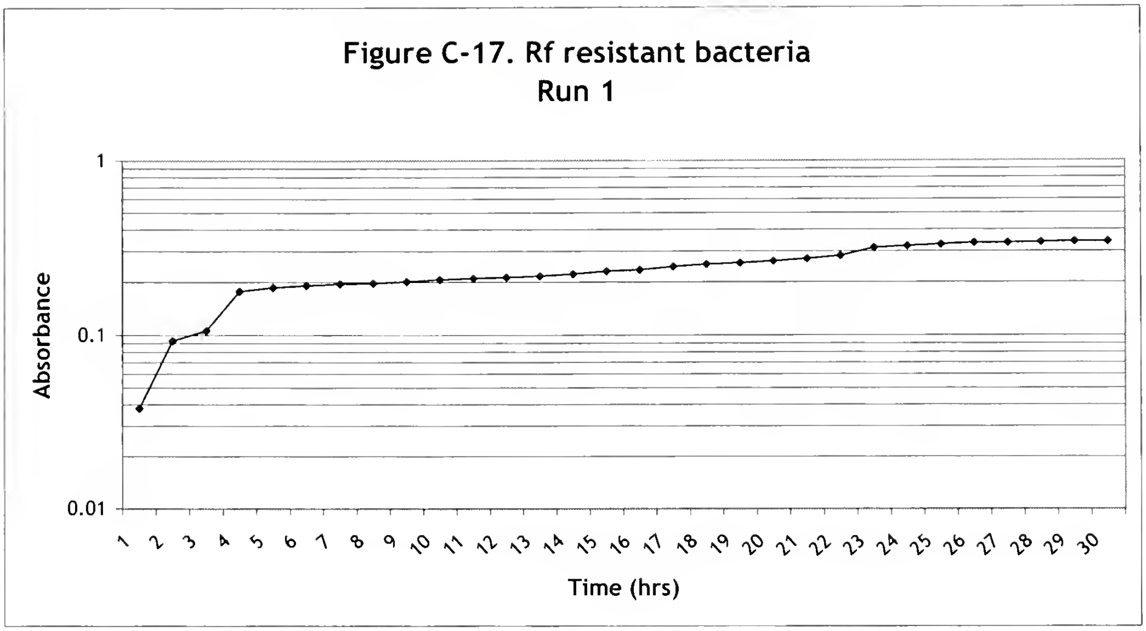


Absorbance increases from 0.109 to 0.216

Actual growth rate = **(114 hrs -66 hrs) = 48 hrs**

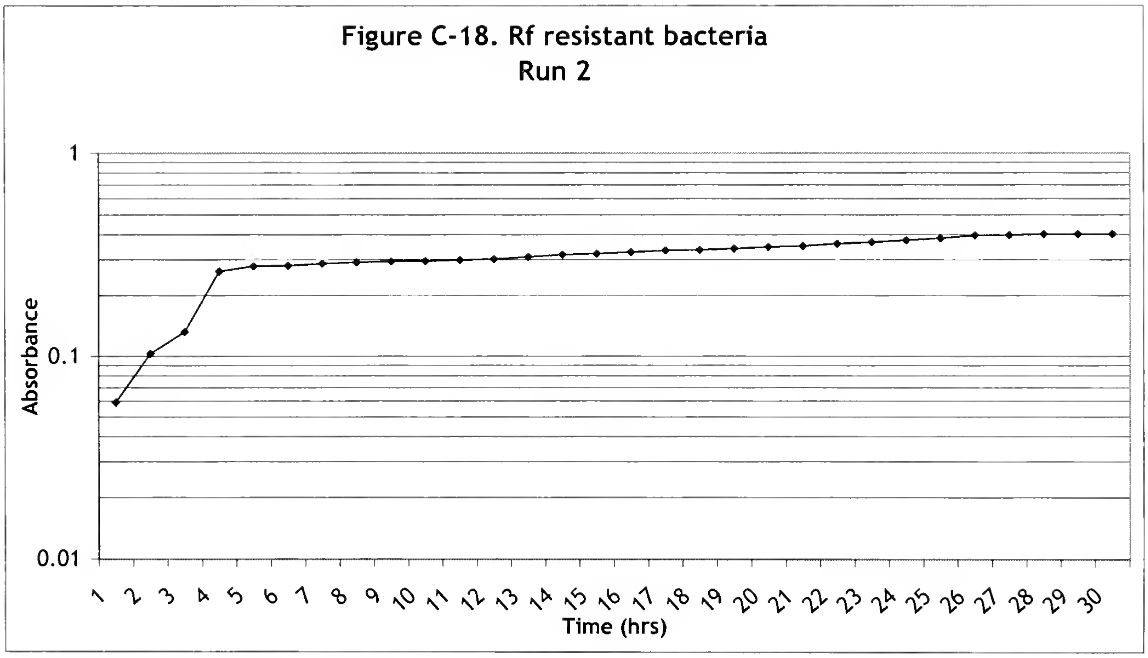
Recipient (Rif resistant *E.coli*) growth rates on LB broth + Rf

Time(min)	Time(hrs)	Run1	Run2	Run3	Average
0					
30					
60	1	0.038	0.059	0.056	0.051
120	2	0.093	0.103	0.099	0.098
180	3	0.106	0.132	0.128	0.122
240	4	0.178	0.263	0.196	0.212
300	5	0.187	0.279	0.236	0.234
360	6	0.192	0.281	0.255	0.243
420	7	0.196	0.288	0.268	0.251
480	8	0.198	0.292	0.275	0.255
540	9	0.202	0.295	0.279	0.259
600	10	0.207	0.296	0.283	0.262
660	11	0.211	0.299	0.291	0.267
720	12	0.214	0.303	0.298	0.272
780	13	0.217	0.311	0.307	0.278
840	14	0.223	0.319	0.314	0.285
900	15	0.232	0.322	0.317	0.290
960	16	0.236	0.328	0.321	0.295
1020	17	0.246	0.334	0.325	0.302
1080	18	0.253	0.337	0.329	0.306
1140	19	0.258	0.341	0.331	0.310
1200	20	0.264	0.348	0.334	0.315
1260	21	0.272	0.352	0.338	0.321
1320	22	0.283	0.361	0.345	0.330
1380	23	0.315	0.367	0.351	0.344
1440	24	0.321	0.376	0.359	0.352
1500	25	0.328	0.384	0.371	0.361
1560	26	0.335	0.396	0.383	0.371
1620	27	0.336	0.398	0.385	0.373
1680	28	0.339	0.402	0.389	0.377
1740	29	0.341	0.403	0.393	0.379
1800	30	0.342	0.402	0.393	0.379



Absorbance increases from 0.093 to 0.187

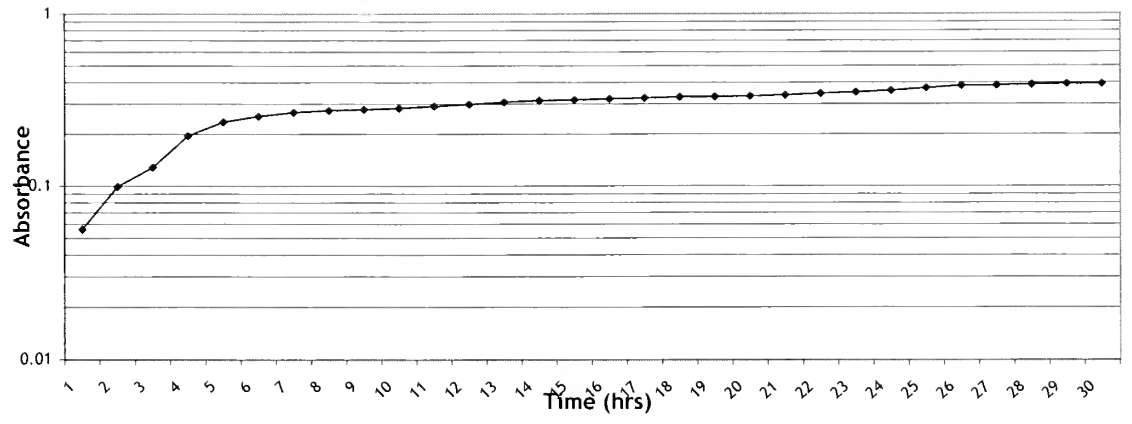
Actual growth rate = **(5 hrs -2 hrs) = 3 hrs**



Absorbance increases from 0.132 to 0.263

Actual growth rate = **(4 hrs -3 hrs) = 1 hrs**

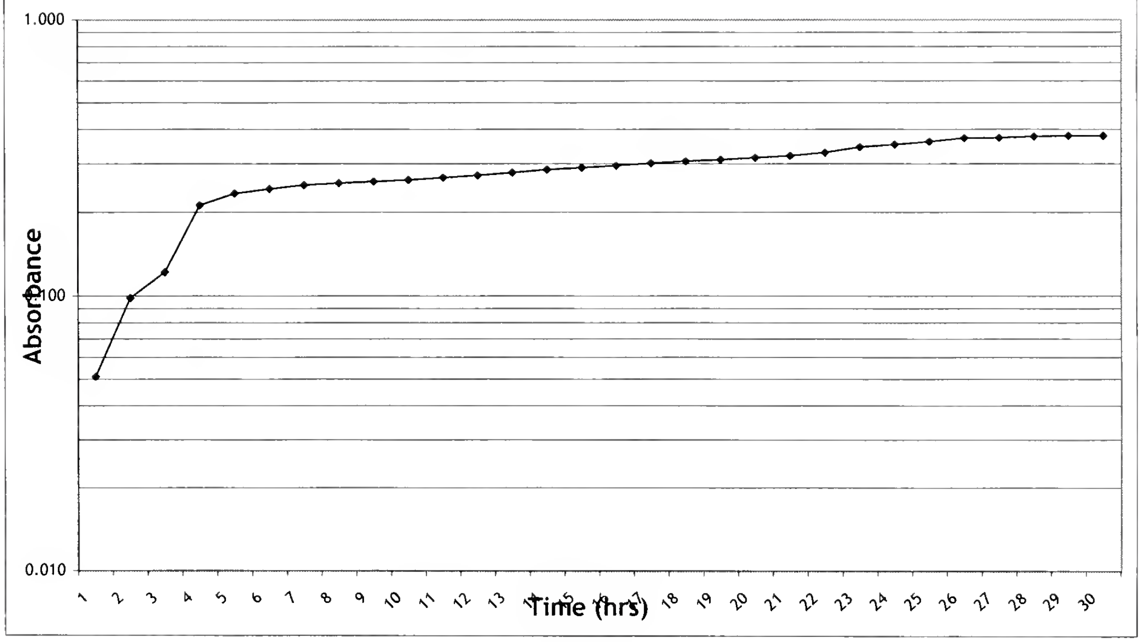
Figure C-19. Rf resistant bacteria
Run 3



Absorbance increases from 0.128 to 0.255

Actual growth rate = (6 hrs -3 hrs) = 3 hrs

Figure C-20. Rf resistant bacteria
Average run

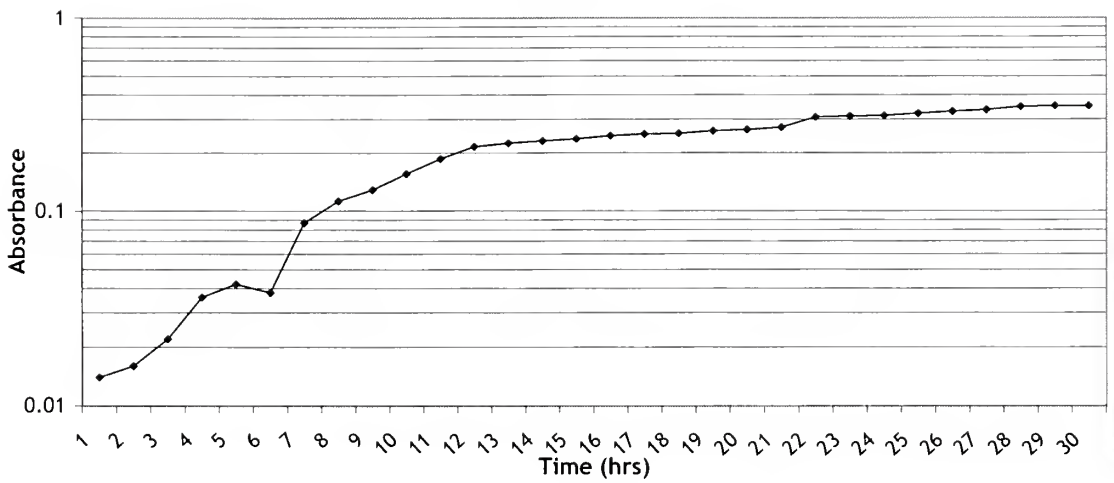


Absorbance increases from 0.122 to 0.243
Actual growth rate = (6 hrs -3 hrs) = 3 hrs

Recipient (Rif resistant *E.coli*) growth rates on LB broth + Rf + Cd

Time(min)	Time(hrs)	Run1	Run2	Run3	Average
0					
30					
60	1	0.014	0.008	0.008	0.010
120	2	0.016	0.01	0.009	0.012
180	3	0.022	0.012	0.014	0.016
240	4	0.036	0.011	0.018	0.022
300	5	0.042	0.018	0.026	0.029
360	6	0.038	0.023	0.059	0.040
420	7	0.087	0.025	0.091	0.068
480	8	0.113	0.057	0.116	0.095
540	9	0.129	0.122	0.121	0.124
600	10	0.156	0.145	0.151	0.151
660	11	0.187	0.169	0.188	0.181
720	12	0.216	0.193	0.192	0.200
780	13	0.226	0.208	0.218	0.217
840	14	0.232	0.249	0.235	0.239
900	15	0.237	0.254	0.246	0.246
960	16	0.246	0.256	0.252	0.251
1020	17	0.251	0.259	0.255	0.255
1080	18	0.253	0.261	0.261	0.258
1140	19	0.261	0.266	0.269	0.265
1200	20	0.265	0.273	0.282	0.273
1260	21	0.272	0.275	0.287	0.278
1320	22	0.308	0.282	0.296	0.295
1380	23	0.311	0.303	0.312	0.309
1440	24	0.313	0.315	0.325	0.318
1500	25	0.322	0.324	0.332	0.326
1560	26	0.329	0.334	0.338	0.334
1620	27	0.336	0.338	0.348	0.341
1680	28	0.349	0.341	0.351	0.347
1740	29	0.352	0.349	0.349	0.350
1800	30	0.351	0.347	0.353	0.350

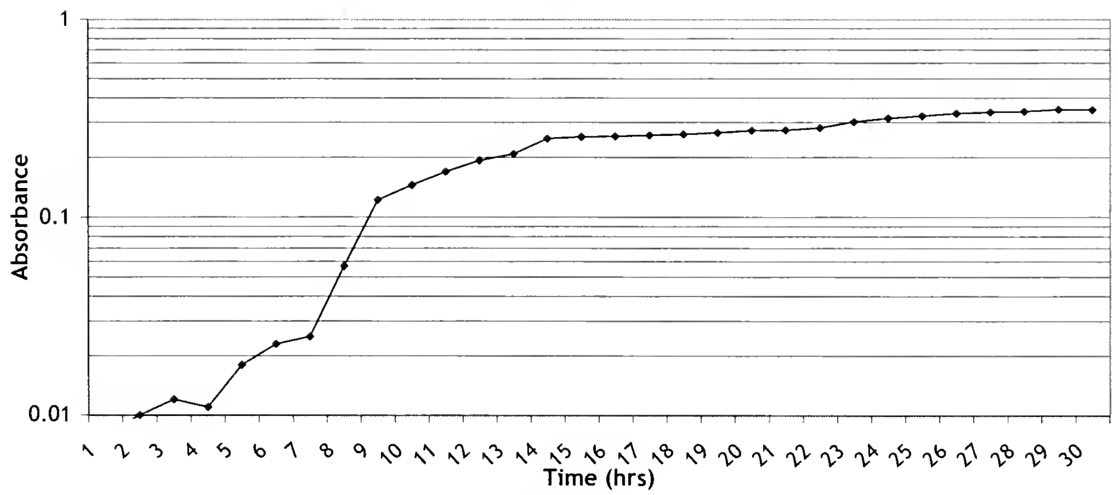
Figure C-21. Rf resistant bacteria + Cd
Run 1



Absorbance increases from 0.113 to 0.226

Actual growth rate = **(13 hrs -8 hrs) = 5 hrs**

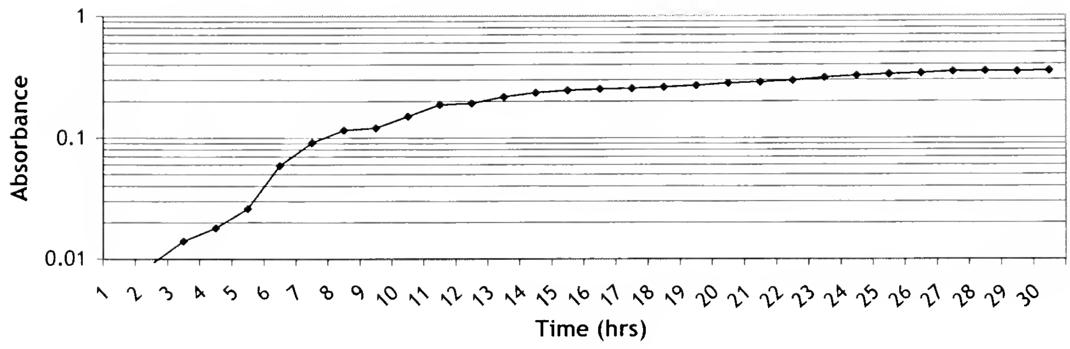
Figure C-22. Rf resistant bacteria + Cd
Run 2



Absorbance increases from 0.122 to 0.249

Actual growth rate = **(14 hrs -9 hrs) = 5 hrs**

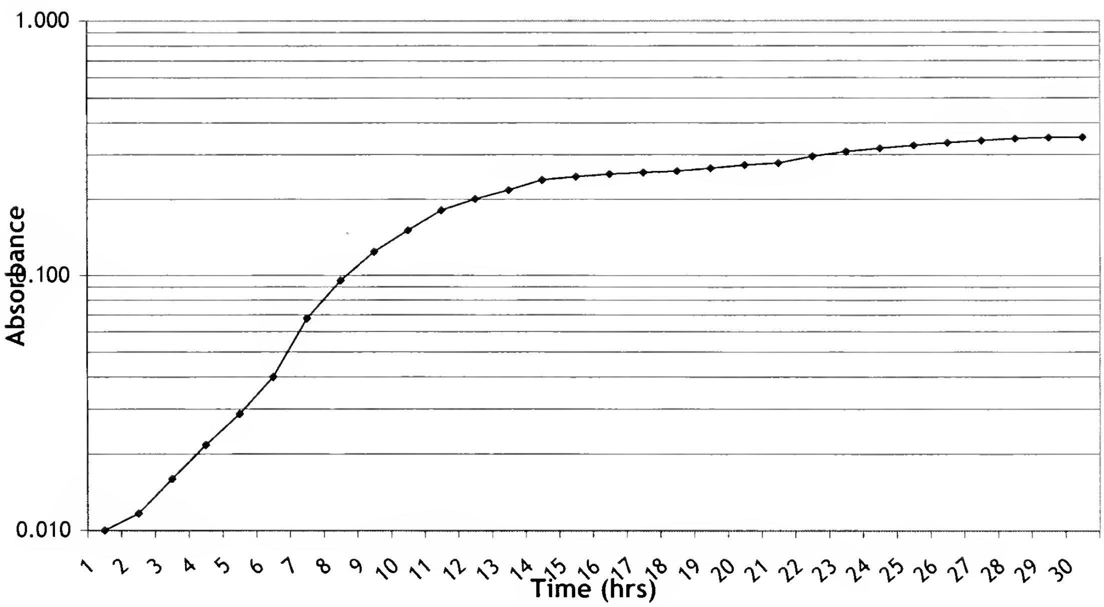
Figure C-23. Rf resistant bacteria + Cd
Run 3



Absorbance increases from 0.116 to 0.235

Actual growth rate = **(14 hrs -8 hrs) = 6 hrs**

Figure C-24. Rf resistant bacteria + Cd
Average Run

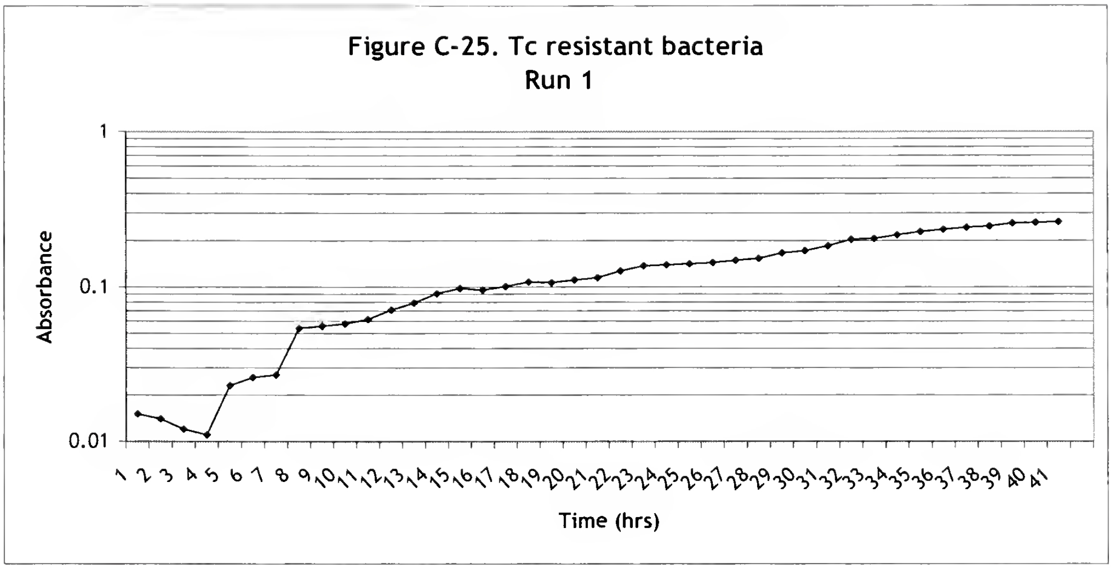


Absorbance increases from 0.124 to 0.246

Actual growth rate = **(15 hrs -9 hrs) = 6 hrs**

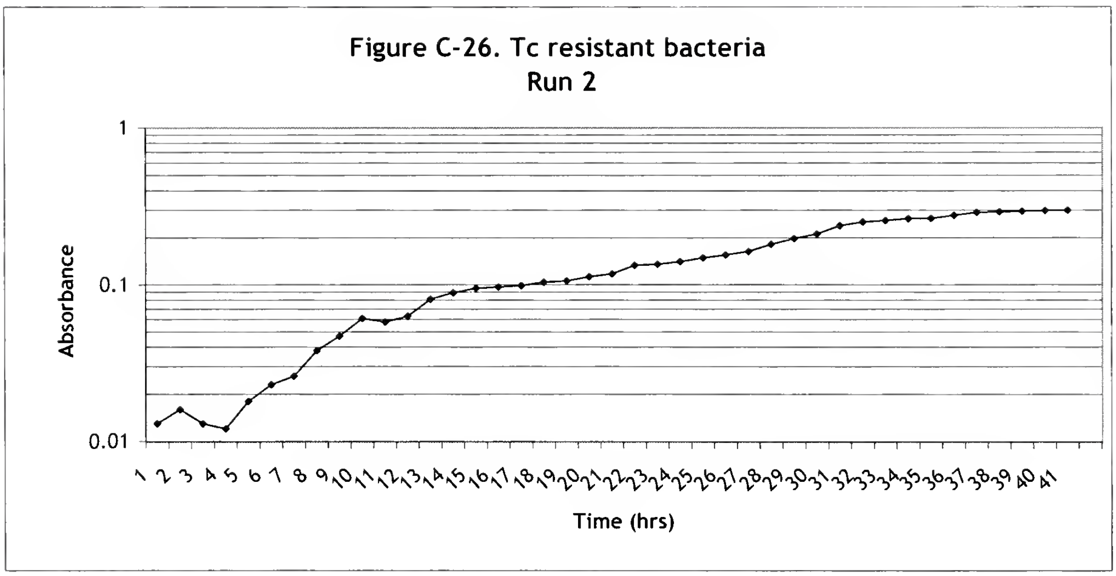
Donor (Tc resistant *E.coli*) growth rate on LB broth + Tc

Time(min)	Time(hrs)	Run1	Run2	Run3	Average
0					
30					
60	1	0.015	0.013	0.018	0.015
120	2	0.014	0.016	0.021	0.017
180	3	0.012	0.013	0.017	0.014
240	4	0.011	0.012	0.014	0.012
300	5	0.023	0.018	0.021	0.021
360	6	0.026	0.023	0.025	0.025
420	7	0.027	0.026	0.024	0.026
480	8	0.054	0.038	0.042	0.045
540	9	0.056	0.047	0.045	0.049
600	10	0.058	0.061	0.052	0.057
660	11	0.06	0.058	0.055	0.090
720	12	0.071	0.063	0.062	0.065
780	13	0.079	0.081	0.071	0.077
840	14	0.091	0.089	0.091	0.090
900	15	0.098	0.095	0.099	0.097
960	16	0.096	0.097	0.098	0.097
1020	17	0.101	0.099	0.103	0.101
1080	18	0.108	0.104	0.105	0.106
1140	19	0.107	0.106	0.103	0.105
1200	20	0.111	0.113	0.109	0.111
1260	21	0.115	0.118	0.114	0.116
1320	22	0.127	0.134	0.129	0.130
1380	23	0.137	0.136	0.133	0.135
1440	24	0.139	0.141	0.139	0.140
1500	25	0.141	0.149	0.145	0.145
1560	26	0.144	0.156	0.159	0.153
1620	27	0.149	0.164	0.167	0.160
1680	28	0.153	0.182	0.178	0.171
1740	29	0.166	0.198	0.193	0.186
1800	30	0.172	0.212	0.205	0.196
1860	31	0.185	0.239	0.211	0.212
1920	32	0.203	0.253	0.232	0.229
1980	33	0.206	0.258	0.248	0.237
2040	34	0.218	0.266	0.255	0.246
2100	35	0.228	0.267	0.265	0.253
2160	36	0.236	0.278	0.269	0.261
2220	37	0.243	0.291	0.272	0.269
2280	38	0.248	0.294	0.278	0.273
2340	39	0.259	0.296	0.283	0.279
2400	40	0.261	0.299	0.288	0.283
2460	41	0.263	0.301	0.294	0.286



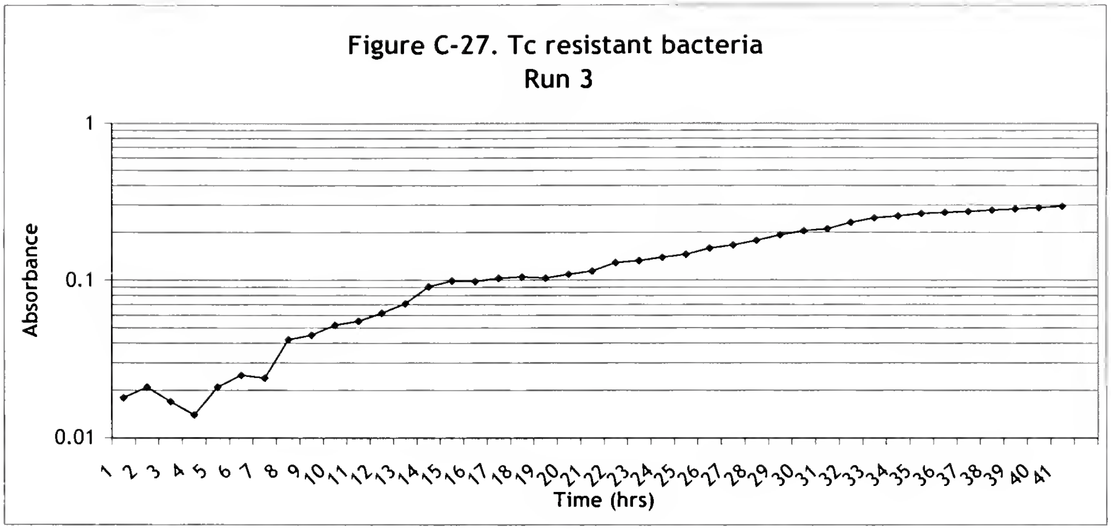
Absorbance increases from 0.101 to 0.203

Actual growth rate = **(32 hrs -17 hrs) = 15 hrs**



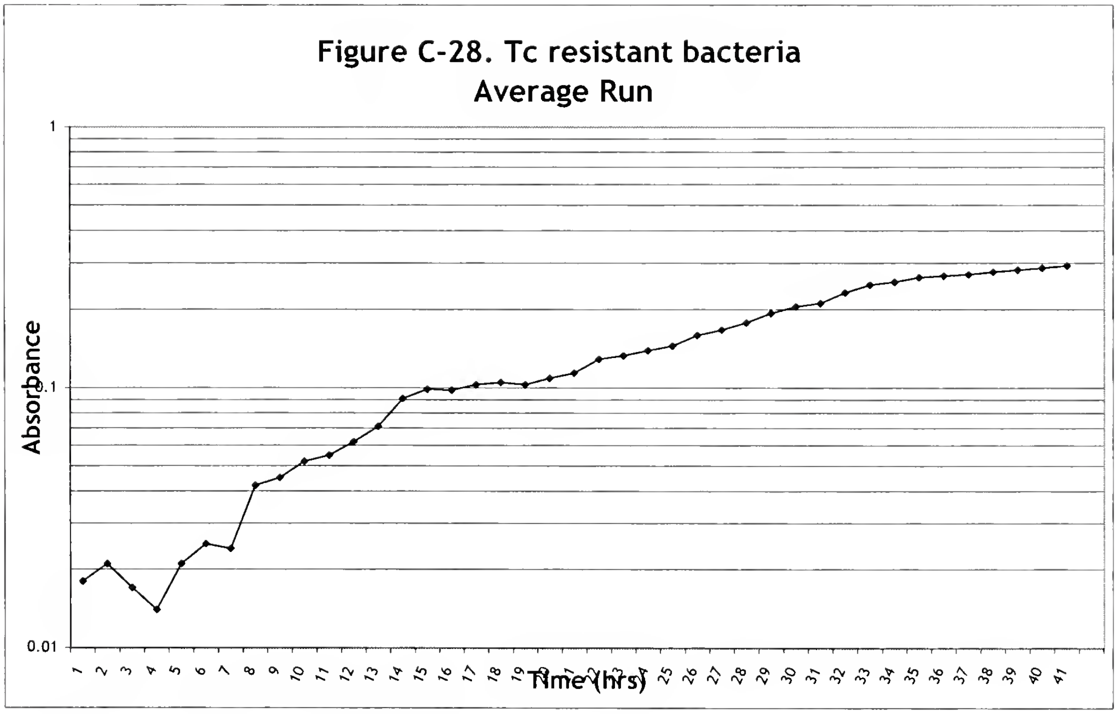
Absorbance increases from 0.106 to 0.212

Actual growth rate = **(30 hrs -19 hrs) = 11 hrs**



Absorbance increases from 0.106 to 0.212

Actual growth rate = **(31 hrs -18 hrs) = 13 hrs**

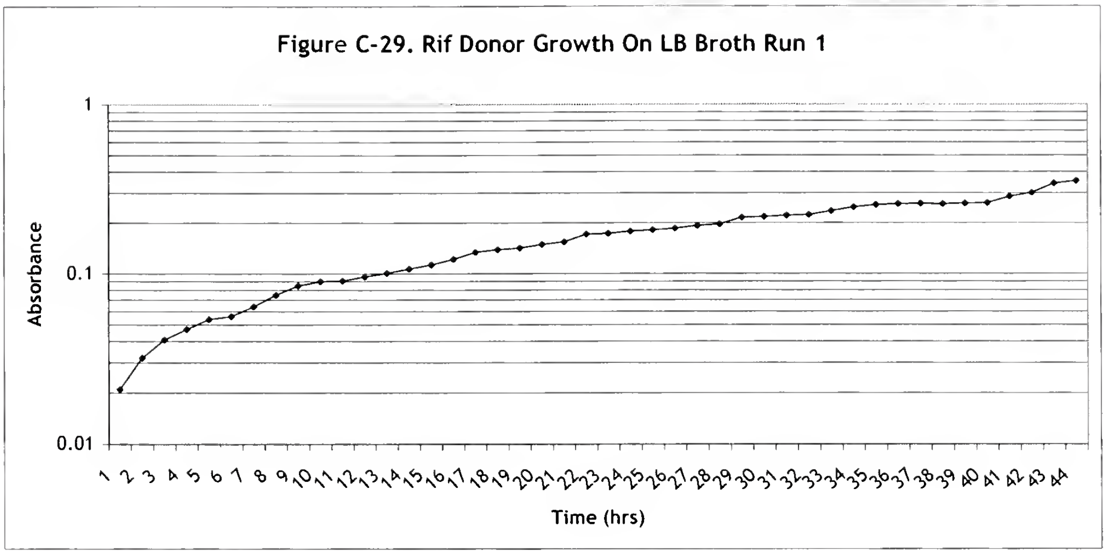


Absorbance increases from 0.106 to 0.212

Actual growth rate = **(31 hrs -18 hrs) = 13 hrs**

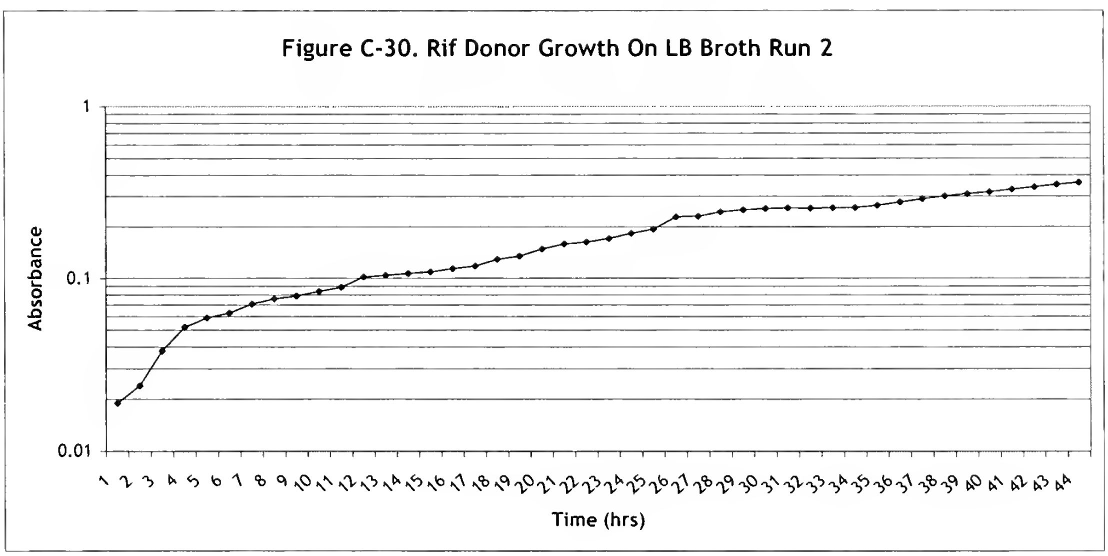
Donor (Rif resistant) growth rate on LB broth + Rif

Time(min)	Time(hrs)	Run1	Run2	Run3	Average
0					
30					
60	1	0.021	0.019	0.018	0.019
120	2	0.032	0.024	0.029	0.028
180	3	0.041	0.038	0.037	0.039
240	4	0.047	0.052	0.045	0.048
300	5	0.054	0.059	0.051	0.055
360	6	0.056	0.063	0.062	0.060
420	7	0.064	0.071	0.068	0.068
480	8	0.075	0.076	0.079	0.077
540	9	0.085	0.079	0.086	0.083
600	10	0.090	0.084	0.097	0.090
660	11	0.091	0.089	0.098	0.093
720	12	0.096	0.102	0.101	0.100
780	13	0.101	0.104	0.105	0.103
840	14	0.107	0.107	0.121	0.112
900	15	0.113	0.109	0.123	0.115
960	16	0.122	0.114	0.132	0.123
1020	17	0.134	0.118	0.139	0.130
1080	18	0.139	0.129	0.147	0.138
1140	19	0.142	0.135	0.153	0.143
1200	20	0.149	0.148	0.159	0.152
1260	21	0.154	0.159	0.163	0.159
1320	22	0.171	0.163	0.168	0.167
1380	23	0.173	0.171	0.185	0.176
1440	24	0.178	0.183	0.189	0.183
1500	25	0.181	0.194	0.193	0.189
1560	26	0.185	0.228	0.212	0.208
1620	27	0.192	0.23	0.228	0.217
1680	28	0.196	0.244	0.231	0.224
1740	29	0.215	0.251	0.236	0.234
1800	30	0.217	0.255	0.243	0.238
1860	31	0.221	0.257	0.253	0.244
1920	32	0.223	0.256	0.255	0.245
1980	33	0.235	0.257	0.259	0.250
2040	34	0.247	0.258	0.261	0.255
2100	35	0.255	0.267	0.265	0.262
2160	36	0.258	0.278	0.269	0.268
2220	37	0.26	0.291	0.272	0.274
2280	38	0.259	0.302	0.278	0.280
2340	39	0.261	0.311	0.283	0.285
2400	40	0.262	0.319	0.299	0.293
2460	41	0.286	0.33	0.312	0.309
2520	42	0.301	0.341	0.314	0.319
2580	43	0.342	0.353	0.321	0.339
2640	44	0.354	0.362	0.331	0.349



Absorbance increases from 0.107 to 0.215

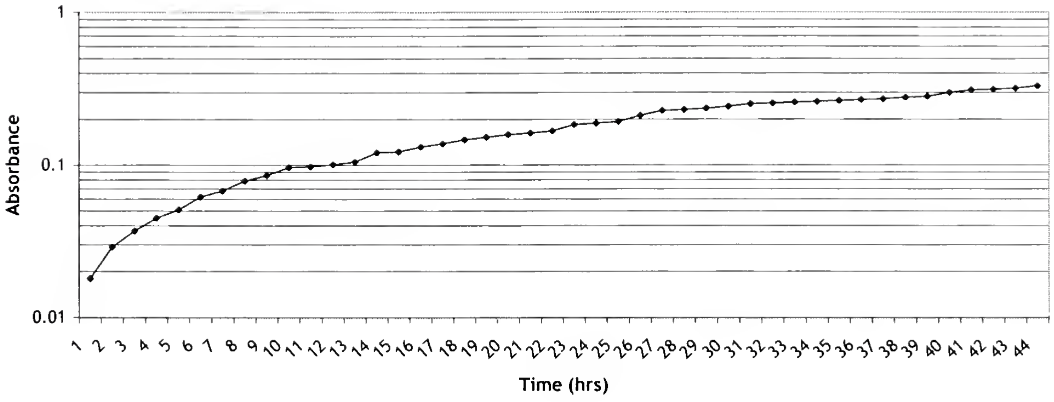
Actual growth rate = **(29 hrs -14 hrs) = 15 hrs**



Absorbance increases from 0.114 to 0.228

Actual growth rate = **(27 hrs -17 hrs) = 10 hrs**

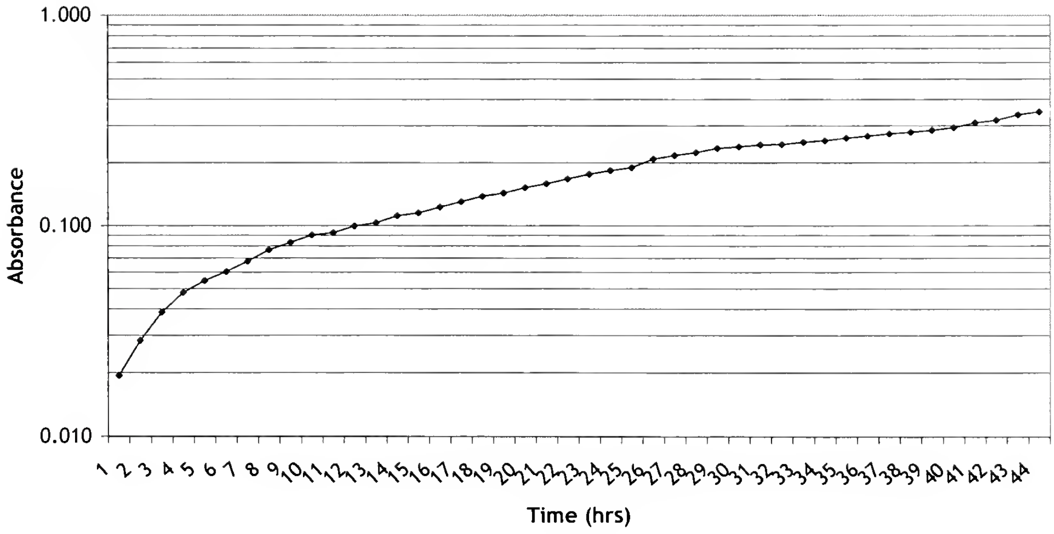
Figure C-31. Mutant Growth On LB Broth Run 3



Absorbance increases from 0.121 to 0.243

Actual growth rate = **(30 hrs -14 hrs) = 16 hrs**

Figure C-32. Average Mutant Growth On LB Broth



Absorbance increases from 0.114 to 0.228

Actual growth rate = **(28 hrs -14 hrs) = 14 hrs**

WILD TYPE, DONOR, RECIPIENT, AND MUTANT GROWTH RATES AT VERY LOW CONCENTRATIONS OF Cd.

TABLE C-1. SUMMARY OF WILD TYPE, Tc and Rif DONOR, RECIPIENT, AND MUTANT AT VERY LOW CONCENTRATIONS OF Cd.

	R1	R2	R3	AVG
WILD TYPE	60min	60min	60min	60min
WILD TYPE + Cd1	60min	60min	60min	60min
WILD TYPE + Cd2	60min	60min	60min	60min
WILD TYPE + Cd3	90min	90min	90min	90min

	R1	R2	R3	AVG
Rif resistant bacteria (recipient)	3 hrs	1 hr	3 hrs	3 hrs
Rif resistant bacteria (recipient) + Cd3	5 hrs	5 hr	6 hrs	6 hrs

	R1	R2	R3	AVG
Tc resistant bacteria(donor)	15 hrs	11 hrs	13 hrs	13 hrs
Tc resistant bacteria(donor) + Cd3	no growth			

	R1	R2	R3	AVG
Rif resistant donor	15 hrs	10 hr	16 hrs	14 hrs
Rif resistant donor + Cd3	no growth			

	R1	R2	R3	AVG
MUTANT on LB	15hrs	11hrs	13hrs	13hrs
MUTANT on LB + Rf + Tc	7hrs	10hrs	10hrs	10hrs
MUTANT + Rf + Tc + Cd1	33hrs	36hrs	32hrs	34hrs
MUTANT + Rf + Tc + Cd2	50hrs	49hrs	48hrs	48hrs
MUTANT + Rf + Tc + Cd3	no growth			

Cd1= $50 * 10^{-6}$ M

Cd2= $100 * 10^{-6}$ M

Cd3= $300 * 10^{-6}$ M

MIC for mutant was $300 * 10^{-6}$ M

Figure C-33. Appearance of plasmids in mutant and donor strain

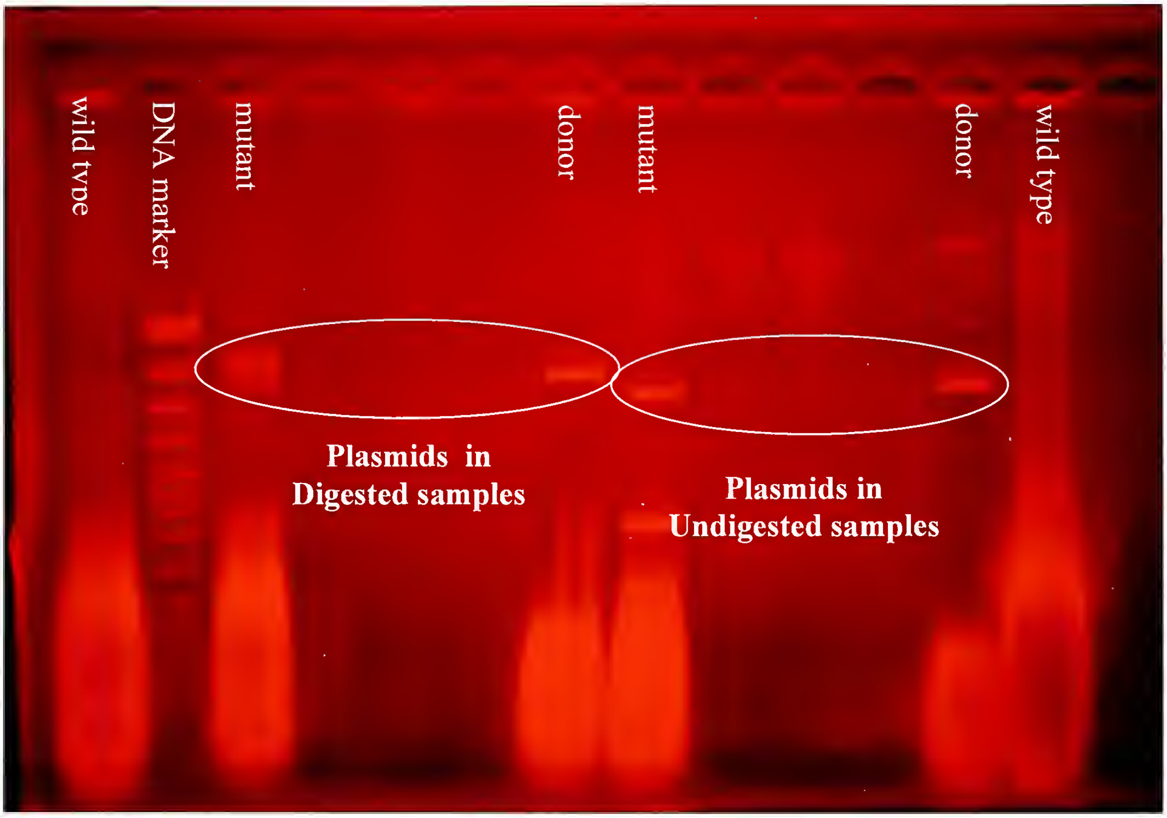


Figure C-34. Appearance of plasmids in mutant and donor strain

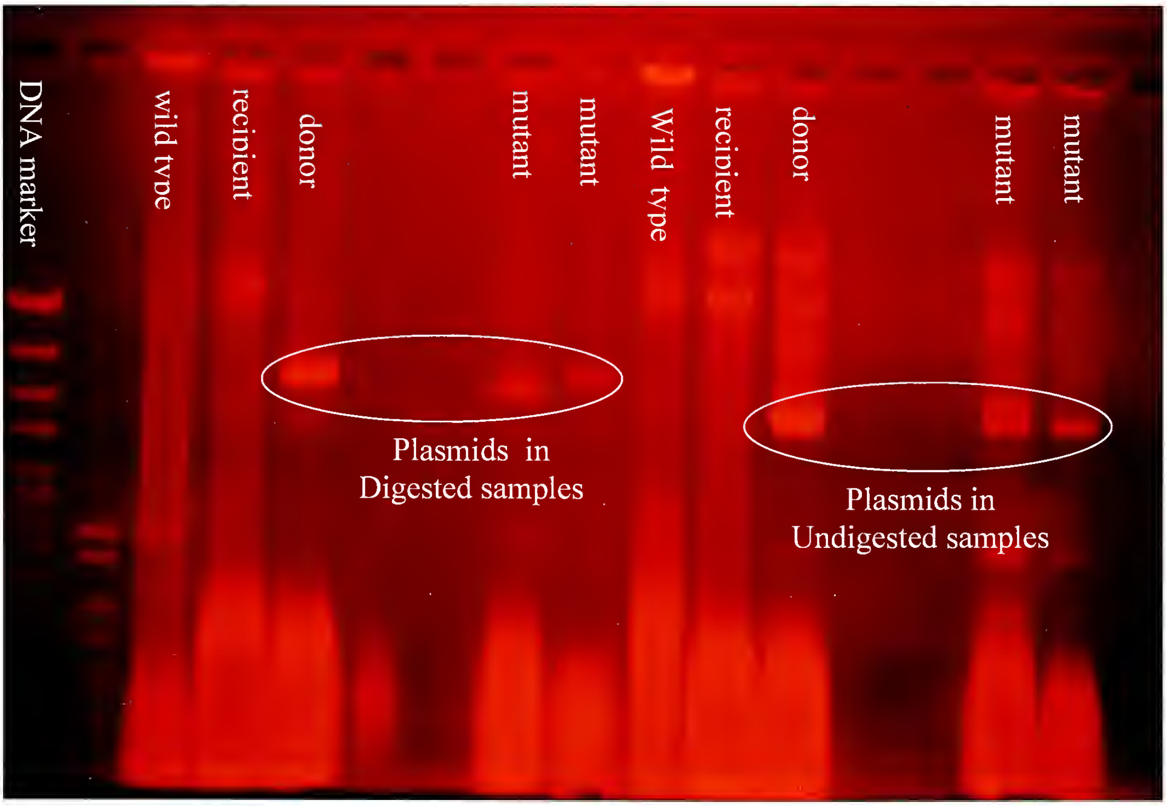


Figure C-35. Appearance of plasmids in mutant and donor strain

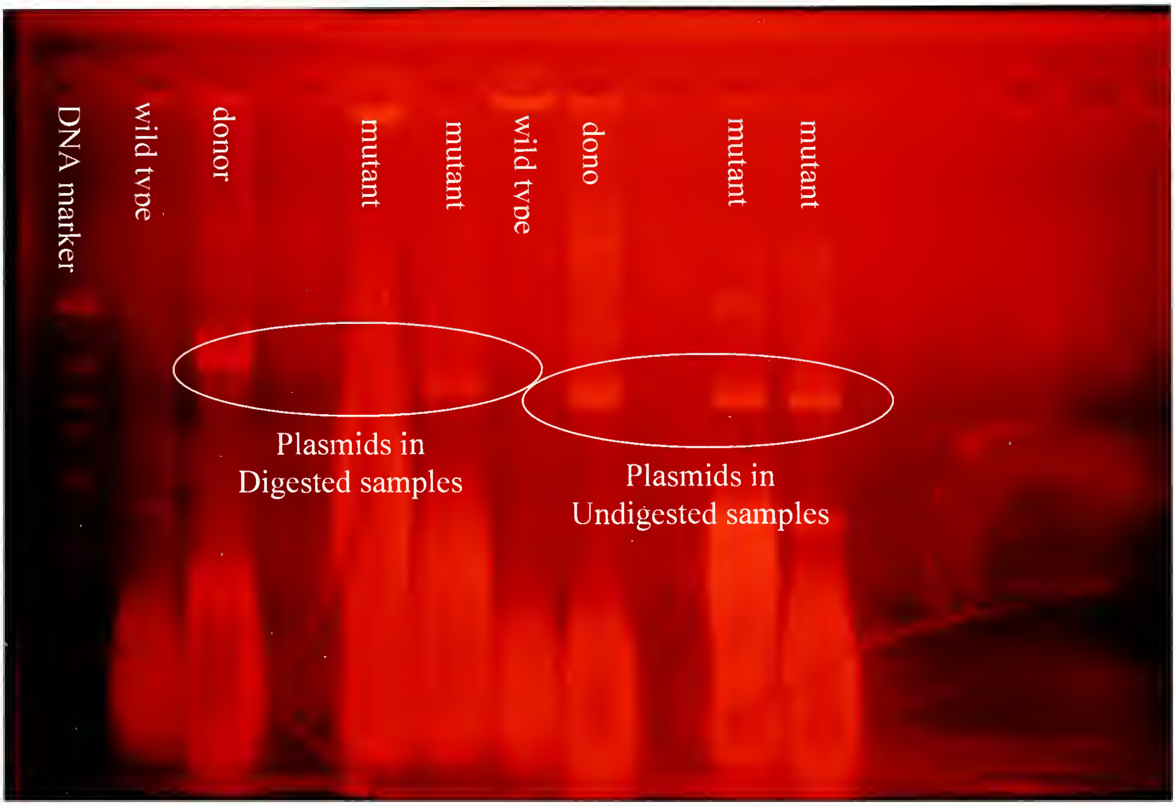


Figure C-36. Appearance of plasmids in mutant and donor strain