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ABSTRACT

Title of Thesis : A STUDY OF BIOLOGICAL DENITRIFICATION OF MUNITION WASTES

Sonia Venugopal, Master of Science in Chemical Engineering, 1991

Thesis directed by: Dr. Basil C. Baltzis, and
Dr. Gordon A. Lewandowski

Kinetics of denitrification were studied using a consortium from the Lawrence, Kansas municipal treatment plant. Experiments were conducted in the batch mode, under controlled oxygen and pH conditions. It was found that the Monod model can successfully perdict the experimental results of nitrate depletion. It was also found that when the pH is maintained at values between 7.0 and 8.5, nitrate can be treated in a batch mode without having any significant nitrite presence in the reactor. The experimentally determined kinetic parameters were used in preliminary calculations for sizing a sequencing batch reactor (SBR). An existing mathematical model for the SBR was slightly modified for the calculations. Due to the slow rates of nitrate depletion, high biomass concentrations should be maintained in the unit in order to achieve effluent quality from a reactor of reasonable size.

A STUDY OF BIOLOGICAL DENITRIFICATION OF MUNITION WASTES

by
Sonia R. Venugopal

Thesis submitted to the Faculty of the Graduate School of
the New Jersey Institute of Technology in partial fulfillment of
the requirements for the degree of
Master of Science in Chemical Engineering
1991

APPROVAL SHEET

Title of Thesis: A STUDY OF BIOLOGICAL DENITRIFICATION OF MUNITION WASTES

Name of Candidate: Sonia R. Venugopal
Master of Science in Chemical Engineering

Thesis and Abstract Approved: _____ _____

Dr. Basil C. Baltzis Date
Associate Professor
Dept. of Chemical Engineering,
Chemistry and Environmental
Science. New Jersey Institute of Technology.

_____ _____

Dr. Gordon Lewandowski Date
Professor
Dept. of Chemical Engineering,
Chemistry and Environmental
Science. New Jersey Institute of Technology.

_____ _____

Dr. Piero M. Armenante Date
Assistant Professor
Dept. of Chemical Engineering,
Chemistry and Environmental
Science. New Jersey Institute of Technology.

VITA

Name : Sonia R. Venugopal

Permanent Address :

Date of Birth :

Place of Birth :

Degree and Date to be conferred : M.S. Che., January 1991

Collegiate Institutions	**Dates**	**Degree**	**Date of Degree**
New Jersey Institute of Technology	1989-1991	M.S.Che	January, 1991
Andhra University, India	1984-1988	B.E.Che	August, 1988

Major : Chemical Engineering

Positions Held

July'89-Present : Research Assistant
Department of Chem. Engg.
New Jersey Institute of
Technology, Newark, NJ

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Chapter 1

Introduction

Sunflower Army Ammunition Plant (SFAAP) is owned by the US Army and operated by Hercules, Inc. to produce nitroguanidine ((NQ) based explosives and propellants. SFAAP generates a significant quantity of wastewaters containing NQ and guanidine nitrate (GN). The wastewaters are pretreated by a lime/steam sparging process and are discharged to evaporative lagoons. The combination of increased wastewater volume and less than expected evaporation from the lagoons has let to the investigation of alternative methods of wastewater handling.

The principal pollutants in the wastewater are nitrogen, mostly in the form of nitrates, and sulfur in the form of sulphates. The primary goal of this study was to understand the kinetics of denitrification of the wastewater under anoxic conditions. Denitrification was accomplished by using a mixed culture. The experiments were conducted in the batch mode and the kinetic parameters obtained, were used in preliminary calculations for the size of a sequencing batch reactor unit that could treat the actual waste.

Chapter 2

Overview of Denitrification

2.1 Biochemistry

Respiration is an ATP generating process in which chemical compounds are oxidized and the final electron acceptor is almost always an inorganic molecule. The energy-yielding process in which the electron transport chain acceptor is an inorganic molecule other than O_2 is called anaerobic respiration. Some bacteria can use nitrate as the final electron acceptor. Nitrate is reduced to nitrite (NO_2), nitrous oxide (N_2O) or nitrogen gas (N_2) in a process known as Denitrification.

Assimilative nitrate reduction is one in which nitrate is reduced to the oxidation level of ammonia for use as a nitrogen source for growth. Denitrification is *dissimilative nitrate reduction* where nitrate is used as an alternative electron acceptor [9]. The enzyme involved in the first step of nitrate reduction, nitrate reductase is a molybdenum- containing enzyme. The product of this step is nitrite, which is further reduced to nitrogen gas with a second enzyme (nitrite reductase).

Denitrification requires the availability of a carbon source which may be used as the reducing agent in the electron-transport chain. Methanol is effective for denitrification and was used as the carbon source in the present study. Its aqueous vapor pressure is sufficiently low so that it should not create a hazard by evolution from the treated water. Methanol is not considered toxic to fish life, as most can tolerate over 10,000 mg/l.

Mc Carty et al. [6] described denitrification as a two step process, the first representing reduction of nitrate to nitrite and the second a reduction of nitrite to nitrogen gas as indicated in the following denitrification reaction with methanol: First step:

$$1/5NO_3^- + 1/15CH_3OH = 1/5NO_2^- + 1/15CO_2 + 2/15H_2O \qquad (2.1)$$

Second step:

$$1/5NO_2^- + 1/10CH_3OH = 1/10N_2 + 1/10CO_2 + 1/10H_2O + 1/5OH^- \qquad (2.2)$$

Overall:

$$1/5NO_3^- + 1/6CH_3OH = 1/10N_2 + 1/6CO_2 + 7/30H_2O + 1/5OH^- \qquad (2.3)$$

Thus, 5/6 moles of methanol are required to reduce one mole of nitrate completely to molecular nitrogen. This only represents the respiration requirement and additional methanol is required for bacterial growth.

Mc Carty et al. [6] estimated the growth requirement according to the following equation:

Cell Synthesis:

$$1/6CH_3OH + 1/84CO_2 + 1/28NO_3^- + 1/28H^+ = 1/28C_5H_7O_2N + 19/84H_2O \quad (2.4)$$

Equation 2.4 indicates some nitrate is used for assimilation. Equations 2.3 and 2.4 are added such that the consumptive ratio is equal to 1.3. The consumptive ratio is defined as the ratio of the total quantity of organic carbon consumed during denitrification to the stoichiometric requirement for respiration alone.This ratio was estimated experimentally from denitrification studies [6]. If Equation 2.4 is multiplied by 0.39 and added to Equation 2.3, an equation is obtained in which the ratio of equivalents of methanol to equivalents of nitrate nitrogen will equal the consumptive ratio of 1.3. Multiplying the equation by 8.8 will normalize it to one mole of nitrate:

Overall nitrate removal:

$$NO_3^- + 1.08CH_3OH + OH^+ = 0.065C_5H_7O_2N + 0.47N_2 + 0.76CO_2 + 2.44H_2O \quad (2.5)$$

The following values can be evaluated from the above equation:

Methanol requirement : 2.47 mg/mg $NO_3^- - N$

Yield coefficient : 0.104 mg/mg NO_3^-

pH change : 1mole OH^-/mole NO_3^-

A material balance for nitrite removal (if present) as required prior to denitrification can be obtained in a similar manner, and will yield the following:

Overall nitrite removal:

$$NO_2^- + 0.67CH_3OH = 0.04C_5H_7O_2N + 0.47CO_2 + 0.48N_2 + 1.7H_2O \quad (2.6)$$

Some nitrate may be assimilatively reduced if dissolved oxygen is also present initially.

2.2 Environmental Factors Influencing Denitrification

Biological denitrification takes place in the presence of nitrate and nitrite reductases. These enzymes must be active under the given environmental conditions. The ability to denitrify can be regulated in part by the ecological selection of the microorganisms capable of denitrification, in part by the regulation of the synthesis of the enzymes involved in the denitrification reactions, and part by the regulation of the activity of the enzymes. An extensive review of the influencing factors was done by Sanyal [11] on . The three factors considered in this study were pH, oxygen and temperature.

2.2.1 pH

Denitrification can occur over a pH range of 5.0-10.0 [8]. It is most rapid in the neutral or slightly alkaline range between 7 and 8.5 [2]. In a batch study by Beccari et al. [1], it has been found that there is a drop in nitrate and nitrite reduction rates above a pH of 7.5. Nitrate reduction rates were more sensitive to pH values above 7.5 whereas nitrite reduction rates were more sensitive to pH values below 7.5. The optimum pH for nitrate-nitrite removal, with methanol as the carbonaceous substrate, was thus found to be 7.5 at a temperature of 25°C.

Timmermans and Haute [15] studied the growth of *Hyphomicrobium spp* in a sequencing batch reactor, under varying conditions of pH. These studies show that the rate of denitrification is maximum at a pH of 8.3 at temperature of 25°C. According to Delwiche [4] elemental N_2 is the end product of denitrification above a pH 7.3 while below this level the nitrous oxide production starts to increase.

2.2.2 Oxygen

Oxygen is known to inhibit the reduction of nitrate and the formation of nitrogen by denitrifying bacteria. Stickland [13] determined the influence of oxygen at various partial pressures in the reduction of nitrate to nitrite by cell suspensions of *Escherichia Coli.* He found that as little as 0.36 % of oxygen caused a 21 % inhibition (i.e., rate of denitrification was decreased by 21 %), and 3.76 % oxygen caused 93 % inhibition. A tenfold increase in nitrate concentration did not modify these results, thus demonstrating that the inhibition was noncompetitive.

Sacks and Baker [10] found that oxygen not only supresses the formation of nitrate and nitrite-reducing enzyme systems, but also when these enzymes are present it also inhibits the rate of reduction. They found that oxygen inhibition of denitrification is almost completely reversible after a 15 minute exposure to oxygen, but only partially reversible after an hour. It is probable that a much more prolonged exposure to oxygen might cause a permanent inhibition, which means that the biomass loses its denitrification capability permanently.

The denitrifying enzymes are generally thought to be inducible. They can be produced by forcing them to use nitrate in the absence of oxygen. Simpkin and

Boyle [12] suggested that the specific enzyme levels could possibly increase and decrease as the microorganisms of an activated sludge go through anoxic/aerobic cycles. It was found that oxygen repression of the synthesis of the denitrifyng enzymes was not complete in the activated sludge, so that the enzymes were synthesized to at least 50% of their maximum level. This implied that inhibition of enzyme activity by oxygen and not repression of enzyme synthesis, must be the most important effect oxygen has on denitrification by an activated sludge.

2.2.3 Temperature

Denitrification can occur at temperatures as low as 5-10°C [8] but the rates are slow. Dawson and Murphy [3] found from laboratory batch tests on a defined medium utilizing a dominant culture of *Pseudomonas denitrificans* that the temperature dependency can be closely approximated by an Arhenius temperature relationship in the range 5 to 27°C. In all studies nitrate removal was characterized initially by periods of relatively slow nitrate removal, during which approximately 25 percent of the nitrate content was removed. They observed this lag or acclimation period to increase with decreasing temperature; from about 5 h for the 27°C reactor, to about 8 days fro the 5°C reactor. They pointed out that cultures at the higher temperature were aleady acclimated to the experimental conditions during their growth sequence, while at 5°C only a small percentage of the original inoculum was probably adapted.

Chapter 3

Experimental Apparatus

3.1 Batch System

All the experiments were conducted in an incubator at a temperature of $31°C$. The inoculated solutions were kept in sealed serum bottles. During a kinetic run samples were taken at equal intervals of time using a syringe.

3.2 Analytical Equipment

- Spectrophotometer - UV-Visible,Varian DMS 200
- pH meter -Orion SA 720
- Ion Chromatograph (IC)- Waters Component System
  - Pump System - System Controller, Waters 600E
  - Auto Sampler - Sample Processor, Waters 715 Ulra Wisp

- Detectors

  1. Tunable Absorbance Detector, Waters 484
  2. Conductivity Detector, Waters 431

- Column - I-C-Pak A HC,150 X 4.6 mm, 10μ
- PC Minichrom, a chromatography data handling system, Software version 1.5, 1990 VG Data System Ltd

- IC Instrument Conditions

  - Eluent : Borate-Gluconate
  - Flow rate : 2.0 ml/min
  - Injection : 100 μl of sample
  - Detection : 431
  - Range : 500 μS
  - Temperature :35^{o}C
  - Polarity : +
  - Background : 220-240 μS

3.3 Chemicals Required

The chemicals required for the experiments are

- KH_2PO_4, F.W.=136.09, 99% purity.

- $Na_2HPO_4.7H_2O$, F.W.=268.07, 98.85% purity.
- KNO_3, F.W. 101.1
- CH_3OH, F.W. 32.04, 99.9% purity.

The chemicals required for the eluent in the IC

- Boric Acid, M.W. 61.83, 99.5% purity.
- Lithium Hydroxide (mono hydrate), M.W. 41.96.
- Glycerin ($C_3H_8O_3.aq$), M.W. 92.10+aq, 86-88% purity.
- D-Gluconic Acid - 50% in water.

Chapter 4

Experimental Procedures

4.1 Batch System

Experiments were carried out in the batch mode using the denitrifying consortium from the Lawrence, Kansas municipal treatement plant. The system had to be maintained under anaerobic conditions and the inoculum was sealed in serum bottles. The experiments were run at room temperature.

A buffer solution of pH 7.5 was originally prepared by mixing 0.01M $Na_2HPO_4 \cdot 7H_2O$ and 0.01M KH_2PO_4. The formation of OH^- ions during denitrification led to an increase in the pH from 7.5 to 10.0 in a very short while. The increase in pH inhibits denitrification as discussed in the previous chapter. Due to these observations, the concentration of KH_2PO_4 was increased to 0.1M while that of $Na_2HPO_4 \cdot 7H_2O$ was kept the same. This way the pH could be maintained constant during the reaction. Phosphorous, as a source of nutrient for the bacteria, was derived from the buffer solution.

The mixed culture was added to the buffer solution such that it made up 20% of the total volume of 50 ml. The active sludge, which was stored in the refrigerator, was brought to room temperature before starting an experiment. The inoculate was then acclimatized to increasing concentrations of KNO_3 from 20 ppm to 1000 ppm of NO_3^-. The acclimatized culture was used for the kinetic run. Denitrification rates were measured at different concentrations of KNO_3. The amount of methanol added was 70 gm CH_3OH/gm $NO_3^- - N$. This was in excess to the required amount to ensure that methanol was not the rate limiting substrate. The solution was purged with N_2 gas before it was sealed to remove any oxygen present.

Ten identical reacting solutions were prepared in sealed bottles with a nitrate concentration of 500 ppm. These bottles were kept in the shaker at a temperature of $31°C$. Each bottle served as a sample for the run. From time zero, samples were taken at equal intervals of time and analysed for NO_3^-, NO_2^- and biomass. Nitrate and nitrite were analysed using the Hach kit, which is described in the next section. There was no accumalation of nitrite and though the concentration of nitrate did reduce it did not come down to zero.

To measure the biomass, 50 ml of the sample were first dried at a temperature of $90°C$. This dried mass was weighed and subsequently fired at $400°C$ in a muffle furnace. The difference between the two dry weights gave the weight of the volatile suspended solids (VSS) driven off by the furnace [14]. The VSS gave a crude measure of the biomass produced during the reaction. This method proved to be an ineffective way to measure the biomass because of the analytical interference

due to calcium carbonate volatilization during firing of the sample. It was not very pracitcal either due to large volumes of sample required each time. Optical density could not be used to measure the biomass due to the turbidity of the mixed liquor.

A volume of 1000 ml of the reacting solution was prepared in a large reactor which was continously purged with N_2 to maintain a low dissolved oxygen level. The objective was to grow the biomass to a higher concentration before starting the kinetic runs. The nitrate concentration was checked every once in a while, and again the rate of denitrification was very slow. It took more than 24hrs for 500 ppm of nitrate to come down to zero. The mixed liquor was spiked with nitrate each time the concentration came down to zero. After about 10 days the denitrification stopped completely. The dissolved oxygen was measured and it was found that the solution was saturated with O_2. The presence of facultative organisms did not seem to help in the removal of O_2. A number of measures were taken to reduce the oxygen including the addition of Na_2SO_3. None of the methods worked and finally it was discovered that the cylinder of N_2 had oxygen. It was decided to run the experiments in sealed bottles.

An inoculate solution of 50 ml was prepared and spiked with different concentrations of KNO_3. NO_3^- and NO_2^- were analysed on the IC which was more accurate than the Hach method. Protein was used as a measure of the biomass.

To check the presence of oxygen, 0.1% rezazurin (a dye) was added to the reactor. The dye turns pink in the presence of O_2. Na_2SO_3 was added as a reducing agent when oxygen was detected in the reactor.

4.2 Analytical Methods

4.2.1 Nitrate/Nitrite Detection

The sample from the reactor was collected in micro centrifuge tubes. A part of it was refrigerated for the protein assay. The other part was centrifuged to separate out the bacteria. The supernatant was used in the nitrate analysis.

Hach Method

This is a colorimetric method where nitrate is measured indirectly by reduction to nitrite by cadmium.The reagents used in this method are: 1. NitraVer 6 (Nitrate reagent powder pillow) 2. NitriVer 3 (Nitrite reagent powder pillow)

Part 1

Step 1. Add 30 μl of the sample to 30 ml distilled water and to this, add the nitrate reagent.Shake the sample cell for 3 minutes to allow the reduction reaction to complete.

Step 2. Decant 25 ml of the prepared sample into a clean sample cell. Add the nitrite reagent to this and let the solution stand for 10 minutes. The absorbance of the solution is read at 500 nm.

Part 2

Step 1. Add 25 μl of sample to 25 ml distilled water. Then add the nitrite reagent to the sample and after 10 minutes read the absorbance at 500 nm.

IC analysis

The supernatant was diluted 10 times to avoid overloading the IC column. As the

nitrate concentration decreased the sample was diluted by a smaller factor. The diluted sample was then filtered and passed through a mili-trap to remove cations and some organics and was ready for injection. 100μl of the sample were injected by the autosampler.

4.2.2 Protein Assay

The total protein formed was assumed to be proportional to the biomass present. The protein analysis would give an idea of the growth rates under different NO_3^- concentrations. The organisms were treated with 1N NaOH so that they underwent lysis and all the intercellular proteins were released into the aqueous medium.

The protein reacts with Cu^{2+} in an alkaline medium to produce Cu^{1+} by the biuret reaction. The interaction of two molecules of BCA (Bicinchoninic acid) with one cuprous ion formed a purple reaction product, which is water soluble and exhibits a strong absorbance at 562 nm. This allows the spectrophotometric quantitation of protein in aqueous solutions.

BCA * protein Assay Reagent, 1000 ml

1. Reagent A: 1000 ml of base reagent which contains: sodium carbonate, sodium bicarbonate, BCA detection reagent and sodium tartrate in 0.2N NaOH

2. Reagent B: 25 ml of 4% Copper Sulfate solution

The working reagent is prepared by mixing 50 parts of Reagent A with 1 part of Reagent B. This reagent is stable for one week.

Protocol

- A set of protein standards of known concentration was prepared by diluting the BSA standard solution (bovine serum albumin) in distilled water.
- 0.01 ml 1N NaOH was added to 0.1ml of the mixed culture. This was done for all samples and the BSA standards solutions.
- The above solution was kept submerged in a boiling water bath for 15 minutes.
- 2 ml of the working reagent was added to the treated solution. They were then kept at 37°C for 30 minutes.
- Absorbance was measured at 562 nm for each sample.

Chapter 5

Mathematical Derivations & Calculations

5.1 Batch Model

The growth of the biomass can be represented by inhibitory or non-inhibitory kinetics. Nitrite is known to have a toxic effect on denitrifying enzymes [8]. However, in the present study there was no accumulation of nitrite. Nitrate, at concentrations above 2000 ppm, is detrimental to the growth of several species [8]. In the course of this study, the highest concentration of nitrate in the medium was not more than 500 ppm. The specific growth rate was thus expressed by the Monod (non-inhibitory) model.

Denitrification in a batch reactor can be expressed by the following equations:

Nitrate balance:

$$\frac{ds}{dt} = -\frac{b}{Y}\mu(s) \tag{5.1}$$

Biomass balance:

$$\frac{db}{dt} = \mu(s)b \tag{5.2}$$

where

$$\mu(s) = \frac{\mu_m s}{K + s}$$

Monod Expression

The symbols appearing in the equations above, stand for the following physical quantities:

- s → Nitrate concentration in the reactor (mg/l)
- b → Biomass concentration in the reactor (mg/l)
- μ_m → Maximum specific growth- rate (hr^{-1})
- K → Saturation constant of the population (mg/l)
- Y → Yield coefficient of the biomass on nitrate.

5.1.1 Determination of Model Parameters

The model parameters to be evaluated are, the specific growth rate (μ_m), the saturation constant (K) and the yield coefficient (Y). The nitrate vs time data can be

used to determine these parameters as well the initial biomass concentration (since it was not possible to measure the latter, as explained previously.

Equations 5.1 and 5.2 give

$$\frac{ds}{dt} = -\frac{1}{Y}\frac{db}{dt} \tag{5.3}$$

or,

$$Y(s_o - s) = b - b_o \tag{5.4}$$

From equations 5.1 and 5.4 one gets:

$$\frac{ds}{dt} = -\frac{\mu_m s}{K+s}\frac{Y(s_o - s) + b_o}{Y} \tag{5.5}$$

$$-\frac{(K+s)Y}{(\mu_m s)(Y(s_o - s + b_o)}ds = dt \tag{5.6}$$

The above equation can be integrated by parts to give

$$t = \frac{1}{\mu_m}\{\ln[\frac{Y(so - s) + b_o}{b_o}] - \frac{KY}{b_o + Ys_o}\ln\frac{sb_o}{s_o[Y(s_o - s) + b_o]}\} \tag{5.7}$$

The batch experiments were run at different initial nitrate concentrations. The nitrate and protein concentrations are tabulated in Tables 1-7. The protein analysis gave very erratic results as can be seen from Figure 9.1, and hence could not be used in the determination of the model parameters or the initial biomass concentration b_o. The nitrate vs time data were regressed using a non-linear regression program.

In order to regress the data it was necessary to have an idea of the order of magnitude of the parameters. P. Timmermann and A.V. Hauk [15] studied

the growth of *Hyphomichrobium* sp under varying environmental conditions. The maximum growth rate was found to be $0.0355h^{-1}$ at a pH = 8.3. This was taken to be the initial guess in the regression. As shown previously from McCarty's results the yield coefficient was calculated to be 0.104 mg cells/mg NO_3 hence, this value was used as the initial guess for Y. The value of K was initially guessed at 10.0, and that of b_o at 1000 mg/l.

The constants were evaluated at each initial nitrate concentration. The values of the yield coefficient and the maximum growth rate were found to converge, and these values were then kept constant while K and b_o were allowed to float. Subsequently K was also kept constant and the initial biomass concentration was evaluated. Since the experiments were done at different times, b_o was different for every run.

The values of the model parameters were finally evaluated as:

$\mu_m = 0.014\ hr^{-1}$

Y = 0.15 mg biomass/mg NO_3

K = 14 mg/l

5.2 SBR Model

The batch process is a discontinous mode of operation and is not practical at the industrial level. In a continous process it is possible to treat larger volumes of waste, more economically. Sequencing batch is a cyclic mode of operating the reactor, and each cycle has five distinct periods; fill, react, settle, draw and idle. Figure 9.2 (a)

shows the volume variation during the cycle, and under the assumption that filling and drawing occurs at constant flow rates (and, thus the volume changes linearly). Sequencing batch reactor (SBR) allows for a variety of operating strategies which can suit various treatement requirements. Since the reaction phase is in a batch mode, the reaction products can be held in the reactor until acceptable discharge levels are acheived.

Denitrification in a SBR has been theoretically investigated by Sanyal [11], and a number of criteria for optimal design have been derived. In the present study, the SBR model proposed earlier [11] has been modified. It is assumed here that the amount of biomass produced is negligible, and that the drawing time is insignificant when compared with the overall cycle time.

Model Derivation

$$\frac{dV}{dt} = Q_f - Q \tag{5.8}$$

where,

- Q_f = Feed flow rate into the reactor
- Q = Effluent flow rate from the reactor

Nitrate balance:

$$\frac{d(Vs)}{dt} = (Q_f s_f - Qs) - \frac{b}{Y}\frac{\mu_m s}{K+s}V$$

or using Equation 5.8 one gets

$$s(Q_f - Q) + V\frac{ds}{dt} = Q_f s_F - Qs - \frac{b}{Y}\frac{\mu_m s}{K+s}V$$

or

$$\frac{ds}{dt} = \frac{Q_f}{V}(s_f - s) - \frac{b}{Y}\frac{\mu_m s}{K+s}V \tag{5.9}$$

For the biomass, since it is assumed to be constant during the cycle:

$$\frac{d(Vb)}{dt} = 0$$

or

$$\frac{db}{dt} = -b\frac{(Q_f - Q)}{V} \tag{5.10}$$

Dimensionless quantities

The following dimensionless quantities are now introduced:

- $u = s/K$ = dimensionless concentration of nitrate
- $u_f = s_f/K$ = dimensionless concentration of nitrate in the feed
- $x = b/Y_1K$ = dimensionless concentration of active biomass
- $Q'_f = Q_f/Q_f\sigma_1$ = dimensionless flow rate of the incoming waste and $Q_f = Q_f$ during the fill phase
- $Q' = Q/(Q_f\sigma_1)$ = dimensionless flowrate of the effluent
- $\sigma_1 = t_1/t_2$ = fraction of the cycle devoted to fill phase
- $V' = V/V_{max}$ = dimensionless volume of the reactor contents
- $\theta = tQ_f\sigma_1/V_{max}$ = dimensionless time
- $\beta = \mu_m V_{max}/(Q_f\sigma_1)$ = measure of the dimensionless hydraulic residence time

- $\delta = V_o/V_{max}$ = fraction of the reactor contents present at the beginning of the fill phase

Using the quantities above, one can rewrite Equations 5.8 to 5.10 as following

$$\frac{dV'}{d\theta} = Q'_f - Q' \tag{5.11}$$

$$\frac{du}{d\theta} = \frac{Q'_f}{V'}(u_f - u) - \beta\frac{u}{1+u}x \tag{5.12}$$

$$\frac{dx}{d\theta} = -\frac{Q'_f - Q'}{V'}x \tag{5.13}$$

During the fill phase $Q' = 0$ and $Q'_f = 1/\sigma_1$. Therefore Equation 5.11 can be written as:

$$V' = \delta + \frac{1}{\sigma_1}\theta \tag{5.14}$$

At the end of the fill phase $(\theta = \theta_1), V' = 1$ and thus Equation 5.14 implies that,

$$1 = \delta + \frac{\theta_1}{\sigma_1} \Rightarrow \theta_1 = (1-\delta)\sigma_1$$

Hence the filling period is

$$0 \leq \theta \leq (1-\delta)\sigma_1$$

During the react phase, $Q'_f = 0 = Q'$. The time interval for this phase is

$$(1-\delta)\sigma_1 \leq \theta \leq \theta_2$$

$$\frac{\theta_1}{\theta_2} = \frac{t_1}{t_2} = \sigma_1$$

$$\theta_2 = \frac{1}{\sigma_1}\theta_1$$

or

$$\theta_2 = 1 - \delta \tag{5.15}$$

The final formulation of the problem is the following:

Filling phase: $0 \leq \theta \leq \sigma_1(1-\delta)$

$$\frac{du}{d\theta} = \frac{1}{\sigma_1\delta + \theta}(u_f - u) - \beta\frac{u}{1+u}x \tag{5.16}$$

$$\frac{dx}{d\theta} = -\frac{x}{\sigma_1\delta + \theta} \tag{5.17}$$

During the react phase: $(1-\delta)\sigma_1 \leq \theta \leq 1-\delta$

$$\frac{du}{d\theta} = -\beta\frac{u}{1+u}x_o\delta \tag{5.18}$$

$$\frac{dx}{d\theta} = 0 \tag{5.19}$$

In order to find the volume of the SBR required to treat a waste with an initial nitrate concentration of 7500ppm, the equations for the fill and react phases have to be solved. These equations are solved by fourth order Runga-Kutta numerical integration using FORTRAN based programs. FORTRAN codings of these programs are listed in Chapter 10.

In solving these equations it was assumed that 10% of the total cycle time was devoted to settling. Hence Equations 5.18 and 5.19 were integrated for θ upto $0.9(1-\delta)$ rather than $1-\delta$. At a paticular feed and initial biomass concentration, the equations were solved at different values of δ to give the required outlet nitrate concentration. The fill period is assumed to be 10% of the total cycle ($\sigma_1 = 0.1$). Beta was evaluated by trial and error, at different values of δ, such that the required extent of denitrification was obtained. The effect of δ and nitrate outlet concentration are tabulated in Table 5.1.

Table 5.1: Variation of β with Outlet Concentration (u_{end}) and δ

$u_f = 20$, $x_o = 450$, $\sigma_1 = 0.1$			
	Beta		
δ	$u_{end} = 1$	$u_{end} = 0.65$	$u_{end} = 0.065$
0.2	0.27869	0.28974	0.33285
0.3	0.19134	0.19958	0.23265
0.4	0.144224	0.15087	0.178534
0.5	0.11809	0.1241	0.15011
0.6	0.10341	0.10961	0.1375
0.7	0.0905	0.09664	0.12613

The greater the value of β, the greater is the required volume and the aim was to find an optimum value of this prameter. Initial biomass concentration also influences reactor volume. Beta was thus evaluated at different initial biomass concentrations, keeping δ constant. The dimensionless concentration of nitrate in the effluent (u_{end}) was taken to be 1.0.

Table 5.2: Effect of Initial Biomass (x_o) on β

$u_f = 530$, $u_{end} = 1$ $\sigma_1 = 0.1$, $\delta = 0.5$	
x_o	β
450	2.67
900	1.335
1200	1.0014
2000	0.6008
4000	0.3004
6670	0.1802

Using the values in Table 5.2, if one wants to treat 400,000 gallons of waste per day with an inlet and outlet nitrate concentration of 7500 mg/l ($u_f = 530$) and 14 mg/l ($u_{end} = 1$) respectively, the following can be evaluated :

From Equation 5.15 we have

$$1 - \delta = \frac{TQ_f\sigma_1}{V_{max}} \tag{5.20}$$

Therefore

$$(1 - \delta)\beta = \mu_{max}T \tag{5.21}$$

Substituting the values of μ_{max} (0.014), δ (0.5) and β (0.3) at initial biomass concentration of 8.4mg/l ($x_o = 4000$):

Total cycle time (T) = 10.7 hrs.

Number of cycles per day = 2.24

Volume treated per day = 400,000 gallons

Volume treated per cycle = 178,571.4 gallons

Fill time (t_1) = 0.1(10.7) = 1.07 hrs

Fill rate (Q_f) = 2,781.5 gpm per reactor

Total volume of reactor (V_{max}) = 357,142.8 gallons

In order to have reactors of reasonable size a number of reactors can be used in parallel, to treat the total volume.

Chapter 6

Results and Discussion

The experimental data was regressed using a program which was a combination of Marquardt and Gauss methods [7]. The calculated and experimental data are compared in Figures 9.3-9.9. The comparison shows that the Monod model was successful in describing the denitrification process. The absence of nitrite accumulation justifies the use of non-inhibitory kinetics to describe the overall reaction.

The model parameters evaluated from the experimental data are of the same order of magnitude as those reported in previous studies. The experimental data gave a value of 0.15 for the yield coefficient, which is in good correlation with the value calculated (0.104) from Equation 2.5. The specific growth rate estimated (0.014) is quite low indicating low reaction rates.

In the formulation of the equations for SBR it was assumed that the biomass concentration is constant with time. The low value of the yield coefficient shows that the assuption is valid. Since the maximum growth rate is such a low value

(0.014), in order to have a reasonable rate of reaction, it is necessary to maintain a high concentration of the biomass. The equations were used to determine the total volume of the reactor required to treat a nitrate waste in an SBR.

In Figure 9.10 β is plotted against δ at different outlet nitrate concentrations. It shows that if the outlet nitrate concentration is allowed to increase, β decreases, in other words the required reactor volume is lowered. The higher the fraction of volume in the reactor before the fill phase, the lower will be the required reactor volume.

Figure 9.11 shows that by increasing the initial biomass, the volume of the reactor can be reduced. If the biomass concentration decreases, the outlet nitrate concentration will rise.Therefore, maintaining a high biomass concentration is critical to maintaining effluent quality.

During the process of denitrification of wastewater nitrite has often been observed to accumulate, most probably because the nitrite reduction rate is slower than the rate of nitrate reduction. The way in which a wastewater treatment plant is designed and operated can have large effects on the phenomenon of nitrite accumulation and the nitrite reduction rate deterioration [16]. Kone and Behrens [5] concluded from the results of mixed cultures, that discontinous operation of denitrification reactors would trigger nitrite to accumulate, and thus steady state conditions are required to maintain low nitrite concentration. Accumulation of nitrite has been observed at a 1200 gallon pilot denitrification unit at the Sunflower AAP in Kansas. However, the present study has shown that nitrite concentra-

tion can be reduced to zero even in cyclical reactors, provided that fill times and operating conditions are adjusted appropriately.

Nitrate reduction is more sensitive to pH values above 7.5 than nitrite (Section 2.1) . The pH during the reaction was maintained between 7.5 and 8.5 which could be one of the reasons for the absence of accumulation of nitrite. Another reason could be the fact that the maximum concentration of nitrate used for the reactions was 500ppm (0.05%). It has been found [8] that if the concentration of nitrate in the media used for the culture of *P. aeruginosa* is greater than 2000ppm (0.2%) then there is accumulation of nitrite.

Chapter 7

Conclusions and Recommendations

The Monod model was very successful in in describing the denitrification process in the batch and SBR mode. The model parameters evaluated from the data were realistic, and of the same order as those cited in previous studies. The values of μ_{max} and Y were critical in developing the model for the SBR. The specific growth rate was very slow, and yield coefficient low (0.15), which proved to be a problem in the enrichment of the denitrifying cultures. This means that more biomass must be retained in the SBR in order to in order to acheive reasonable denitrifying rates.

The experiments should be conducted under highly controlled conditions of pH and oxygen in order to have a constant viable culture. This also prevents nitrite from accumulating during denitrification. Oxygen can be prevented from entering the reactor by continously sparging the system with pure nitrogen gas. The results from the protein analysis were quite erratic. One of the ways to improve the

method would be to wash the biomass thoroughly before the analysis. The final treated solution should be centrifuged before reading the absorbance, reducing the possibility of any particulate interference.

Chapter 8

Tables

Batch Data

Table 8.1: Denitrification with Initial NO_3^- = 30 ppm (set 1)

Time hr	Nitrate mg/l	Protein absorbance
0.000	32.365	2.630
0.333	15.29	2.680
0.666	9.325	2.735
1.000	3.305	2.570
1.333	0.655	-
1.666	0.00	2.697

Table 8.2: Denitrification with Initial NO_3^- = 30 ppm (set 2)

Time hr	Nitrate mg/l	Protein absorbance
0.0	28.370	2.628
0.333	14.195	2.650
0.666	8.440	2.730
1.000	2.880	2.738
1.666	0.00	2.720

Table 8.3: Denitrification with Initial NO_3^- = 45 ppm

Time hr	Nitrate mg/l	Protein absorbance
0.0	45.90	0.782
1.0	33.30	0.892
2.0	21.20	1.073
3.0	7.85	0.992
4.0	0.59	1.287

Table 8.4: Denitrification with Initial NO_3^- = 100 ppm

Time hr	Nitrate mg/l	Protein absorbance
0.0	106.19	2.254
0.5	58.88	2.356
1.0	26.55	2.189
1.5	4.42	2.650
2.0	0.00	2.817

Table 8.5: Denitrification with Initial NO_3^- = 120 ppm

Time hr	Nitrate mg/l	Protein absorbance
0.0	120.51	1.632
0.5	76.63	1.979
1.0	49.96	1.888
1.5	16.24	2.185
2.0	0.00	2.525

Table 8.6: Denitrification with Initial NO_3^- = 250 ppm

Time hr	Nitrate mg/l	Protein absorbance
0.0	250.970	2.682
0.333	224.210	2.756
0.833	208.410	2.606
1.333	172.940	2.715
1.833	148.866	2.803
2.333	118.510	2.826
2.833	80.000	2.794
3.833	41.100	2.770
4.833	15.930	2.760
5.833	0.000	2.600

Table 8.7: Denitrification with Initial NO_3^- = 270 ppm

Time hr	Nitrate mg/l	Protein absorbance
0.0	274.39	2.785
0.333	249.31	2.738
1.333	195.48	2.780
1.833	161.71	2.803
2.333	123.63	2.815
3.833	63.40	2.800
4.833	17.83	2.819
5.833	0.32	2.79

Chapter 9

Figures

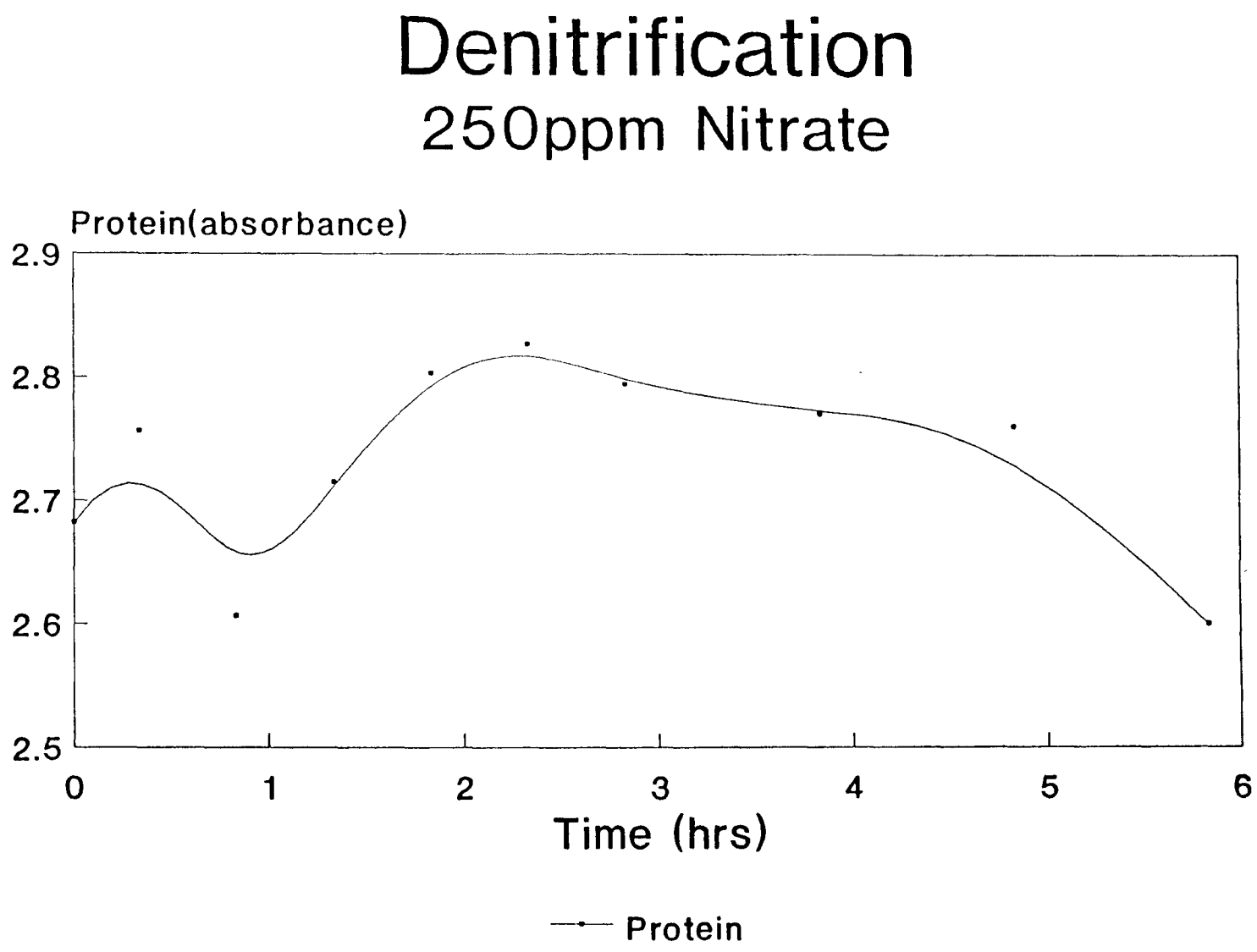

Figure 9.1: Protein Concentration Vs Time (NO_3 = 250ppm)

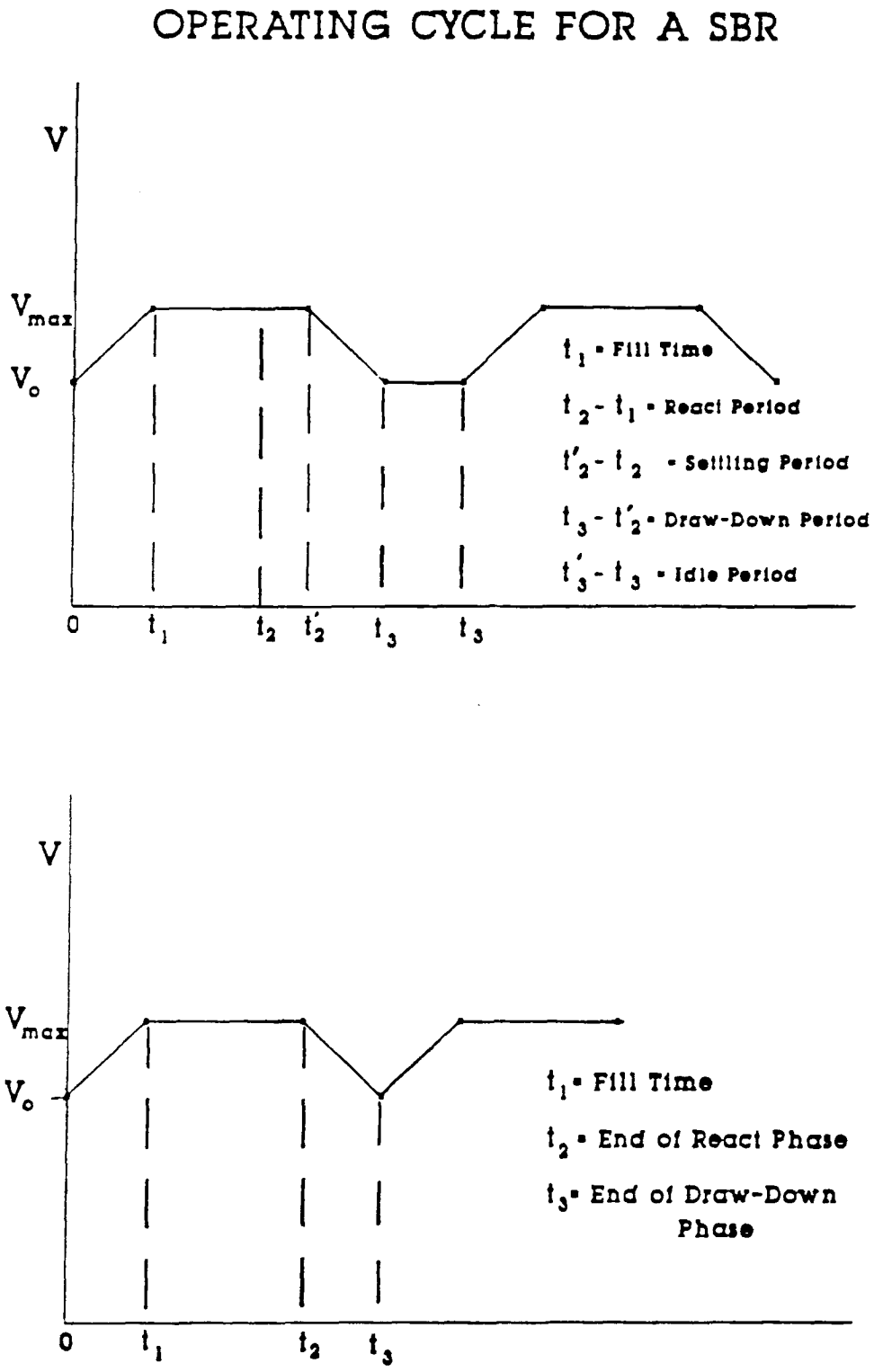

Figure 9.2: Cyclic Operation of A Sequencing Batch Reactor

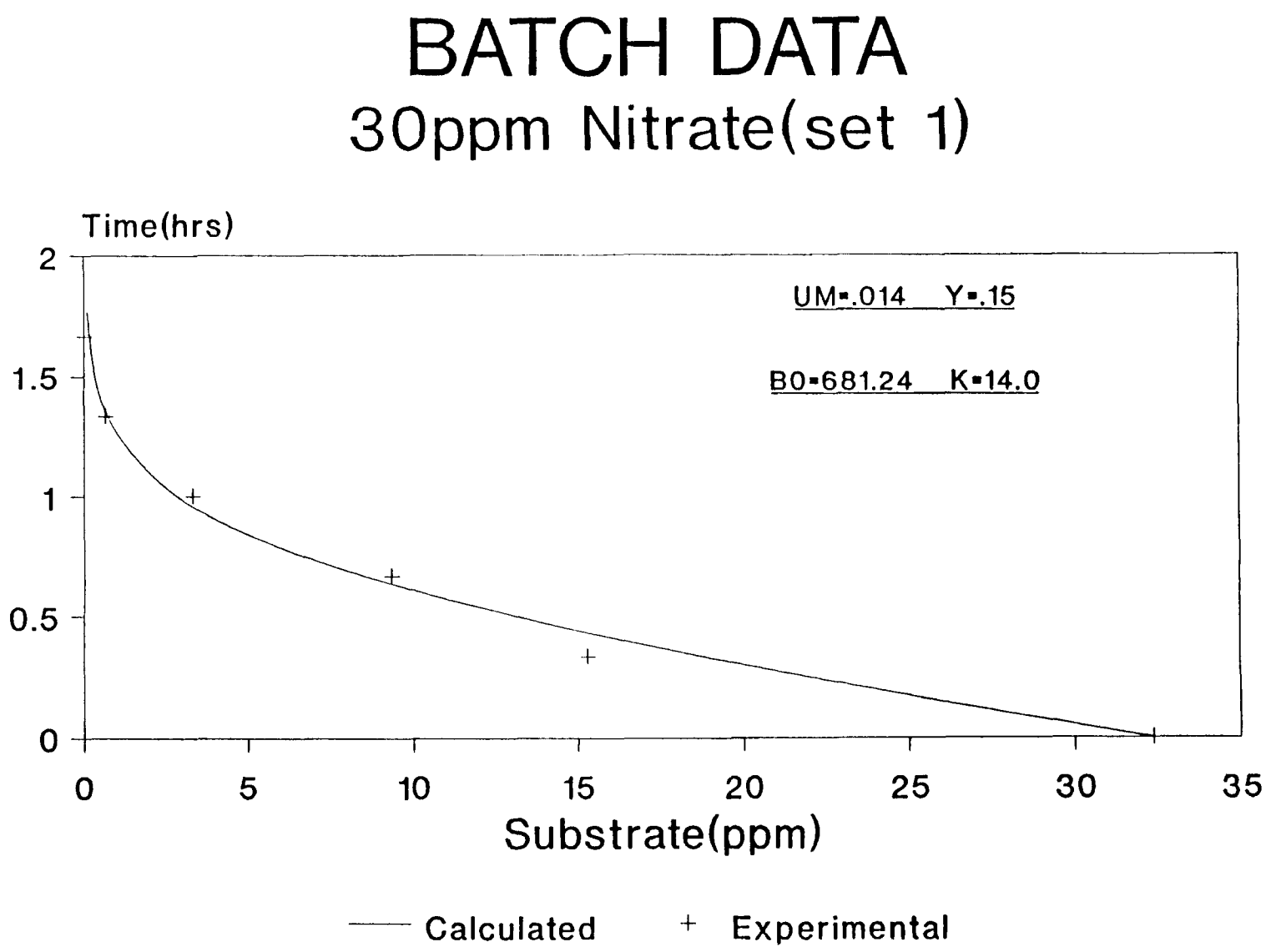

Figure 9.3: Comparison of Experimental Data with Model Predictions (set 1)

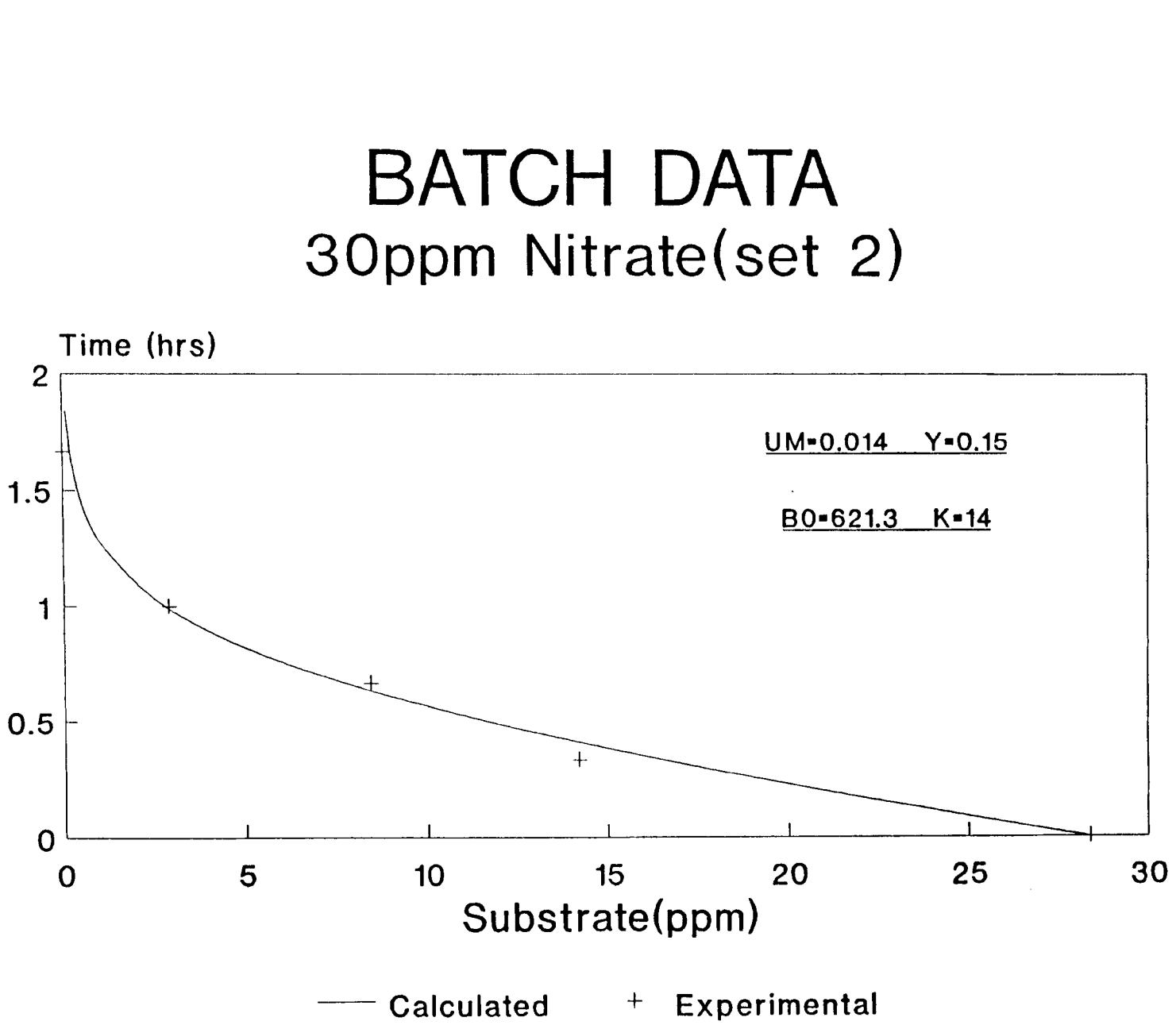

Figure 9.4: Comparison of Experimental Data with Model Predictions (set 2)

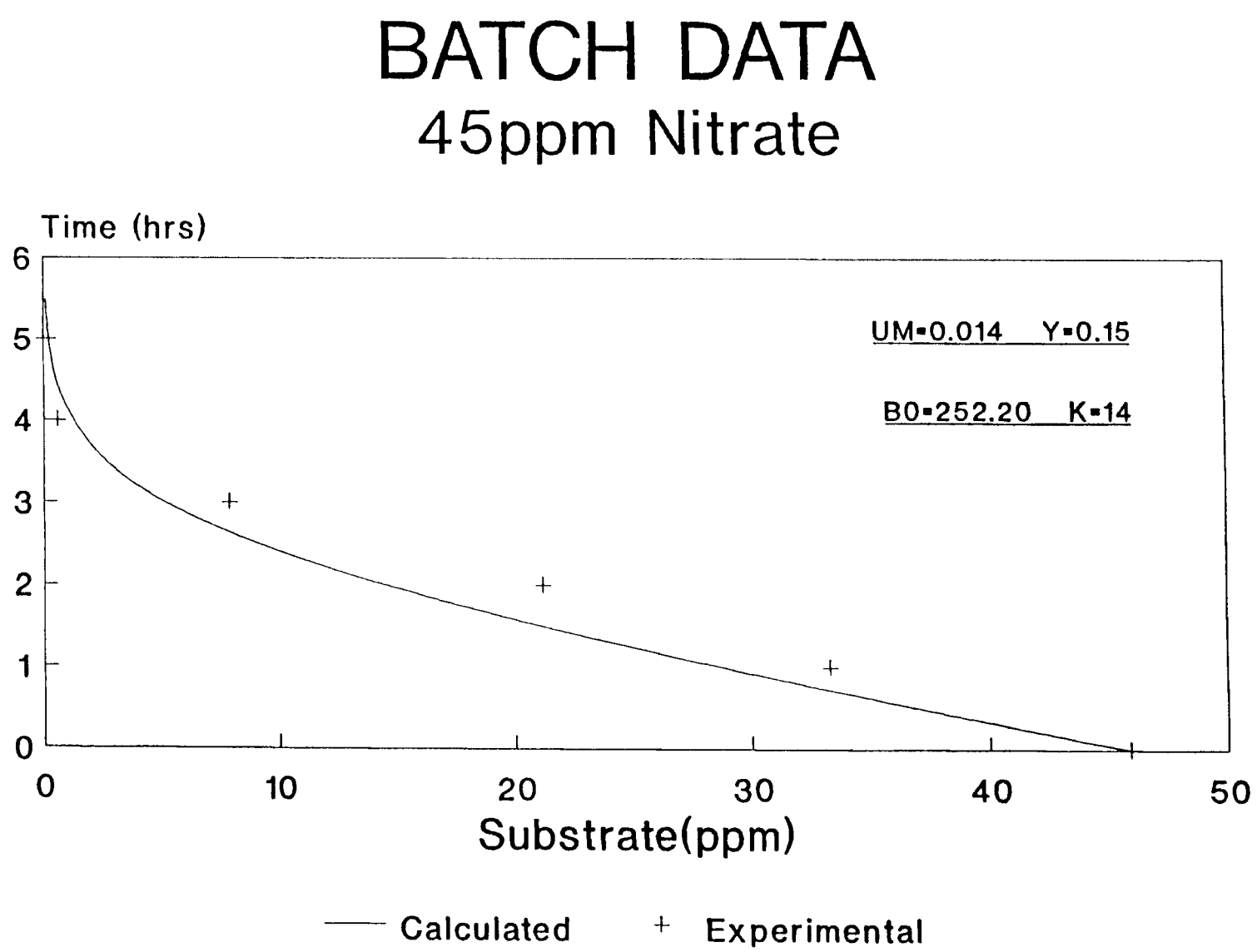

Figure 9.5: Comparison of Experimental Data with Model Predictions (set 3)

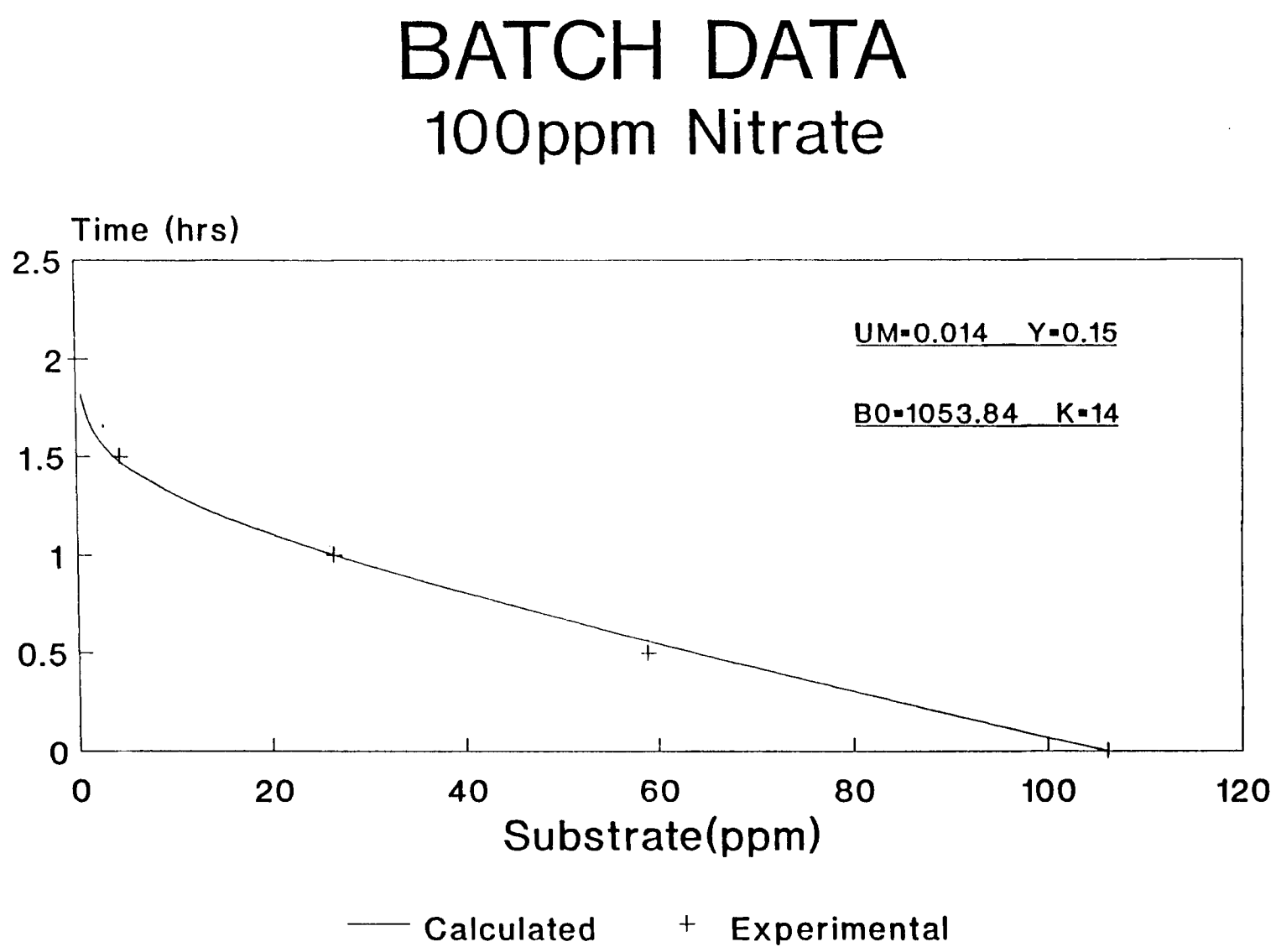

Figure 9.6: Comparison of Experimental Data with Model Predictions (set 4)

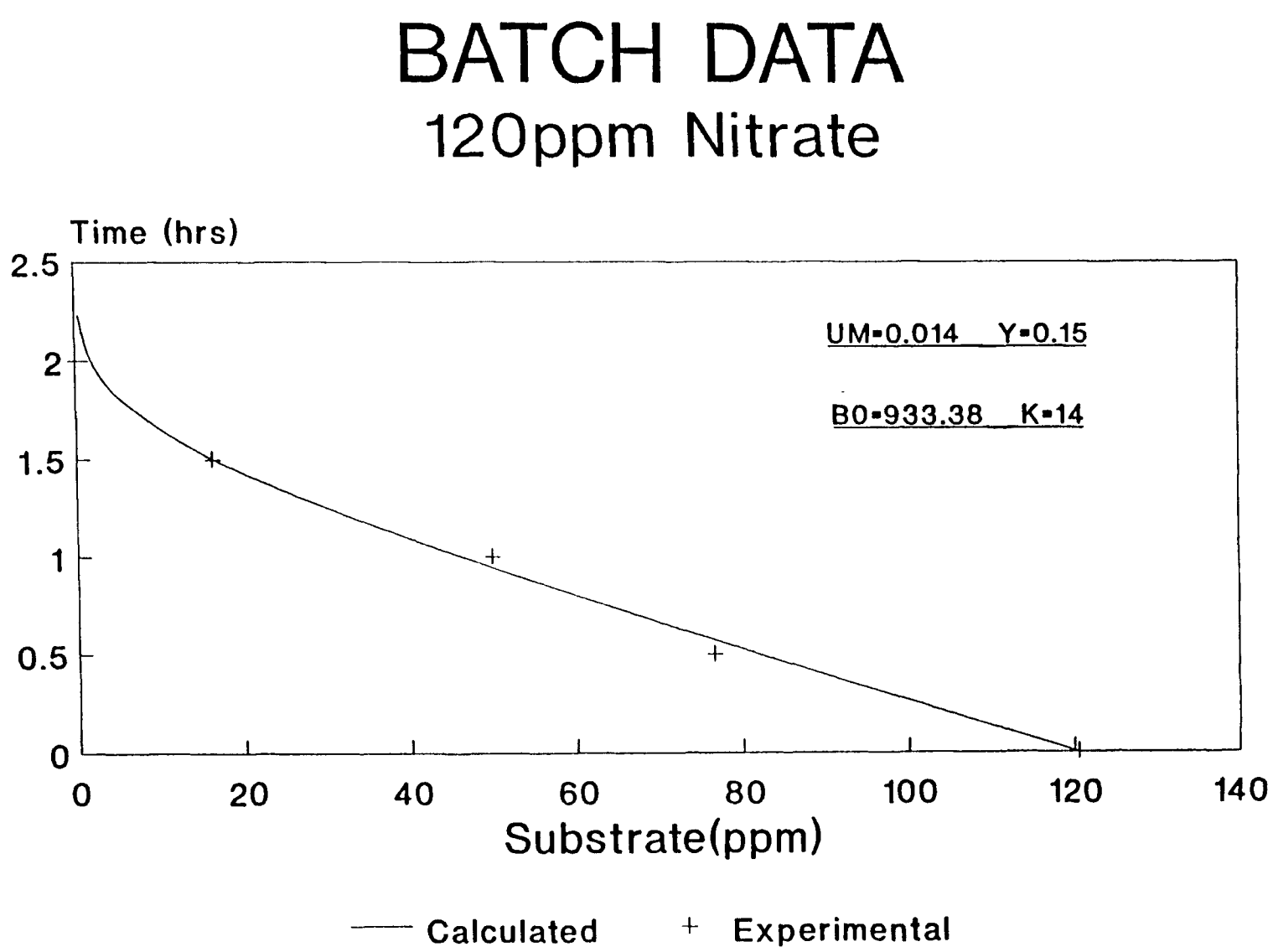

Figure 9.7: Comparison of Experimental Data with Model Predictions (set 5)

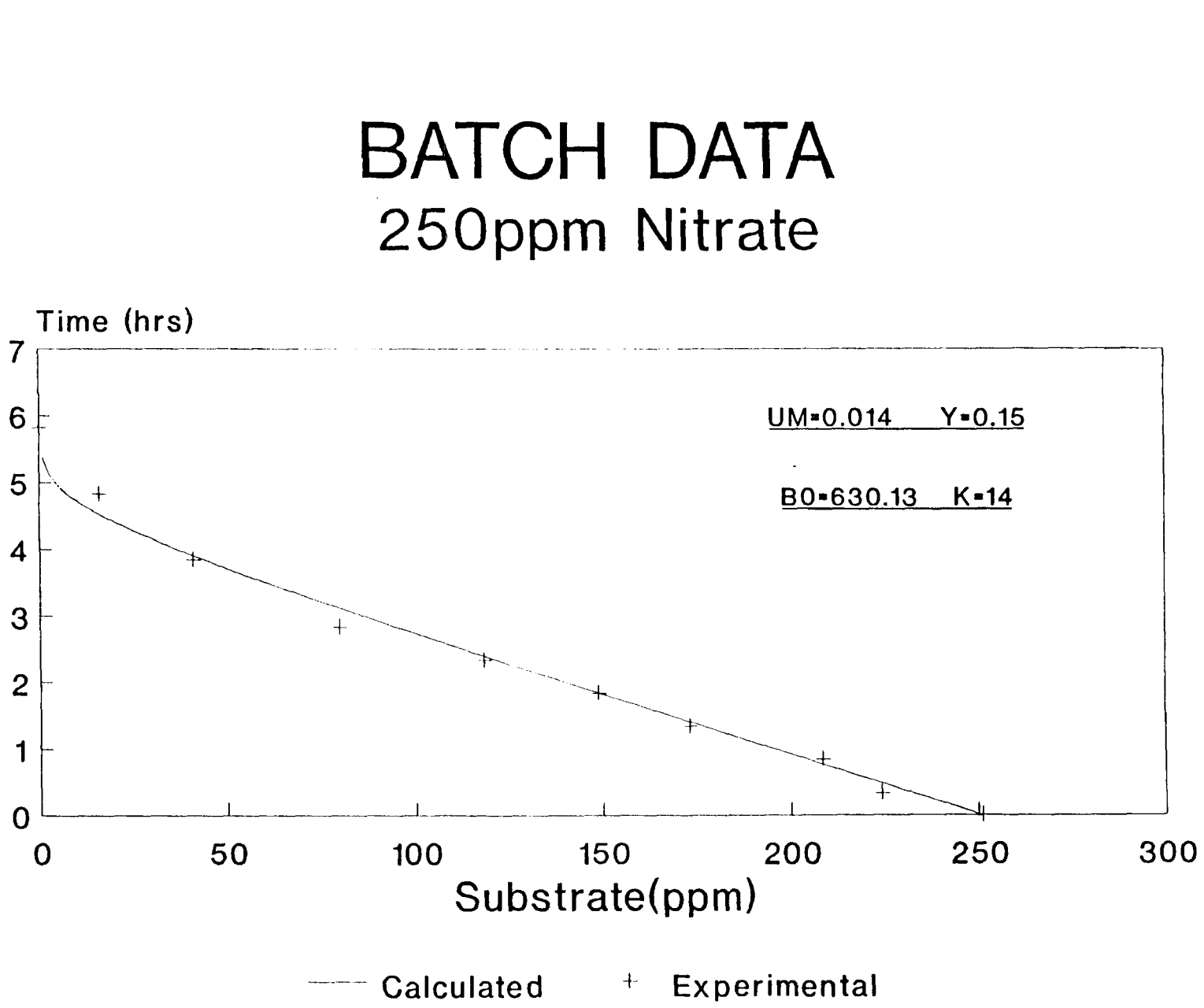

Figure 9.8: Comparison of Experimental Data with Model Predictions (set 6)

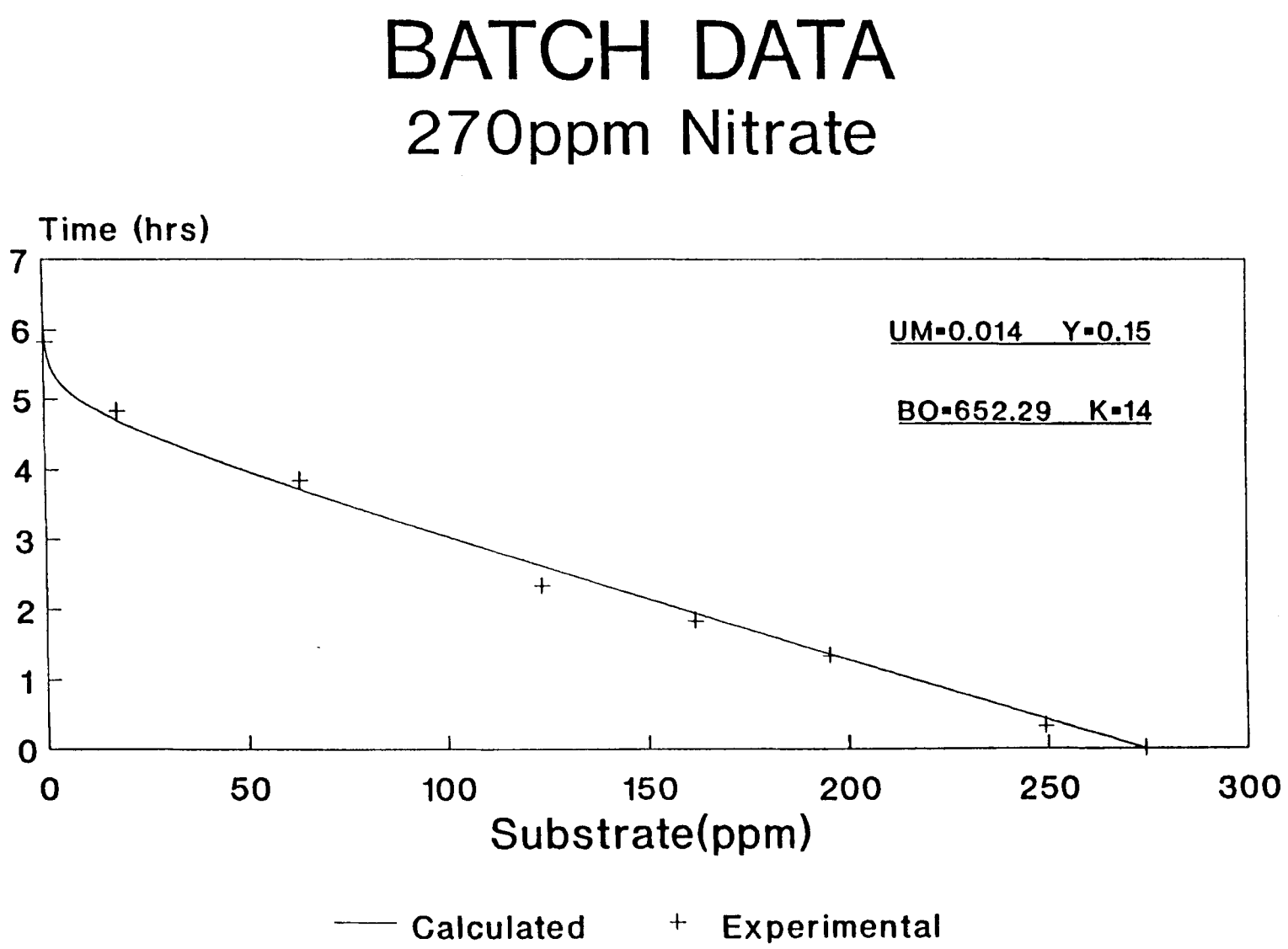

Figure 9.9: Comparison of Experimental Data with Model Predictions (set 7)

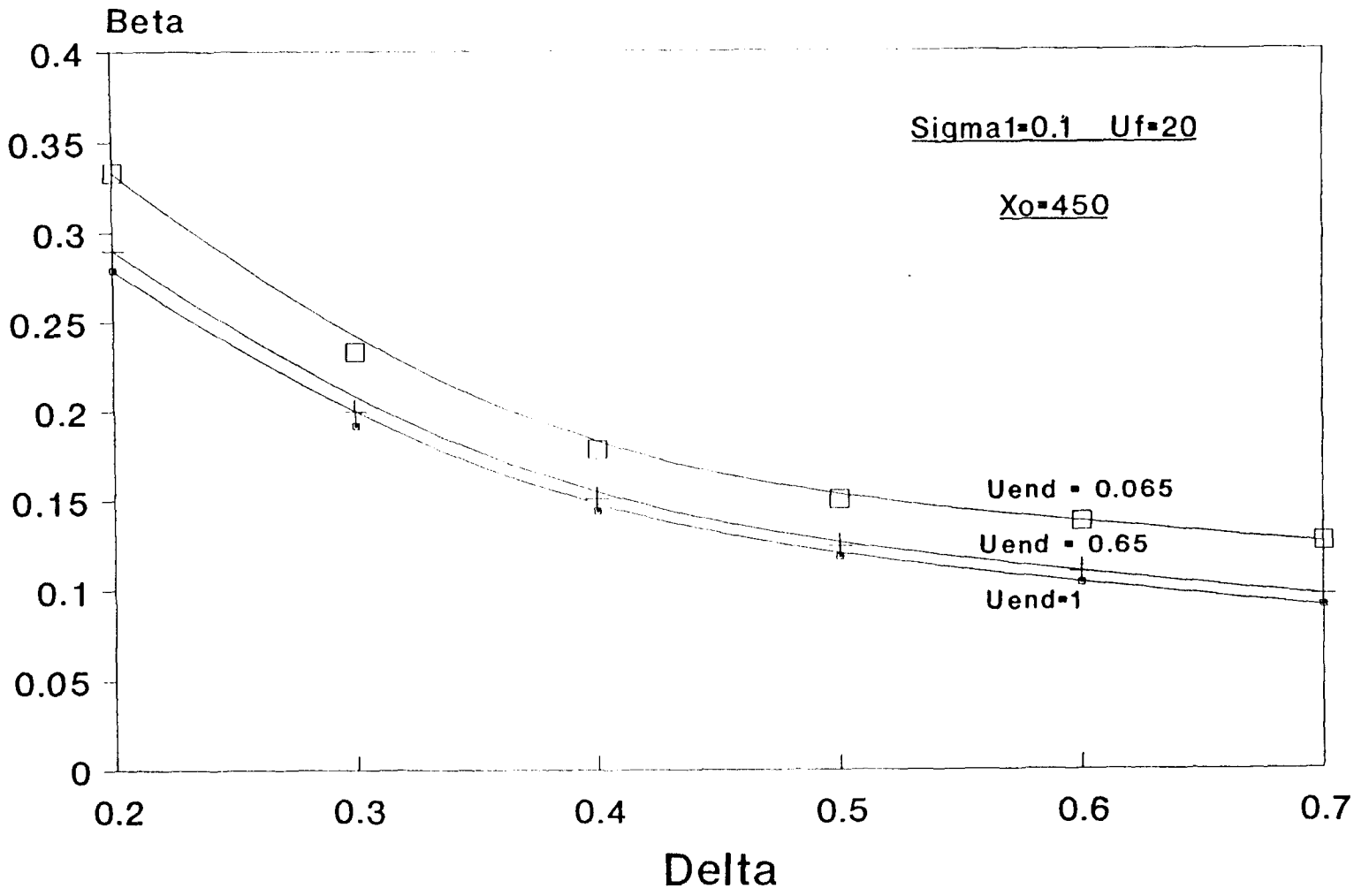

Figure 9.10: Effect of u_{end} and δ on Beta

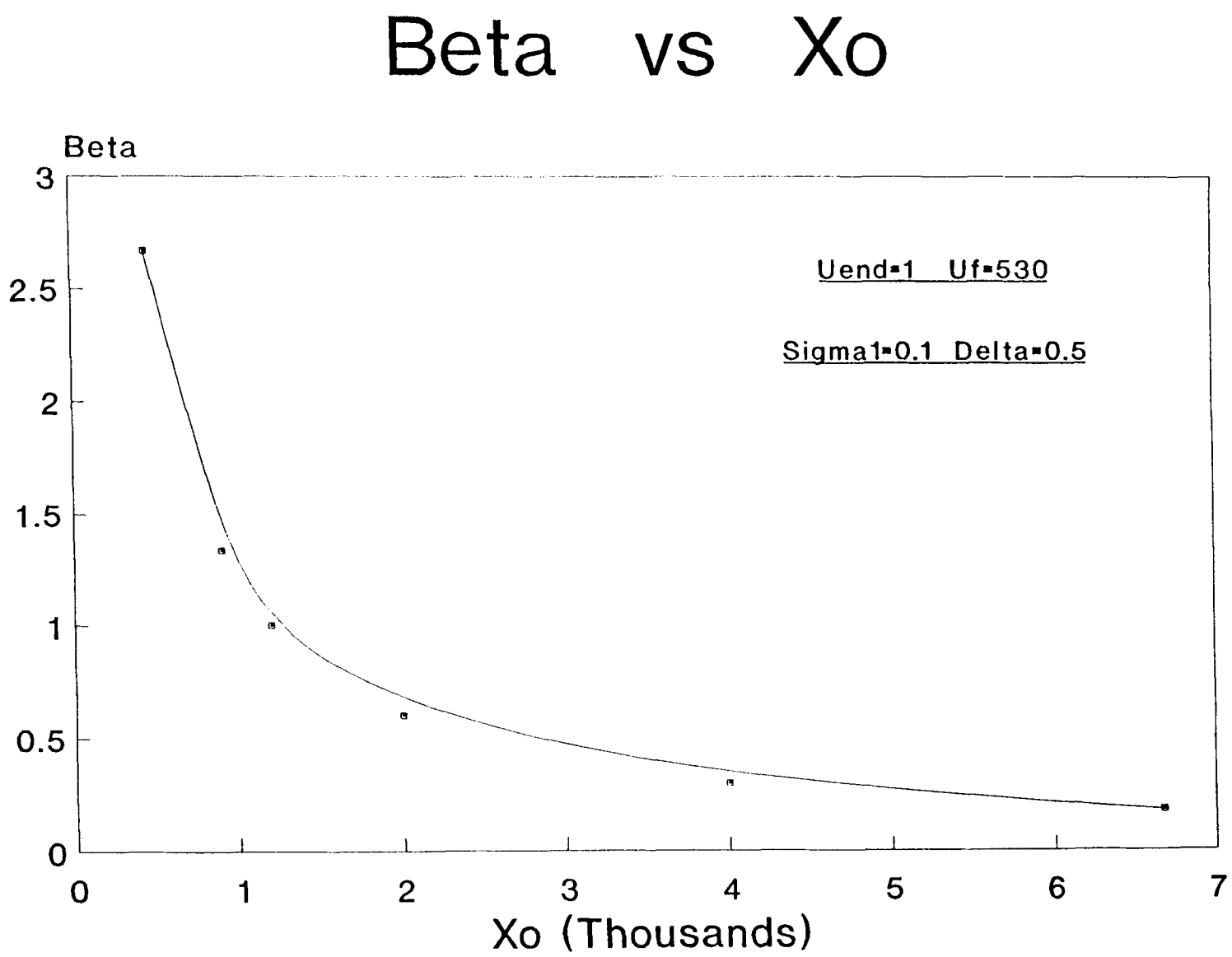

Figure 9.11: Effect of Initial Biomass on Beta

Chapter 10

Program Listing

```
c*****************************************************************
c                                                                 :
c     THIS PROGRAM GIVES THE CONCENTRATION OF SUBSTRATE AND BIOMASS :
c                                                                 :
c                              IN A SBR                           :
c                                                                 :
c                          WITH RESPECT TO TIME.                  :
c                                                                 :
c       HERE RUNGA KUTTA NUMERICAL METHOD IS USED TO  SOLVE  A  SET  OF :
c                                                                 :
c                 NONLINEAR ORDINARY DIFFERENTIAL EQUATIONS.      :
c                                                                 :
c*****************************************************************
c
c_________________________________________________________________
c
c      INITIALIZATION
c
       implicit double precision (a-h,o-z)
c
c
       common tm(100000),um(100000),tmt(100000),umt(100000)
c
c
       open(10,file='[jxw7025.bo]bo.dat', status='old')
       open(20,file='[jxw7025.bo]bo1.dat', status='new')
       open(30,file='[jxw7025.bo]bo2.dat', status='new')
       open(40,file='[jxw7025.bo]bo3.dat', status='new')
c
c----------------INPUT DATA-------------------------------------
c
c
       read(10,*) delta,beta
       read(10,*) uf,x0
       read(10,*) n_pcycle,n_scycle
       read(10,*) step,last
       read(10,*) sigma1
       read(10,*) dif
       read(10,*) ans
c
```

```
c
      time3=(1.0-delta)*0.9
     np=time3/step
c
c
      u0=0.0
      u=u0
c
      u_init=u0
c
c
      if(ans.eq.0.0) then
      n_scycle=1
      endif
c
c
      if (n_pcycle.gt.n_scycle) then
        ncycle=n_pcycle
      else
         ncycle=n_scycle
      endif
c
c
      do 100 icycle=1,ncycle+1,1
c
c
      tm(1)=0.0
      um(1)=u0
c
c
      call process(delta,ncycle,beta,last,u_init,np,icycle,
     &  step,time3,uf,x0,u,sigma1,dif,n_pcycle,n_scycle,u_last)
c
c
      if (ncycle.eq.1) then
      goto 400
      endif
c
```

```
c
      if(icycle.eq.ncycle) then
      goto 100
      endif
c
c
      call new_values(u_last,u0)
c
c--------------here u0 become the initial value for the next cycle----
c
c
      u=u0
c
c-----------------------------------------------------------------------
c
      if(ans.eq.0.0) then
      goto 100
      endif
c
c
      if(icycle.eq.1) then
      goto 32
      endif
c
c
      do 31 ii=1,np+1,1
            if(abs(tmt(ii)-tm(ii)).gt.dif) then
            goto 32
        else  if(abs(umt(ii)-um(ii)).gt.dif) then
            goto 32
c
        endif
c
c
c
      if(ii.eq.np) then
```

```
      call print_3(delta,uf,x0,last,tm,um,u_init,np,n_pcycle,
      &   beta,dif,step,n_scycle,icycle,sigma1)
c
      goto 400
c
      endif
c
c
   31 continue
c
c
   32 do 33 jj=1,np+1,1
c
            tmt(jj)=tm(jj)
            umt(jj)=um(jj)
c
   33 continue
c
c
  100 continue
c
c
  400 stop
      end
c
c
c------------END OF MAIN PROGRAM-----------------------------------
c
c
c---------------SUBROUTINE PROCESS BEGINS HERE--------------------
c
c
c
      subroutine process(delta,ncycle,beta,last,u_init,
     &  np,icycle,step,time3,uf,x0,
     &  u,sigma1,dif,n_pcycle,n_scycle,unxt)
c
c
      implicit double precision(a-h,o-z)
c
c
      common tm(100000),um(100000)
```

```
c
      j=1
c
c
      do 30 time=0.0000000001,time3+1,step
c
c
            j=j+1
c
c
c-------------here we check whether the fill period is over--------------
c
         a=0.0
         b=1.0
      if (time.le.(sigma1*(1.0-delta))) then
         a=1.0
         b=0.0
      endif
c
c-----------------------------------------------------------------------
c
c-----------here we determine the values of substrate and biomass--------
c
          call RungaKutta1(a,b,delta,beta,sigma1,step,time,uf,x0,u,unxt)
c
c-----------------------------------------------------------------------
c
c----------here we store the instantaneous values----------------------
c
c
            um(j)=unxt
            tm(j)=time+step
c
c-----------------------------------------------------------------------
            u=unxt
c
   30 continue
c
c
      if (icycle.eq.1) then
      call print_1(delta,uf,x0,last,tm,um,u_init,np,n_pcycle,
         &   beta,dif,step,n_scycle,sigma1)
      endif
```

```
c
  500 if(icycle.eq.n_pcycle) then
c
      call print_2(delta,uf,x0,last,tm,um,u_init,np,n_pcycle,
     &  beta,dif,step,n_scycle,sigma1)
c
      endif
c
  600 return
      end
c
c
c--------------SUBROUTINE_1 RUNGAKUTTA BEGINS HERE----------------------
c
c
      subroutine RungaKutta1(a,b,delta,beta,sigma1,step,t,uf,x0,u,unxt)
c
      implicit double precision (a-h,o-z)
c
c
c
      fun1(t1,u1) = a*(1/(t1+delta*sigma1))*((uf-u1)-
     &                 (beta*u1*sigma1*delta*x0)/(1.0+u1))
     &             - b*beta*delta*x0*u1/(1.0 + u1)
c
      uk1=step*fun1(t,u)
c
      uk2=step*fun1((t+step/2.0),(u+uk1/2.0))
c
      uk3=step*fun1((t+step/2.0),(u+uk2/2.0))
c
      uk4=step*fun1((t+step),(u+uk3))
c
      unxt=u+(1.0/6.0)*(uk1+2.0*uk2+2.0*uk3+uk4)
c
      return
      end
c
c--------------------END OF RUNGAKUTTA SUBROUTINE----------------------
c
c------------------------------------------------------------------------
```

```
c
c---------------------SUBROUTINE NEW_VALUUES BEGINS HERE----------------
c
c
      subroutine new_values(u_last,u0)
c
c
      implicit double precision (a-h,o-z)
c
c
      u0=u_last
c
c
      return
      end
c
c---------------------SUBROUTINE NEW_VALUUES ENDS HERE-------------------
c
c
c---------------------SUBROUTINE PRINT-1 BEGINS HERE---------------------

c
c
      subroutine print_1(delta,uf,x0,last,tm,um,u_init,np,n_pcycle,
     &  beta,dif,step,n_scycle,sigma1)
c
c
      implicit double precision (a-h,o-z)
c
      dimension tm(100000),um(100000)
c
C-------PRINT INPUT DATA ON OUTPUT FILE-----------------------------
c
c
      WRITE(20,140)
  140 FORMAT('**********************************
     &*****************************************')
      WRITE(20,160)
  160 FORMAT(//10X,' S E Q U E N C I N G   B A T C H   R E A C T O R ')
      WRITE(20,150)
  150 FORMAT(//10X,'CONCENTRATION OF SUBSTRATE AND BIOMASS '//)
```

```
      WRITE(20,140)
      WRITE(20,151)
  151 FORMAT(//10X,'              DURING Ist CYCLE'//)
      WRITE(20,140)
      WRITE(20,201)delta,beta
  201 FORMAT(/1x,'DELTA=',F10.5,4X,'BETA=',F10.5)
      WRITE(20,203)uf,x
  203 FORMAT(/1x,'UF=',F10.5,4X,'X0=',F10.5)
      WRITE(20,204)u_init
  204 FORMAT(/1x,'U0=',F10.5)
      WRITE(20,205) np,step
  205 FORMAT(/1x,'NUMBER OF POINTS IN THE TIME DOMAIN=',I10,10X//
     &        1x,'STEP SIZE =',F10.6//)
      WRITE(20,140)
      WRITE(20,206) sigma1
  206 FORMAT(//1x,'  SIGMA1 = ', F10.5//)
      WRITE(20,140)
      WRITE(20,207)
  207 FORMAT(/1x,'TIME',15X,' U ')
c
C-------PRINT COMPUTED DATA INTO OUTPUT FILE-------------------------
c
c
      do 40 i=1,np+1,last
c
c
           write(20,210) tm(i),um(i)
```

```
  210 format(1X,f10.5,5x,f10.5)
   40 continue
c
c
      return
c
      end
c
c
c---------------------SUBROUTINE PRINT-1 ENDS HERE-----------------------
c
c
c
c
c---------------------SUBROUTINE PRINT-2 BEGINS HERE---------------------
c
c
      subroutine print_2(delta,uf,x0,last,tm,um,u_init,np,n_pcycle,beta,
     &   dif,step,n_scycle,sigma1)
c
c
      implicit double precision (a-h,o-z)
c
      dimension tm(100000),um(100000)
c
c
c-------PRINT INPUT DATA ON THE SECOND OUTPUT FILE---------------
c
c
      WRITE(30,1400)
 1400 FORMAT('**********************************
     &************************************')
      WRITE(30,1600)
 1600 FORMAT(//10X,' S E Q U E N C I N G   B A T C H   R E A C T O R ')
      WRITE(30,1500)
 1500 FORMAT(//10X,'CONCENTRATION OF SUBSTRATE AND BIOMASS '//)
      WRITE(30,1400)
      WRITE(30,1550)n_pcycle
 1550 FORMAT(//10x,'              DURING ',I5,4x,'CYCLES'//)
```

```
      WRITE(30,1400)
      WRITE(30,2010)delta,beta
 2010 FORMAT(/1x,'DELTA='F10.5,4X,'BETA=',F10.5)
      WRITE(30,2030)uf,x0
 2030 FORMAT(/1x,'UF=',F10.5,4X,'X0=')
      WRITE(30,2040)u_init
 2040 FORMAT(/1x,'U0=',F10.5)
      WRITE(30,2050) np,step
 2050 FORMAT(/1x,'NUMBER OF POINTS IN THE TIME DOMAIN=',I5//
     &        1x,'STEP SIZE =',F10.6//)
      WRITE(30,1400)
      WRITE(30,2055) sigma1
 2055 FORMAT(//1x,'  SIGMA1 = ', F10.5//)
      WRITE(30,1400)
      WRITE(30,2060)
 2060 FORMAT(/1x,'TIME',15X,' U ')
c
C-------PRINT COMPUTED DATA INTO OUTPUT FILE-------------------------
c
c
      do 41 i=1,np+1,last
c
c
           write(30,2100) tm(i),um(i)
 2100 format(1X,f10.5,5x,f10.5)
   41 continue
c
c
      return
c
      end
c
c--------------------SUBROUTINE PRINT-2 ENDS HERE---------------------
```

```
c
c---------------------SUBROUTINE PRINT-3 BEGINS HERE--------------------
c
c
      subroutine print_3(delta,uf,x0,last,tm,um,u_init,np,n_pcycle,
     &   beta,dif,step,n_scycle,icycle,sigma1)
c
      implicit double precision (a-h,o-z)
c
      dimension tm(100000),um(100000)
c
c
c-----------PRINT INPUT DATA ON OUTPUT FILE-----------------------------
c
c
      WRITE(40,1401)
 1401 FORMAT('**********************************
     &**************************************')
      WRITE(40,1601)
 1601 FORMAT(//10X,' S E Q U E N C I N G   B A T C H   R E A C T O R ')
      WRITE(40,1501)
 1501 FORMAT(//10X,'CONCENTRATION OF SUBSTRATE AND BIOMASS '//)
      WRITE(40,1401)
      WRITE(40,2052) icycle
 2052 FORMAT(//1x,'STEADY STATE IS REACHED AFTER',I6,4X,'CYCLES'//)
      WRITE(40,1401)
      WRITE(40,2011)delta,beta
 2011 FORMAT(/1x,'DELTA='F10.5,4X,'BETA=',F10.5)
      WRITE(40,2031)uf,x0
 2031 FORMAT(/1x,'UF=',F10.5,4X,'X0=',F10.5)
      WRITE(40,2041)u_init
 2041 FORMAT(/1x,'U0=',F10.5)
```

```
      WRITE(40,2051) np,step
 2051 FORMAT(/1x,'NUMBER OF POINTS IN THE TIME DOMAIN=',I10//
     &        1x,'STEP SIZE =',F10.6//)
      WRITE(40,1401)
      WRITE(40,2053) sigma1,dif
 2053 FORMAT(//1x,'  SIGMA1 = ',F10.5,10x,'DIF=',F10.5//)
      WRITE(40,1401)
      WRITE(40,2061)
 2061 FORMAT(/1x,'TIME',15X,' U ')
c
C-------PRINT COMPUTED DATA INTO OUTPUT FILE-------------------------
c
c
      do 44 i=1,np+1,last
c
c
           write(40,2101) tm(i),um(i)
 2101 format(1X,f10.5,5x,f10.5)
   44 continue
c
c
      return
c
      end
c
c------------------END OF SUBROUTINE PRINT-3-----------------------------
c
```

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