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Kinetic Formulations for Growth and Substrate Uptake in Biological Wastewater Treatment

by
Qiuzi Zhang

A thesis
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To my parents

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ABSTRACT

The Monod or hyperbolic kinetic formulation became the de facto kinetic descriptor in activated sludge models in the ‘80s-‘90s. It still plays a prominent role in activated sludge models; however, a dual hyperbolic formulation (with respect to both substrate and active biomass) that implicitly accommodates storage as well as more rationally describes metabolic kinetics is more prominent in governing metabolic functions in activated sludge models (ASMs) promulgated by working groups under the aegis of the International Water Association (IWA). There are other kinetic formulations used in the models of this group as well as models of the activated sludge process formulated by others. There is lack of consistency in the choice of kinetic formulations for various processes even within the IWA family of models.

This thesis examined the basis for the common Monod and dual-hyperbolic formulations and puts forward theoretical justifications of them based on various considerations of mass transfer, storage and metabolic rate formulation. Other models were developed based on major governing principles.

Models used in biological wastewater treatment are Eulerian gross descriptors of a process involving mass transfer, many substrates, and metabolic pathways and their enzymes, contained within many microorganisms. Any model at this level is merely a fit of mathematical formulations to data. As number of processes in biotreatment models increases along with mathematical descriptors and their associated coefficients, fitting an overall model improves to a point. But beyond this there has been little justification of the Monod or other kinetic expressions. Since a variety of formulations have been proposed for two of the primary metabolic processes which are hydrolysis of complex substrates and then metabolism of resulting readily degradable (or simple) substrates, the primary objective of this study was to examine these processes by experiment and determine the most appropriate models for each step.

A chemostat was used in this study to culture active biomass acclimatized to a feed containing starch, a complex substrate requiring hydrolysis, and glucose which is readily biodegradable and also a product of starch hydrolysis.

Active mass samples were taken from the chemostat and placed in batch reactors where varying concentrations of active mass were exposed to varying concentrations of either glucose or starch. Before adding any substrate to the batch reactors the active mass was aerated for a period of time until DO change was not observed to ensure that any stored or extraneous substrate was metabolized. After addition of one or the other substrate, the rate of dissolved oxygen (DO) uptake was monitored over the initial 15 minutes in the batch culture. Rapidly changing conditions dictated the necessity of using DO as a surrogate for either starch or glucose. Over 249 models were examined for their ability to describe glucose metabolism for 16 different batch experiments. The Monod model gave a good fit to the data. Other models that were equally applicable were too complex or made no scientific sense.

Another series of batch tests were conducted using only starch as a substrate. Again substrate concentration and active mass concentration were varied in the batch tests in six different batch runs. The results from these experiments were again used to determine the adequacy of 249 starch hydrolysis models. The Monod type relationship and a dual hyperbolic relation again proved to be the most reasonable choices. Also a first-order model based on starch concentration was applicable. However no general set of coefficients that applied to all experiments was found for any model; it is necessary to calibrate either model to environmental conditions. All of these models are fairly simple in terms of concept as well as determination of parameters and have scientific sensibility in describing hydrolysis of SBCOD.

Keywords: wastewater, activated sludge, dissolved oxygen, modeling.

TABLE OF CONTENTS

ACRONYMS

ASM	Activated sludge model
COD	Chemical oxygen demand
$CBOD_5$	*5-day carbonaceous biochemical oxygen demand*
MOE	Ministry of Environment
CSTR	Continuous stirred- tank reactor
DO	Dissolved oxygen
HRT	Hydraulic retention time
IAWQ	International Association on Water Quality
IWA	International Water Association
MLSS	Mixed liquor suspended solids
nbd	Nonbiodegradable
OUR	Oxygen uptake rate
SOUR	Specific oxygen uptake rate
SCOD	Soluble COD
SIC	Starch- iodine complex
RBCOD	Readily biodegradable COD
SBCOD	Slowly biodegradable COD
SRT	Solids retention time
SBR	Sequencing batch reactors
ROPEC	Robert O Pickard Environmental Centre
UCT	University of Cape Town
TSS	Total suspended solids
VSS	Volatile suspended solids
VFA	Volatile fatty acid
APAH	American Public Health Association
SM	Standard Methods
MS	Residual Mean Squares

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background

In May 2008, the City of Ottawa was fined $562,000 and convicted under the Ontario Water Resources Act for allowing a spill that could impair water quality and for failing to notify the province's Environment Ministry of the spill in a timely fashion. The Ontario's Ministry of Environment (MOE) has kept the provincial wallet open for Ottawa's sewage treatment plants, despite the worldwide economic free fall. Sewage treatment really matters.

Wastewater treatment processes may be conveniently categorized as physical, chemical, and biological unit operations. Biological wastewater treatment has been used to treat the waste effluents of society for ages. Compared to other treatments, biological wastewater treatment has the following benefits:

- Low capital and operating cost
- True destruction of organics
- A wide variety of organic compounds can be removed
- Removal of inorganic compounds at the same time, such as nitrogen and phosphorus

All biological treatment processes take advantage of bacterias' remarkable ability to use different wastewater constituents to provide energy for microbial metabolism and the building blocks for cell synthesis. A typical process schematic of biological wastewater treatment is shown in Fig. 1.1.

The activated sludge process, including its variations, is the most commonly used secondary treatment process in Ontario. Atmospheric air or pure oxygen (not used very often bacause it is expensive) is bubbled through primary treated sewage (or industrial wastewater) combined with microorganisms to develop a biological floc which reduces the organic content of the sewage. Suspended growth systems (including, lagoons, etc.) and fixed-film systems are also capable of meeting secondary effluent quality [25 mg/L

5-day carbonaceous biochemical oxygen demand ($CBOD_5$) and 15 mg/L total suspended solids (TSS)].

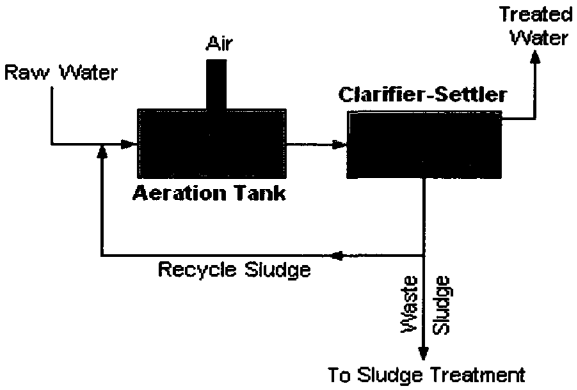


Figure 1.1 A typical biological wastewater treatment process

A process model is a mathematical description of a physical, chemical or biological state or process. All models are deficient in some respects because they are simplifications of reality and many assumptions underlie their formulation. Even though they are imperfect, they are often useful and provide a fairly accurate description and prediction of process behaviour if the major phenomena are correctly incorporated into the model. Using a model can help one understand better process variations.

Modelling is considered to be an important part of design and operation of a wastewater treatment plant. Models can range from very simple ones, such as first-order reactions that describe complex situations, to the International Water Association's (IWA's) complex activated sludge models (ASMs). Selecting the best model is a large issue in designing and running any wastewater treatment process. The goal in using a model is not trying to describe the system perfectly, because this would require too many parameters to be useful. It is better to find the simplest model that comes close to describing the system; the model does not have to be perfect.

The major objectives of this study were to obtain an improved understanding of how active biomass hydrolyzes complex molecules and metabolizes readily degradable substrate produced from hydrolysis. An improved understanding of this behaviour will allow engineers to be more confident in models used to design and operate wastewater treatment plants.

1.2 Scope of the Investigation

Various kinetic formulations for readily biodegradable substrate metabolism and hydrolysis of complex molecules have been used in mathematical models. The theoretical basis of these models varies from formulations that are purely empirical to others that incorporate storage, diffusion or other considerations. The primary objectives of this study were to characterize mathematical expressions that adequately describe hydrolysis and metabolism of readily degradable substrate yielded by hydrolysis. In order to meet these objectives, a combination of lab-scale studies and statistical analyses were carried out. Two substrates were used in this study: starch and glucose to examine the rate of hydrolysis of a complex molecule (starch) into glucose, a readily degraded substrate by one hundred percent active biomass, cultivated in a continuous stirred-tank reactor (CSTR). From 2008 to 2009, approximately 100 samples were taken and analyzed to investigate the most appropriate models that described hydrolysis and substrate uptake rates in an aerated biological wastewater treatment process.

The samples were analyzed for oxygen uptake rate (OUR), by measuring dissolved oxygen (DO) using an Orion 4-Star Plus benchtop pH/DO meter equipped with a polarographic oxygen probe. In addition the samples were characterized with respect to TSS, volatile suspended solids (VSS), pH, total chemical oxygen demand (COD), soluble COD (SCOD) and other wastewater characteristics that impact the behaviour of biomass. Biomass yield factor was calculated by its definition.

CHAPTER 2

LITERATURE REVIEW

2.1 Basic Model

The development of a mathematical model involves compromises to balance conflicting needs. A model has to cover all the major steps occurring within the system. However, the model cannot be so complicated that it may be not solvable. The more complicated the model is, probably the more close it is to reality. In many cases, this may require many assumptions in order to simplify the situation. COD is the superior measure of the concentration of organic material in wastewater because it provides a link between electron equivalents in the organic substrate, the biomass and the oxygen. In other words, a mass balance can be made in terms of COD. The organic matter can be subdivided in to numbers of categories.

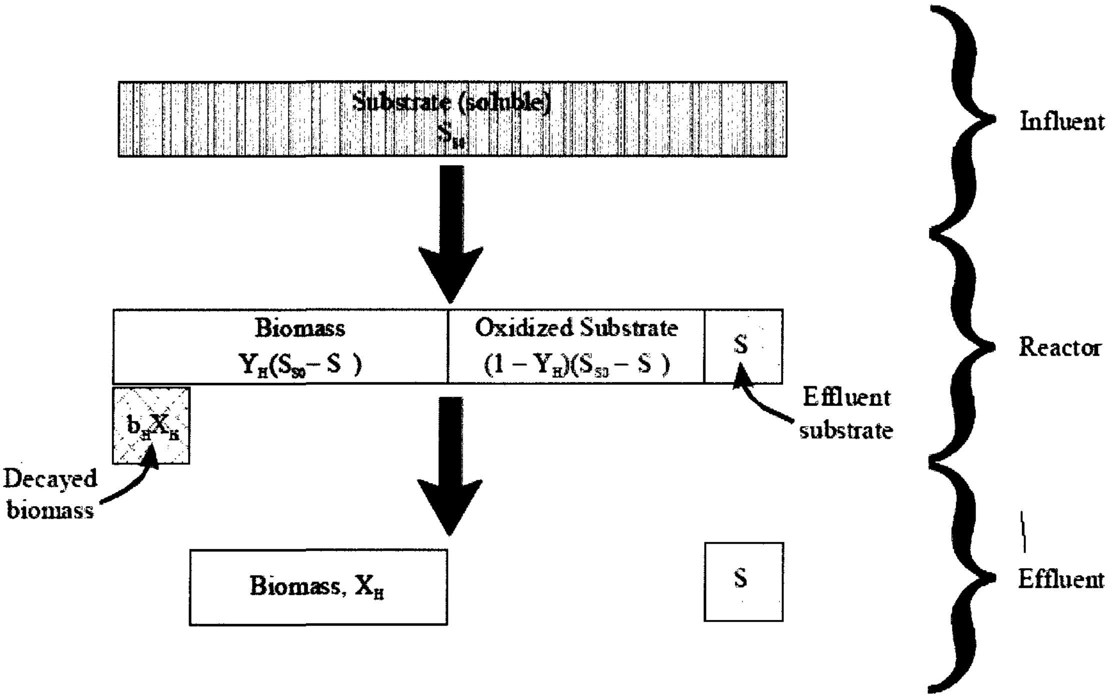


Figure 2.1 Substrate and Biomass Transformations for the Basic Model

A basic model for an activated sludge processes removing organics will be discussed here. It assumes, which is far from the reality, that there are no particulate organics in the influent and all soluble organics are degradable. The processes are depicted in Fig. 2.1. This basic model consists of two parts: biodegradable substrate removal and biomass growth based on substrate removals which are the two major phenomena in a biotreatment process. The matrix shown in Table 2.1 can be used to describe all the variables and processes. The basic model was used to describe biotreatment processes up until the 1970s.

Table 2.1 Basic Model of the Activated Sludge Process for Carbon Removal

Component →	i	1	2	3[a]	4	5	Process Rate[b]
j	Process ↓	S_S	X_H	S_O	S_{NH}	S_{PO4}	ρ_j $[ML^{-3}T^{-1}]$
1	Aerobic growth of heterotrophs	$-\frac{1}{Y_H}$	1	$\frac{1-Y_H}{Y_H}$	$-i_{NBM}$	$-i_{PBM}$	$\frac{\mu_{Hm} S_S}{K_S + S_S} X_H$ [c]
2	Decay of heterotrophs		-1	1	i_{NBM}	i_{PBM}	$b_H X_H$
Observed conversion rates $[ML^{-3}T^{-1}]$		$r_i = \sum_j \nu_{ij}\rho_j$					

a. Oxygen is negative COD and therefore the stoichiometric coefficients refer to oxygen consumption.

b. Any appropriate rate formulation may be used for any process.

c. For all notations, refer to Appendix A

2.2 First Generation Advanced Models

Advanced models were developed to improve the basic models. The most famous one is the University of Cape Town (UCT) model (Wentzel et al., 1992). The UCT model adapted a bisubstrate hypothesis and a death-regeneration hypothesis. It was proposed that the biodegradable COD in the influent wastewater consisted of two fractions: readily biodegradable COD (RBCOD) and slowly biodegradable COD (SBCOD) which necessitated the introduction of a hydrolysis function. The RBCOD consists of simple molecules that can easily pass through a cell wall. The SBCOD, which consists of larger complex molecules, are adsorbed onto the surface of biomass and need to be hydrolyzed before they can be used for metabolism. When microorganisms die, they go into an endogenous decay phase. The model is given in Table 2.2 and shown schematically in Fig. 2.2.

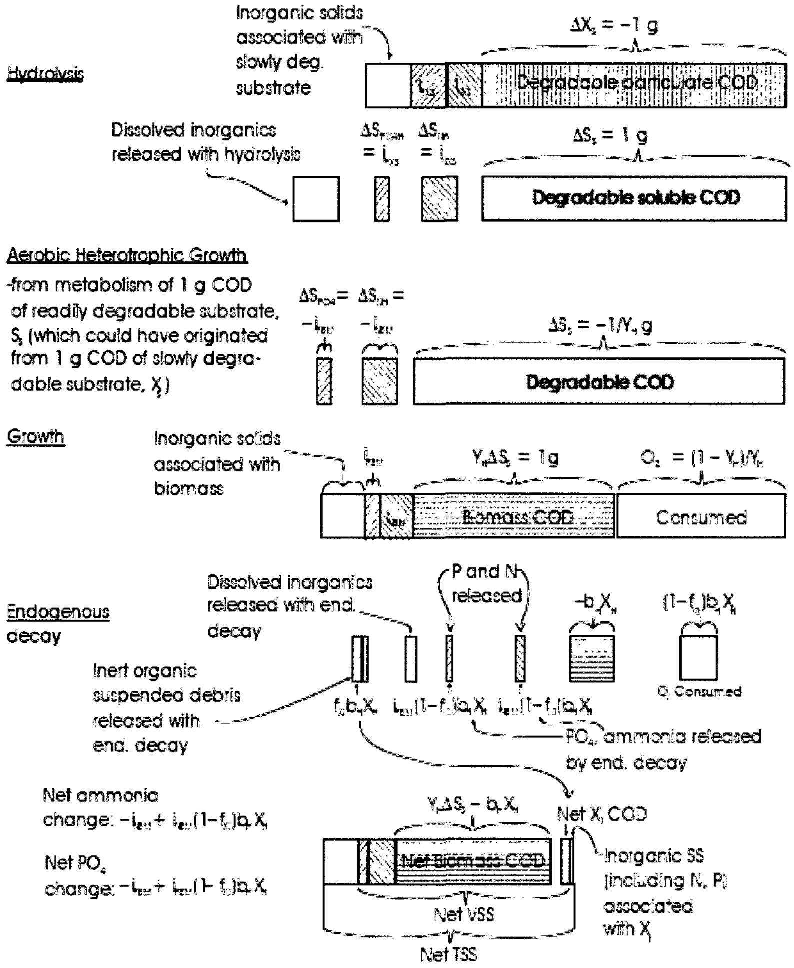


Figure 2.2 Advanced Model Transformations

Initial formulations of the advanced model used Monod kinetics for substrate metabolism and a first-order formulation of hydrolysis. The adoption of these expressions was as much due to chance as theoretical considerations.

Table 2.2 Advanced Model of the Activated Sludge Process for Carbon Removal

Component →	i	1	2	3	4	5	6	7	8	Process Rate
j	Process ↓	S_I	S_S	X_I	X_S	X_H	S_O	S_{NH}	S_{PO4}	ρ_j [$ML^{-3}T^{-1}$]
1	Hydrolysis of particulate organics		1		-1			i_{NXS}	i_{PXS}	$k_h X_S$
2	Aerobic growth of heterotrophs		$-\frac{1}{Y_H}$			1	$\frac{1-Y_H}{Y_H}$	$-i_{NBM}$	$-i_{PBM}$	$\frac{\mu_{Hm} S_S}{K_S + S_S} X_H$
3	Decay of heterotrophs			f_{XI}		-1	$1 - f_{XI}$	$i_{NBM}(1 - f_{XI})$	$i_{PBM}(1 - f_{XI})$	$b_H X_H$

2.3 Literature Review on Activated Sludge Models

The traditional endogenous respiration concept, based on net decay of microorganisms was not sufficient to describe some phenomena, (e.g., rapid OUR that occurred when a reactor was made aerobic after an anaerobic period). The death-regeneration hypothesis was introduced where a major proportion of cells under go lysis and those remaining are able to grow on the degradable lysis products. The International Association on Water Quality (IAWQ which has now become the IWA) task group also proposed some modifications such as substrate storage and enmeshment concepts (See Fig. 2.3). Also, a switching function was introduced into their models to adjust to varying environmental conditions (e.g., between aerobic and anoxic conditions). This is very important for processes depending on different type of electron acceptors. For example, if there is no oxygen available, bacteria, which are responsible for nitrification, are not working, regardless of the concentration of their energy yielding substrate. The switch function adopted by the task group is in the form of $\frac{S_O}{K_S + S_O}$, where S_O is the concentration of DO. Since it is difficult to follow the conceptualization and complex system components, the task group introduced a matrix format, based on the work of Peterson (Henze et al., 2000), for the presentation of stoichiometric and kinetic information of biological reactions, which is easy to read and understand. Also, it allows rapid and easy recognition of the fate of each component. Basically, there are two main fundamental processes that occur: the biomass concentration increases by cell growth and decreases by cell decay. Based on the COD balance, the substrate COD lost from solution due to growth minus the amount converted into new cells must equal the oxygen used for cell synthesis.

Monod kinetics were used for RBCOD metabolism and a dual hyperbolic model was adopted for hydrolysis. Historical developments and a theoretical basis for the dual hyperbolic model as well as the Monod formulation are given in the next chapter. Non-biodegradable organic matter is biologically inert, and passes through an activated sludge system unchanged in form. The fractions of COD can be further divided into soluble and particulate components. Inert soluble organic matter leaves the system with outflow and the concentration does not change. Inert particulate substrate is enmeshed in the floc and is removed by sludge settling and removal. Biodegradable organic matter can be divided into two fractions as well: readily biodegradable and slowly biodegradable. Some slowly biodegradable material may be soluble. Conversion of slowly biodegradable substrate into readily biodegradable substrate does not require energy and thus there is no utilization of an electron acceptor associated with it. Because the hydrolysis rate is lower than substrate utilization rate, it is the rate limiting step. There are 13 components in the ASM No 1 matrix. They are shown across the top and bottom of Table 2.3. Soluble and particulate inert organic matters are not involved in the whole process. However soluble and particulate inert organic matter are in fact important to the performance of the process. Column i = 2, the readily biodegradable substrate, S_S, is removed by heterotroph growth under either an aerobic or anoxic conditions. As well, it is being formed by hydrolysis of entrapped organics. The i = 4 column indicates that slowly biodegradable substrate is removed by hydrolysis of entrapped organics and is being formed by decay. Columns i = 5 and i = 6 represent the heterotrophic and autotrophic biomass in the system. Heterotrophic biomass can be formed by aerobic and anoxic growth. On the other hand, autotrophic biomass can only be formed at aerobic conditions. They are both destroyed by decay. Column i = 7 is the particulate substrate coming from decay of both heterotrophic and autotrophic biomass. The hydrolysis rate is very low. The inert particulate component is introduced into this model because not all the biomass is active. Column i = 8 are phenomena affecting the oxygen concentration in the reactor. Since the matrix only considers biological processes, only the oxygen in solution is being calculated.

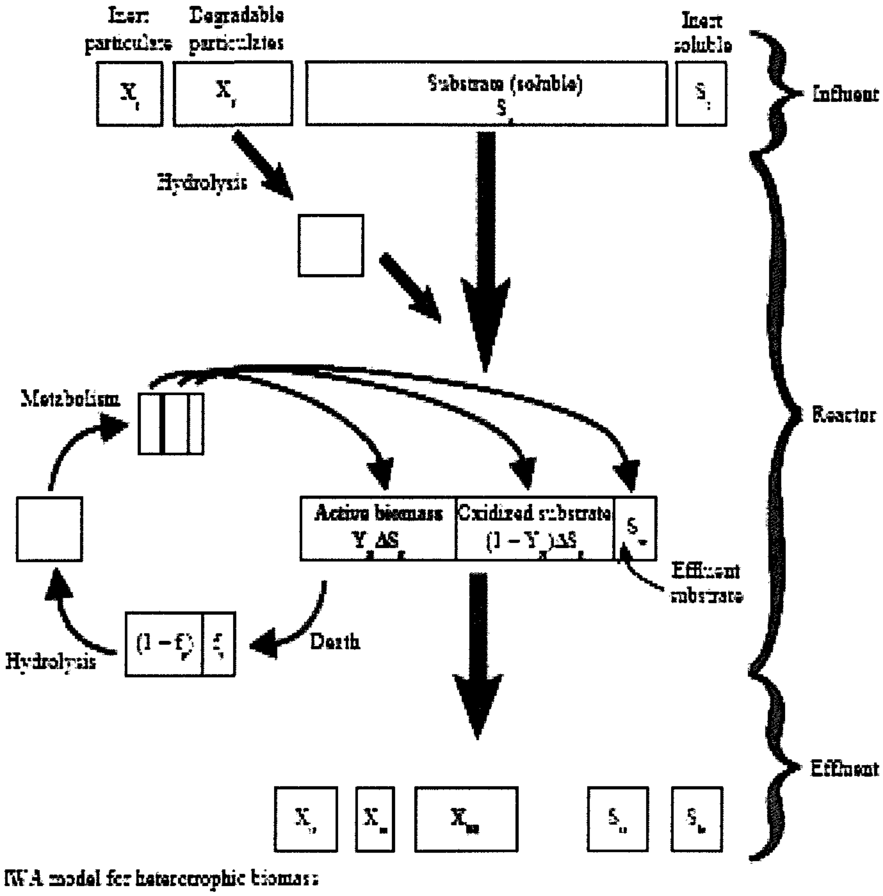


Figure 2.3 IWA Modell (ASM1)

Table 2.3 ASM1, Process Kinetics and Stoichiometry (Henze et al., 2000)

Component →	i	1	2	3	4	5	6	7	8	9	10	11	12	13	Process Rate
j	Process ↓	S_I	S_S	X_I	X_S	X_{BH}	X_{BA}	X_P	S_O	S_{NO}	S_{NH}	S_{ND}	X_{ND}	S_{ALK}	ρ_j [$ML^{-3}T^{-1}$]
1	Aerobic growth of heterotrophs		$-\frac{1}{Y_H}$			1			$-\frac{1-Y_H}{Y_H}$		$-i_{XB}$			$-\frac{i_{NBM}}{14}$	$\mu_{Hm}(\frac{S_S}{K_S+S_S})(\frac{S_O}{K_{OH}+S_O})X_{BH}$
2	Anoxic growth of heterotrophs		$-\frac{1}{Y_H}$			1				$-\frac{1-Y_H}{2.86Y_H}$	$-i_{XB}$			$\frac{1-Y_H}{14*2.86Y_H}-\frac{i_{XB}}{14}$	$\mu_{Hm}(\frac{S_S}{K_S+S_S})(\frac{S_{OH}}{K_{OH}+S_O})(\frac{S_{NOX}}{K_{NOX}+S_{NOX}}$ $\eta_g X_{BH}$
3	Aerobic growth of autotrophs						1		$-\frac{4.57-Y_A}{Y_A}$	$\frac{1}{Y_A}$	$-i_{XB}-\frac{1}{Y_A}$			$-\frac{i_{XB}}{14}-\frac{1}{7Y_A}$	$\mu_{Am}(\frac{S_{NH}}{K_{NH}+S_{NH}})(\frac{S_O}{K_{OA}+S_O})X_{B}$
4	Decay of heterotrophs				$1-f_P$	−1		f_P					$i_{XB}-f_P i_{XP}$		$b_H X_{BH}$
5	Decay of autotrophs				$1-f_P$		−1	f_P					$i_{XB}-f_P i_{XP}$		$b_A X_{BA}$
6	Ammonification of soluble org. nitrogen										1			$\frac{1}{14}$	$k_a S_{ND} X_{BH}$

Table 2.3 continued

7	Hydrolysis of entrapped organics		1		−1							−1			$k_h(\frac{X_S/X_{BH}}{K_X+(X_S/X_{BH})})[(\frac{S_O}{K_{OH}+S_O}) + \eta_h(\frac{K_{OH}}{K_{OH}+S_O})(\frac{S_{NO}}{K_{NO}+S_{NO}})]X_{BH}$
8	Hydrolysis of entrapped organic nitrogen											1	−1		$\rho_7(X_{ND}/X_S)$
Observed conversion rates $[ML^{-3}T^{-1}]$		$r_i = \sum_j v_{ij}\rho_j$													
Units		M [COD/L^3]	M [COD/L^3]	M [COD/L^3]	M [COD/L^3]	M [COD/L^3]	M [COD/L^3]	M [COD/L^3]	M [COD/L^3]	M [(N)/L^3]	M [(N)/L^3]	M [(N)/L^3]	M [(N)/L^3]		

The basic processes incorporated in ASM1 are listed on the left hand side. Basically, there are four processes: biomass growth, biomass decay, nitrification and hydrolysis. Row 1 shows that biomass growth occurs at the expenses of soluble substrate. This step consumes oxygen. Rows 2 and 3 represent anoxic growth and autotrophic biomass growth, which is not being discussed in this paper. Row 4 is the death-regeneration concept. It can be seen that the rate expression is very simple: first-order with respect to biomass concentration. No loss of COD and electron acceptor is involved. In an activated sludge system, increase in sludge age will result in decrease of sludge production. This phenomenon is related to endogenous respiration processes. In activated sludge models, cell lysis is incorporated. The particulate substrate is formed and then converted to soluble substrate. After this, it is used by biomass. Soluble substrate biosorption, accumulation, storage and regeneration mechanisms are not included in ASM1. In ASM3 (see Table 2.4), bacteria can store substrate as polymers and use it when there is an absence of external substrate. The conversion of stored substrate is described as endogenous respiration- oxidation of their own tissue.

Orhon (2003) presented the scientific basis for the design of small activated sludge systems. The study presented an evaluation of the oxygen requirement and sludge production based on mass balance. Most of the models nowadays define substrate utilization and endogenous decay as major processes. The electron acceptor, such as oxygen and nitrogen, and sludge production are essential to activated sludge system design. Even though the ASM1 is used widely, it cannot distinguish SBCOD presented in the influent and produced by the decay process. The endogenous decay mechanism, which is defined as a process consuming electron acceptor and producing soluble and particulate products, has been adapted in ASM1.

Table 2.4 ASM3, Process Kinetics and Stoichiometry (Henze et al., 2000)

Component →		1	2	3	4	5	6	7	8	9	10	11	12	13	Process Rate, □$_j$ $[ML^{-3}T^{-1}]$
j	Process ↓	S_O	S_I	S_S	S_{NH}	S_{N2}	S_{NO}	S_{HCO}	X_I	X_S	X_H	X_{STO}	X_A	X_{TS}	
1	Hydrolysis		f_{SI}	$1-f_{SI}$	$i_{NXS} - f_{SI}i_{NSI}$ $- (1 - f_i)i_{NSS}$			$\frac{i_{NXS}-f_{SI}i_{NSI}-(1-f_I)i_{NSS}}{14}$		-1				$-i_{TSXS}$	$k_h\left(\frac{X_S/X_H}{K_X+(X_S/X_H)}\right)X_H$
2	Aer. storage of COD	$-1+Y_{STO}$		-1	i_{NSS}			$\frac{i_{NSS}}{14}$				$Y_{STO,O2}$		$0.6Y_{STO,O2}$	$k_{STO}\left(\frac{S_S}{K_S+S_S}\right)\left(\frac{S_O}{K_O+S_O}\right)X_H$
3	Anox. storage of COD			-1	i_{NSS}		$-\frac{1-Y_{STO,NO}}{2.86}$	$-\frac{i_{NSS}}{14}+\frac{1\quad Y_{STO,NO}}{14\times 2.86}$				$Y_{STO,NO}$		$0.6Y_{STO,NO}$	$k_{STO}\eta_{NO}\left(\frac{S_S}{K_S+S_S}\right)\left(\frac{K_O}{K_O+S_O}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)X_H$
4	Aerobic growth of heterotrophs				$-i_{NBM}$			$-\frac{i_{NBM}}{14}$			1	$-\frac{1}{Y_{H,O2}}$		$i_{TSBM}-\frac{0.6}{Y_{H,,O2}}$	$\mu_H\left(\frac{S_O}{K_O+S_O}\right)\left(\frac{S_{NH}}{K_{NH}+S_{NH}}\right)\left(\frac{S_{HCO}}{K_{HCO}+S_{HCO}}\right)$ $\times\left(\frac{X_{STO}/X_H}{K_{STO}+(X_{STO}/X_H)}\right)X_H$
5	Anoxic growth of heterotrophs				$-i_{NBM}$		$\frac{1-1/Y_{H,NO}}{2.86}$	$-\frac{i_{NBMS}}{14}+\frac{1\quad 1/Y_{H,NO}}{14\times 2.86}$			1	$-\frac{1}{Y_{H,NO}}$		$i_{TSBM}-\frac{0.6}{Y_{H,NO}}$	$\mu_H\eta_{NO}\left(\frac{K_O}{K_O+S_O}\right)\left(\frac{S_{NH}}{K_{NH}+S_{NH}}\right)\left(\frac{S_{HCO}}{K_{HCO}+S_{HCO}}\right)$ $\times\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\left(\frac{X_{STO}/X_H}{K_{STO}+(X_{STO}/X_H)}\right)X_H$
6	Aer. endog. resp. of heterotrophs	$f_I - 1$			$i_{NBM}- f_Ii_{NSI}$			$\frac{i_{NBM}-f_Ii_{NSI}}{14}$	f_I		-1			$f_Ii_{TSXI} - i_{TSBM}$	$b_{HO2}\left(\frac{S_O}{K_O+S_O}\right)X_H$
7	Anox. endog. resp. of heterotrophs				$i_{NBM}- f_Ii_{NSI}$		$-\frac{1-f_I}{2.86}$	$\frac{i_{NBM}-f_Ii_{NSI}}{14}+\frac{1\quad f_I}{14\times 2.86}$	f_I		-1			$f_Ii_{TSXI} - i_{TSBM}$	$b_{HNO}\left(\frac{S_O}{K_O+S_O}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)X_H$

Table 2.4 continued

8	Aer. resp. of stored organics	−1										−1		−0.6	$b_{STO,O2}\left(\frac{S_O}{K_O+S_O}\right)X_{STO}$ $b_{STO,O2} > b_{H,O2}$
9	Anoxic resp. of stored organics						$-\frac{1}{2.86}$	$\frac{1}{14\times 2.86}$				−1		−0.6	$b_{STO,NO}\left(\frac{K_O}{K_O+S_O}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)X_{STO}$ $b_{STO,NO} > b_{H,NO}$
10	Aer. growth of autotrophs	$-\frac{4.57-Y_A}{Y_A}$			$-\frac{1}{Y_A}+i_{NBM}$		$\frac{1}{Y_A}$	$-\frac{1}{14Y_A}+\frac{i_{NBM}}{14}$					1	i_{TSBM}	$\mu_A\left(\frac{S_O}{K_{AO}+S_O}\right)\left(\frac{S_{NH}}{K_{ANH}+S_{NH}}\right)\left(\frac{S_{HCO}}{K_{HCO}+S_{HCO}}\right)X$
11	Aer. endog. resp. of autotrophs	f_I-1			$i_{NBM}-f_I i_{NSI}$			$\frac{i_{NBM}-f_I i_{NSI}}{14}$	f_I			$i_{XB}-f_P i_{XP}$	−1	$f_I i_{TSXI}-i_{TSBM}$	$b_{AO2}\left(\frac{K_O}{K_O+S_O}\right)X_A$
12	Anox. endog. resp. of autotrophs				$i_{NBM}-f_I i_{NSI}$	$\frac{1-f_I}{14\bullet 2.86}$	$-\frac{1-f_I}{2.86}$	$\frac{i_{NBM}-f_I i_{NSI}}{14}+\frac{1\ \ f_I}{14\times 2.86}$	f_I				−1	$f_I i_{TSXI}-i_{TSBM}$	$b_{ANO}\left(\frac{K_O}{K_O+S_O}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)X_A$

2.4 Respirometry

Respirometry, as a tool, can be used to measure characteristics of wastewater and several other things. Spanjers and Vanrolleghem (1995) presented methodology to use a respirometor to determine wastewater and sludge characteristics. The procedure is based on respirometric measurements at low substrate to biomass ratio (*S/X*). They demonstrated that the most important parameter in batch experiments is the ratio of initial substrate to initial biomass concentration. An optimal ratio needs to be obtained because a very low S/X ratio may lead to low measuring frequencies, and a very high S/X ratio results in growth of biomass so that the obtained kinetic parameters are no longer representative of the initial biomass.

The respirometer based test can also be used for determining influent COD, which normally requires a large dataset for calibration. Witteborg et al. (1996) developed a method for a respiration meter which can be used to determine influent substrate concentration, and the results were compared with classical lab analysis. Three different kinds of measurements were considered: endogenous conditions and intermediate and high substrate load conditions. There are three respiration rate measurements, 4 equations and 6 unknown variables. Relatively simple methods were proposed by Kappeler and Gujer (1992) to determine YH and KS based on 4 unknowns. Numerical computer software was used to solve these equations. Xu (1996) used a single OUR method (based on one single measurements of oxygen utilization) compared with batch OUR method to measure RBCOD in wastewater. It was observed that the DO dropped dramatically at the beginning of the test, which reflects the utilization of RBCOD. However, Xu (1996) pointed out that methods for characterizing the organic fractions in wastewater are still under development, and are not standardized. In Xu's experiments, all the activated sludge was subjected to pre-aeration for such a long time that the OUR of sludge reached a constant (OUR does not change with time).

Boursier (2004) used respirometry to calibrate ASM1. Nowadays, ASM1 is widely used to describe aerobic industrial wastewater treatment and municipal wastewater treatment systems. However, there has been no research using ASM1 to simulate agriculture wastewater treatment processes. The simulation of a specific treatment process using ASMs require (1) the characterization of the influent organic and nitrogen fractions, (2) the determination of the physical characteristics and operating conditions of the process, and (3) knowledge of the kinetic and stoichiometric constants involved in the model equations.

Orhon (1998) stated that almost all the activated sludge models for organic carbon removal involve a hydrolysis step to quantify the fate of SBCOD as a model component. Since there are several kinds of SBCOD in municipal or industrial wastewaters, it is sometimes misleading to have only one model to describe all hydrolysis processes. As a result, dual hydrolysis models (incorporating readily hydrolysed and slowly hydrolysed substrates) need to be used.

Boursier et al. (2004) tested three different models to describe the hydrolysis process. Respirometric tests were conducted with a pure readily biodegradable substrate [volatile fatty acid (VFA)] and piggery wastewater at various temperatures and pH. The determination of the sludge yield factor was determined by using a synthetic substrate solution containing acetic, propionic and butyric acids at respective concentration of 50, 25 and 25 mg COD/L, respectively. The proportions used for the preparation of the synthetic solution corresponded approximately to the VFA proportions in piggery wastewater. The aerobic respirometric tests were performed using a 10 L reactor and a 400 mL DO measuring chamber. The oxygen concentration was monitored with a DO probe and the pH and temperature were also recorded. pH was adjusted by adding acid (1M H_3PO_4) and base (1M NaOH) solutions. Three hydrolysis models (simple to complex) were used to simulate the aerobic respirometric curves and the most appropriate one was chosen (see Table 2.5 and Fig. 2.4).

Table 2.5 Hydrolysis Models Tested (Boursier et al. (2004))

Model Reference	Hydrolysis Equation	
Model 1	$\frac{dX_S}{dt} = k_h \frac{X_S / X_{BH}}{K_X + X_S / X_{BH}} X_{BH}$	Surface-limited hydrolysis (Henze et al. 2000)
Model 2	$\frac{dX_S}{dt} = -K_H X_S$	Simplified hydrolysis (case $X_S << X_{BH}$))
Model 3	$\frac{dS_H}{dt} = -K_{HR} S_H$ [a]	Dual hydrolysis (derivated from Orhon et al., 1998)
	$\frac{dX_S}{dt} = -K_{HS} X_S$ [b]	

a K_{HR} is readily hydrolysis substrate rate constant

b K_{HS} is slowly hydrolysis substrate rate constant

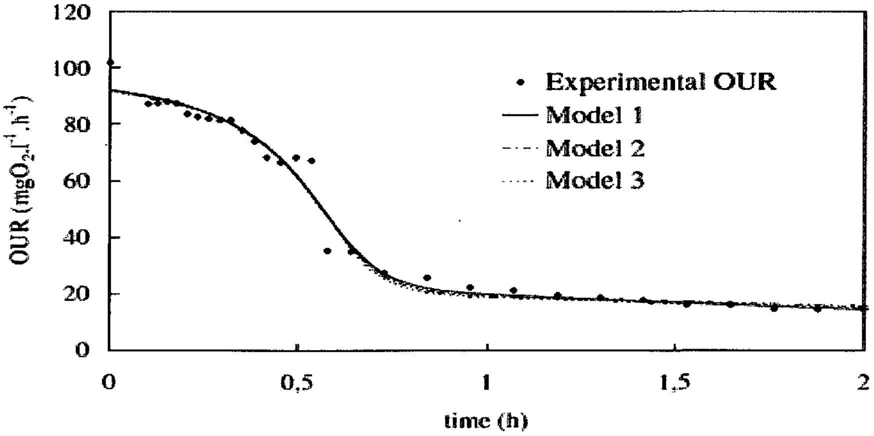


Figure 2.4 Model Calibration of the Respirometric Curve

It can be seen from the curve that there are no large differences among the three models, which all simulated the experiments successfully. Consequently, model No. 2 was selected due to its simplicity.

Delfin (1994) stated that SBCOD are large organic molecules that are degraded slowly because they need to be hydrolysed before they can be metabolized. He used starch as SBCOD and found that after activated sludge has been well acclimatized to starch, the hydrolysis rate was strongly affected by the final electron acceptor. Two laboratory scale sequencing batch reactors (SBR) were used to acclimatize the raw sludge. The operation sequence and configuration of the SBR are shown at Table 2.6 and Fig. 2.5 respectively.

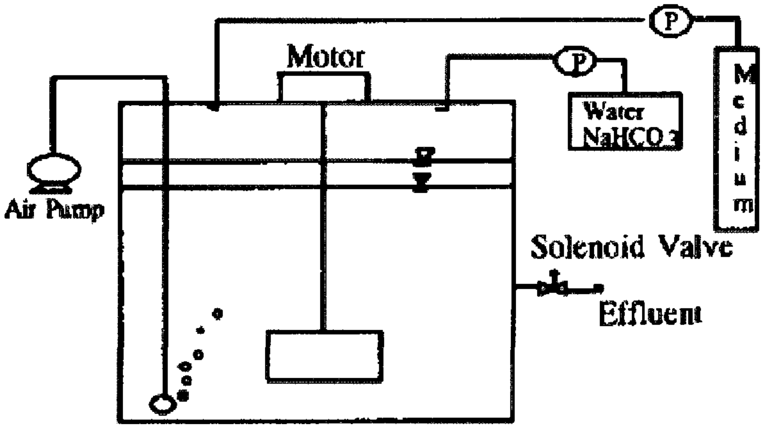


Figure 2.5 Configuration of SBR Reactor (adapted from Delfin 1994) configured to cycle the following events: (1) Fill (2) Mix (anoxic or Anaerobic) (3) Aerate (4) Settle and (5) Decant modes

Table 2.6 SBR Reactor Cycle times

Cycle	1	2	3	4	5
Duration (h)	0.2	1.75	3.0	0.8	0.25

The determination of starch was carried out by using the starch- iodine complex formation (SIC) method (Stauffer et al., 1989). The Anthrone method (Hamilton 1956) was used to determine glucose concentration. Figure 2.6 shows the fractionations and measurements schemes and Table 2.7 shows the possible implication of the fractions determined by the SIC and Anthrone methods.

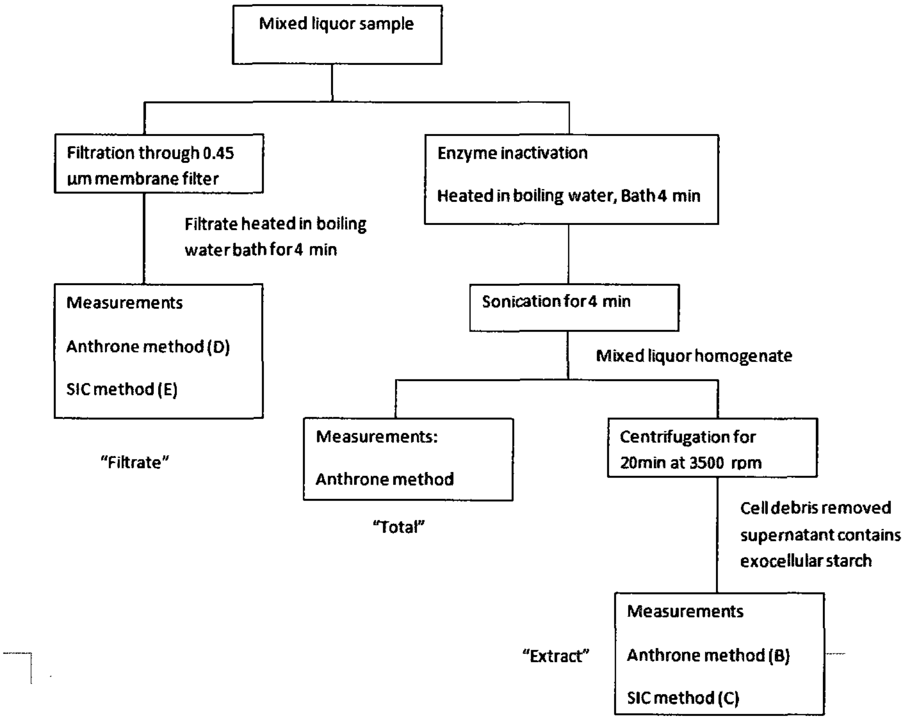


Figure 2.6 Schematic of Sample Treatments and Measurements

Table 2.7 Fractions Determined by SIC and Anthrone Methods

	Fraction	Analysis	Bulk		Exocellular		Intracellular		
			Starch	Glucose	Starch	Glucose	Glucose	Glycogen	Cell
A	Total	Anthrone	0	0	0	0	0	0	0
B	Extract	Anthrone	0	0	0	0	0	PA	NA
C		SIC	0	NA	0	NA	NA	PA	NA
D	Filtrate	Anthrone	0	0	NA	NA	NA	NA	NA
E		SIC	0	NA	NA	NA	NA	NA	NA

Note: 0- accounted NA- not accounted PA- maybe partially accounted

Filtrate starch was no longer in the bulk solution. This indicated that the starch was adsorbed to the biomass rapidly. However, there was almost no accumulation of extracellular starch observed in the sludge. This indicated that the adsorbed sludge was instantaneously hydrolysed to RBCOD, like glucose. This may be attributed to extracellular enzymes. Figure 2.7 conceptualizes how starch was being utilized.

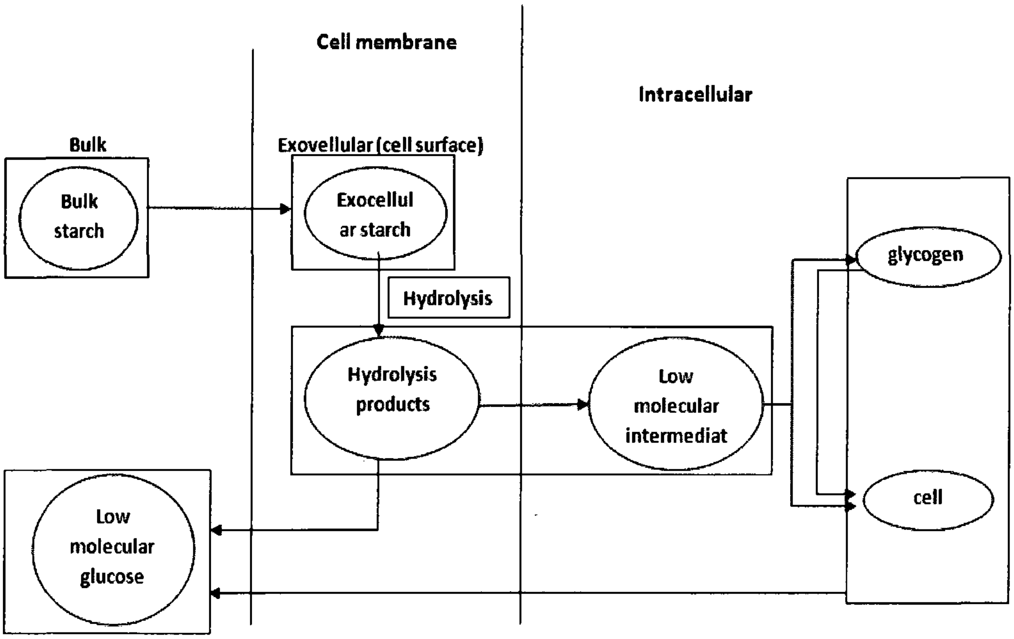


Figure 2.7 Hydrolysis- Utilization Mechanism of Starch by Activated Sludge

Delfin (1994) stated that during the hydrolysis of starch, a fraction of hydrolysis products is utilized for oxidation and cell synthesis, and the other part is transformed into intracellular reserve glycogen. The hydrolysis rate was evaluated from the profile of total starch. It was concluded that the hydrolysis rate of starch by activated sludge followed first-order kinetics with respect to starch concentration and biomass had no effect on it.

CHAPTER 3

THEORY

3.1 The Monod Equation

The hyperbolic kinetic expression proposed by Monod (1949) is omnipresent in models of biological wastewater treatment, becoming the de facto standard. It well-describes biomass growth of mixed cultures in a batch environment in the growth and substrate limited phases of the culture where endogenous decay is of minor importance. Monod simply proposed a hyperbolic model based on empirical evidence from his vast experience. In the '60s-'80s other kinetic formulations were used in models but by the '90s, the Monod model had become dominant. Endogenous decay was incorporated into overall models through a separate equation; thus, use of the Monod model for metabolism had a logical basis.

Models used in biological wastewater treatment are Eulerian gross descriptors of a process involving mass transfer, many substrates, and metabolic pathways and their enzymes, contained within many microorganisms. Any model at this level is merely a fit of mathematical formulations to data. As number of processes in biotreatment models increases along with mathematical descriptors and their associated coefficients, fitting an overall model improves to a point. But beyond this there has been little justification of the Monod expression. The formulation has been roundly criticized by many authors as being inadequate for dynamic simulations in particular (Blackwell, 1971).

This chapter examines conceptualizations that include hyperbolic metabolic expressions of Monod or Michaelis-Menten forms but also incorporate specific equations related to mass transfer.

Hyperbolic expressions arise in a number of engineering situations (e.g., Langmuir adsorption isotherms). A simple conceptual justification for the Monod hyperbolic model can be obtained from conjunction theory based on surface area of bioparticles combined with simple first-order kinetics for metabolism. Defining n_S as primary particles (substrate molecules), no./L, to be removed by bioparticles, n_H, also with units of no./L. The subscript “H” is used because heterotrophs are the predominant active agent in bioparticles. Bioparticles can and usually do, consist of many microorganisms aggregated together. The total surface area of a bioparticle is A_b. The capture rate of substrate is proportional to the available bioparticle surface area and a factor that is related to mixing conditions in the basin.

$$r_{ni} = -\alpha n_S n_H (A_b - A_{bS})\Omega \qquad (3.1)$$

where r_{ni} is rate of substrate molecule capture (no. $\mathrm{L}^{-3}\mathrm{T}^{-1}$); α is probability of capture for a collision; A_{bS} is surface area of a bioparticle occupied by captured substrate; Ω is a collision frequency factor related to turbulence and mixing conditions that affect particle size and number in the vessel

Assuming a first-order rate of substrate metabolism for a bioparticle:

$$r_m = -k_m n_H A_S \qquad (3.2)$$

where k_m is a substrate processing velocity factor

But in a steady state situation, the two rates are equal.

$$-\alpha n_S n_H (A_b - A_{bS})\Omega = -k_m n_H A_S$$

Noting that n_S and n_H are proportional to concentrations of substrate (S) and active biomass (X_H), respectively, the above equation can be solved for A_S which is then substituted into Eq. (3.2) to find the following form for the substrate removal expression.

$$r_S = -\frac{\mu_{HM} X_H S}{K_S + S} \qquad (3.3)$$

where μ_{HM} is maximum rate (velocity) constant; K_S is the half-velocity constant; r_S is rate of substrate removal ($\mathrm{ML}^{-3}\mathrm{T}^{-1}$)

Biomass growth and substrate removal are always related by the bioenergetics of the situation that dictates the yield factor in the overall stoichiometric formulation of organics transformation.

3.2 The Dual Hyperbolic Expression

Probably the most important advancement in modeling biotreatment was incorporation of a dual hyperbolic kinetic expression into ASM1 (IWA, 2000) to describe hydrolysis of slowly degraded organics. The expression is hyperbolic with respect to both substrate and viable biomass:

$$r_{\mathrm{h}} = -\frac{k_h X_H X_S}{K_X X_H + X_S}$$

where k_h is hydrolysis rate constant; K_X is the half-velocity constant; r_h is rate of hydrolysis; X_S is concentration of slowly degraded substrate

In ASM3 this expression plays a much more prominent role in substrate metabolism kinetic formulations (Gujer et al., 1999; IWA, 2000).This expression evolved from work of Blackwell (1971) who conducted a series of carefully designed dynamic batch studies to examine and model response of an activated sludge system to dynamic feed patterns. Blackwell's work was seminal to current models of the activated sludge processes. He incorporated accumulation and degradation of storage products into his model which was another fundamental improvement in describing microbial metabolism. His formulation for conversion of exogenous substrate (which was glucose) to stored substrate was

$$r_{sto} = X_{TSS}K_F(F_{max} - F) \tag{3.4}$$

where r_{sto} is rate of substrate removal for accumulation of stored products ($ML^{-3}T^{-1}$); K_F is a kinetic constant (T^{-1}); X_{TSS} is mixed liquor total suspended solids; F and F_{max} are fraction and maximum fraction of biomass that can be composed of storage products, respectively

Blackwell used experimental data from Takii (1970) who studied a glucose fed system to establish that the maximum value of F (F_{max}) was 0.51. Blackwell also used a glucose feed supplemented with nutrients. He also noted that Takii's data clearly indicated that there was a lower value of F (F_{min}), approximately 0.22, below which storage product concentration did not influence substrate uptake. The value of F_{min} was not well-defined but the principle was evident.

Blackwell modelled his system according to the substrate flow pattern in Fig. 3.1. Blackwell also noted that relative changes in the availability of exogenous substrate to the microorganisms would influence substrate diversion to storage products and concomitantly use of storage products for growth as outlined in Fig. 3.1. Blackwell used a Monod model to describe growth.

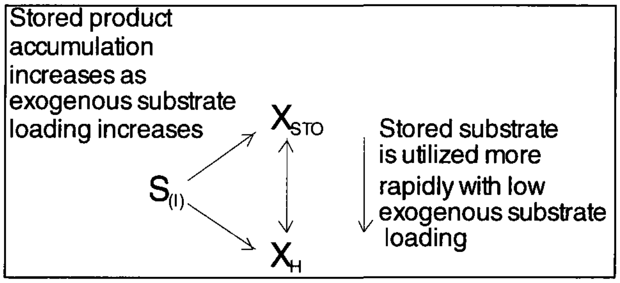


Figure 3.1 Blackwell's Conceptualization of the Activated Sludge Process. $S_{(i)}$ is Exogenous Substrate That is Taken Up by Biomass

Jacquardt et al. (1973) also found that incorporating storage products into the metabolic scheme was essential to successfully modelling dynamic activated sludge processes. Their model conceptualization used biomass accumulation of both particulate and dissolved storage reserves. Biomass growth only occurred as a result of utilization of stored reserves. Monod expressions were used for utilization of both particulate and dissolved storage reserves albeit with different parameters for each type of reserve.

Stenstrom (1975) examined work of two other researchers who successively modified Blackwell's Eq. (3.4) (merely changing kinetics to the Monod standard of the day) to firstly

$$r_{\mathrm{sto}} = k\left(\frac{X_S}{X_{TSS}}\frac{S}{K_S+S} - \frac{X_S}{X_{TSS}}\right)X_{TSS} \tag{3.5a}$$

where X_{TSS} is mixed liquor total suspended solids

In the second modification, for simplification, Jacquardt et al.'s (1973), conceptualization of two types of storage reserves was changed to a single stored substrate and the kinetic formulation was applied to growth from stored substrate. Also the expression within the parentheses was changed to a Monod formulation based on stored substrate (X_{sto}). X_{TSS} was kept as the variable describing biomass because of other work indicating that total surface area of suspended mass was operative in removing substrate.

$$r_g = -\frac{k_{sto} X_{Sto} X_{TSS}}{K_S + X_{Sto}} \qquad (3.5b)$$

where r_g is growth rate

The important contribution of Stenstrom was to note that Eq. (3.5b) could not be used for low sludge ages where at a low value of X_H (near washout conditions), mass of stored products relative to the liquid in Eq. (3.5b) would be low because X_H is low, but, in fact, growth rate of biomass would be high. Thus the equation was modified to reflect concentration of stored substrate in the biomass itself.

$$r_g = -\frac{k_{sto}(X_{Sto}/X_H)X_H}{K_S + (X_{Sto}/X_H)} \quad \text{or} \quad r_g = -\frac{k_{sto} X_{Sto} X_H}{K_S X_H + X_{Sto}} \qquad (3.6)$$

The importance of this modification cannot be understated. It not only better describes growth when biomass concentration is low, but also it is a more realistic descriptor of kinetics when substrate concentration is low. As biomass concentration is increased with little substrate present, it is unlikely that rate of substrate metabolism (referred to the reactor volume) increases directly proportional to biomass concentration, if it increases at all. This point was noted by Blackwell (1971) that at some point, further increase in active biomass does not result in any increase in rate of substrate uptake.

Dold et al. (1980) adopted Eq. (3.6) as proposed by Stenstrom for hydrolysis and synthesis of particulates based on Stenstrom's proposal and logic. They also incorporated a mass transfer expression for adsorption of particulate or slowly degradable substrate by active biomass. Understanding of biotreatment had made numerous other advances that influenced their model, defining substrate as two entities: slowly degradable (X_S) matter that required hydrolysis into rapidly degradable substrate (S_S). As Dold et al. (1980) point out, hydrolysis and storage of slowly degraded matter occur in tandem and one expression describes both processes. They modelled simple substrate metabolism and growth from it with a standard Monod expression.

In ASM1, Eq. (3.6) was only used to describe hydrolysis with all simple substrate produced by hydrolysis along with exogenous simple substrate used for growth being modelled by a Monod expression (IWA Task Group, 2000). ASM3 was a significant modification of ASM1 where some changes were made for issues of parameter identifiability and other reasons, although the number of parameters increased in ASM3 compared to ASM1. Storage was explicitly incorporated into ASM3 as a state variable. The dual hyperbolic control kinetic expression also played a more prominent role in ASM3. Flow charts of ASM1 and ASM3 are shown in Fig. 3.2.

Bioparticle surface area plays a governing role in substrate transport. The justification for the Monod equation above assumes first-order metabolism and incorporates mass transfer but it is too simplistic. Presence of readily degradable substrate must be taken into consideration in the development based on the conceptualization of the two major classes of substrate degradability used nowadays. Substrate being hydrolyzed occupies some of the bioparticle surface (and therefore it is "stored" there), making that portion of the surface area unavailable to either simple or particulate substrate.

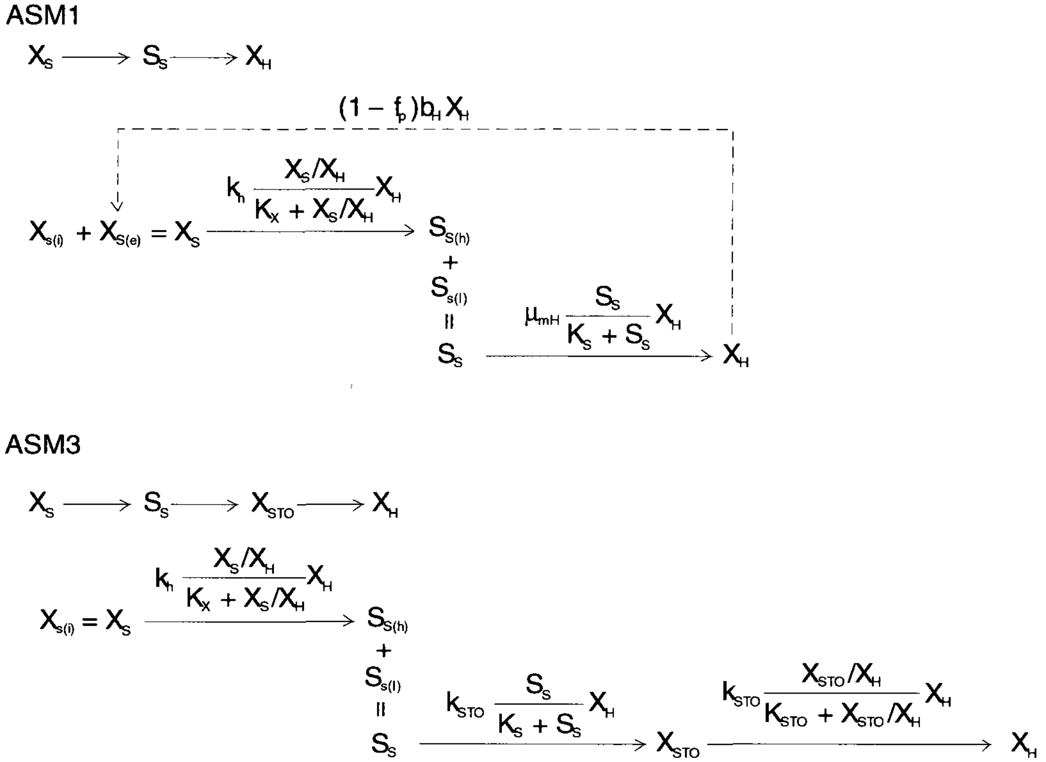


Figure 3.2 Susbtrate Flow Charts and Kinetic Expressions in ASM1 and ASM3. Subscript i refers to influent entities.

Dold et al. (1980) assumed that simple substrate passes unfettered by mass transfer limitations to the active biomass and it is metabolized. This does not appear to be consistent. Surface area of a bioparticle occupied by substrate of any form will present diffusional resistance to passage of simple as well as complex molecules. Mass transfer is totally ignored in ASM1 and ASM3. The task group that formulated ASM1 eschewed adsorption of large molecules by biomass as a significant step in its metabolism (Dold and Marais, 1980). Large molecules were thought to all be entrapped or adsorbed into the floc matrix. This does not seem to be tenable on a physical basis. Turbulence will limit growth of flocs and thus affect their ability to trap substrate.

The commercial model Biowin has a mass transfer function for adsorption of colloidal substrate onto flocs but no mass transfer is considered for other types of substrate (Envirosim, 2005).

Mass transfer or substrate capture is a process that is independent of metabolism. Captured substrate is metabolized according to a reasonable kinetic expression. The substrate flow chart proposed here is given in Fig. 3.3.

All ASMs use standard Monod formulations for some metabolic steps. The dual hyperbolic expression is a more reasonable formulation for all metabolic phenomena within cells for reasons given above and because of the development below. The Michaelis-Menten (hyperbolic) equation was developed from theory and experiment for enzymes and substrate in a volume V. In the case of biotreatment, reactions proceed in the volume of each bioparticle which has its concentrations of enzymes and substrate, respectively. Volume of a bioparticle is V_b. However, concentrations are measured with respect to the reactor volume, V. The following definitions are used.

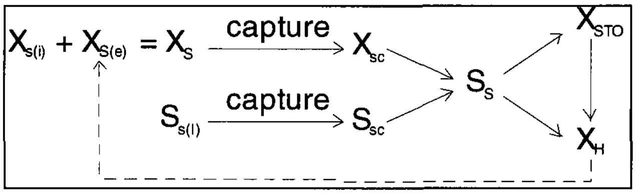


Figure 3.3 Substrate Flow in The Proposed Model. X_{SC} and S_{SC} are Captured Slowly Degraded and Simple Substrates, respectively

$$x_{cb} = \frac{m_c}{V_b} \quad (3.7a) \qquad X_c = \frac{M_c}{V} \quad (3.7b) \qquad X_b = \frac{M_b}{V} \quad (3.7c) \qquad n_b = \frac{M_b}{m_b} \quad (3.7d)$$

where x_{cb} is the concentration of captured substrate in a bioparticle; m_c is the mass of captured substrate by a single bioparticle; M_c is the total mass captured by bioparticles; X_c is the concentration of captured mass in the reactor volume, V; X_b is mass concentration of bioparticles in the reactor; M_b is total mass of bioparticles in the reactor; n_b is number of bioparticles in the reactor; m_b is mass of a single bioparticle

The two concentrations are related by

$$x_{cb} V_b \frac{n_b}{V} = X_c \quad (3.8a) \qquad \text{and} \qquad x_{cb} V_b \frac{M_b}{V m_b} = X_c \quad (3.8b)$$

Thus, $$x_{cb} = \frac{X_c}{X_b}\frac{m_b}{V_b} = \frac{X_c}{X_b}\rho_b \tag{3.9}$$

where ρ_b is density of a bioparticle

The density of activated sludge solids is 1.01-1.10 g/L (Dammel and Schroeder, 1991) and therefore ρ_b can be eliminated from the above equation (even if it is significantly different from 1, it can be factored into parameters in the hyperbolic expression and thus ignored). x_{cb} is the concentration that should be used in the hyperbolic metabolic expression but it can be replaced with X_c/X_b as shown in Eq. (3.9). Equations (3.10-3.12) describe capture of slowly degradable substrate (r_{XS}), capture of simple substrate (r_{SS}), and hydrolysis of slowly degradable substrate (r_{SS}) in the proposed model. As noted above, not only capture of large complex molecules (X_S) but also capture of simple substrates (S_S) is limited by the capacity or available surface area of the bioparticles. The mass transfer and capture of both types of substrate is controlled by the degree of saturation of the bioparticles with captured large molecules (which are one form of stored substrate). If the microorganisms become fully saturated, their capture rate of any type of substrate drops to zero.

The Michaelis-Menten or dual hyperbolic expression is applied to hydrolysis of captured slowly degradable substrate.

$$r_{SS} = k_1{}' S_S X_H(A_b - A_S) \tag{3.10}$$

$$r_{XS} = k_2{}' X_S X_H(A_b - A_S) \tag{3.11}$$

$$r_{\mathrm{h}} = -\frac{k_h X_H X_{Sc}}{K_X X_H + X_{Sc}} \tag{3.12}$$

where k_1' is a capture rate coefficient ($LM^{-1}T^{-1}$); k_2' is a capture kinetic constant ($LM^{-1}T^{-1}$); k_h is a hydrolysis rate coefficient ($L^{-2}T^{-1}$); K_X is a saturation constant for X_{Sc}/X_H

To relate A_b and A_S the following relation is used.

$$\frac{m_c}{A_S} = \frac{m_{cm}}{A_b} \quad and \quad \frac{A_S}{A_b} = \frac{m_c}{m_{cm}} = \frac{X_c}{X_b} \tag{3.13}$$

where m_{cm} is maximum mass of captured substrate

Using the relation in Eq. (3.13) with appropriate subscripts, Eqs. (3.10) and (3.11) become

$$r_{SS} = k_1 S_S X_H \left(1 - \frac{X_{SC}}{X_H}\right) \tag{3.14}$$

$$r_{XS} = k_2 X_S X_H \left(1 - \frac{X_{SC}}{X_H}\right) \tag{3.15}$$

A_b has been incorporated into k_1' and k_2' to form k_1 and k_2, respectively.

Equation (3.14) is the same equation as used by Dold et al. (1980) except that 1 within the parentheses was replaced by a factor that described the maximum ratio of stored substrate to biomass in their model. Equations (3.14) and (3.15) could also be modified if experimental data reflect that the maximum storage capacity is greater than 1.

The above development separates mass transfer and metabolic functions. The dual hyperbolic metabolic expression does not incorporate mass transfer; it can be considered as a Michaelis-Menten description of metabolism within a bioparticle. A single expression incorporating both mass transfer and metabolic activity using the dual hyperbolic expression is complicated, but with modern computers both formulations are easily handled as individual expressions.

In both ASM1 and ASM3 simple substrate is metabolized according to a standard hyperbolic formulation. This also appears to be contrary to reason. The concentration of substrate is referred to the reactor volume and the formulation suffers the errors noted above that it will not properly describe biomass growth in low sludge age situations and predicts that substrate removal rate will linearly increase with biomass even when the substrate concentration is far below the concentration of biomass where all degradable simple substrate molecules would be instantaneously taken up and metabolized.

In ASM3 all substrate is converted into storage products with, of course, loss of energy to achieve a degree of modeling simplification. This simplification can be used in the conceptualization of Fig. 3.3 if identifiability problems dictate the necessity. But this approach appears to be a gross oversimplification of reality. It would be highly inefficient for microorganisms to convert all simple substrate to glycogen or other storage polymers then cleave them to simple substrates for growth without utilizing available simple substrates to the maximum extent for growth.

The conceptualization of Blackwell (1971) is more reasonable for describing biomass growth. Or since storage products are cleaved back to simple substrates for metabolism and growth (Lehninger et al., 1993), his conceptualization or that in Fig. 3.3 could be modified to reflect this. Diversion of simple substrate to storage products and vice-versa could be controlled by the ratio of simple substrate metabolism to maximum specific growth rate of biomass. When this ratio exceeds 1, storage products are formed; when it is less than 1, storage products are metabolized, if there are any.

Use of hyperbolic expressions is functional by the success with using ASMs to model activated sludge systems even though these types of expressions have not been found to accurately describe metabolism of storage products (Dircks et al., 2001). Dircks et al. have shown that degradation of one of the major storage products, glycogen, best followed second-order kinetics. The success of the ASMs is as much due to their identification of the major steps in metabolism as the formulation of the kinetic expressions.

3.3 Various Conceptualizations of Kinetic Expressions

A justification for the dual hyperbolic expression is given. A model where mass transfer affects both slowly degraded and simple substrates is also proposed for exploration.

This first approach assumes that readily degradable substrate is able to be directly taken into a cell. Transport of readily degradable into the cell and metabolism are lumped into a single coefficient.

For a continuous flow CM reactor the mass balance on rapidly degraded substrate is

$$Qn_{SS0} - Qn_{SS} + [f_1 b_H n_H - k_1 n_{SS} n_H]V = 0 \tag{3.16}$$

where f_1 is the rapidly degradable fraction of COD released from endogenous decay
k_1 is a metabolic rate coefficient

The above expression assumes that there is some release of readily degradable substrate from endogenous decay. This is not unreasonable considering that cells die and lyse (i.e., the death-regeneration concept).

The mass balance on slowly degraded substrate is:

$$Qn_{XS0} - Qn_{XS} + [f_2 b_H n_H - k_2 n_{XS} n_H (A_b - A_S)]V = 0 \tag{3.17}$$

where f_2 is the slowly degradable fraction of COD released from endogenous decay.k_2 is a capture rate coefficient that depends on the degree of mixing and capture efficiency of the heterotrophs

The death-regeneration approach of releasing slowly degradable substrate through endogenous decay is used. A_S is area occupied by slowly degraded substrate. Slowly degradable substrate occupies a portion of cell surface area while it is hydrolyzed.

$$-Qn_H + [Y_H(k_1 n_{SS} n_H + k_3 n_H A_S) - b_H n_H]V = 0 \tag{3.18}$$

where k_3 is a metabolic rate coefficient (it is also a rate of hydrolysis coefficient)

Slowly degraded substrate is transformed into readily degraded substrate which is then metabolized.

$$r_{XS} = k_3 n_H A_S \tag{3.19}$$

$$r_{SS,total} = k_1 n_{SS} n_H + k_3 n_H A_S \tag{3.20}$$

$$\frac{dA_S}{dt} = k_2 n_{XS} n_H (A_b - A_S) - k_3 n_H A_S = 0 \tag{3.21}$$

From Eq. (3.21) at steady state:

$$k_2 n_{XS} (A_b - A_S) = k_3 A_S \tag{3.22}$$

$$k_2 n_{XS} A_b - k_2 n_{XS} A_S = k_3 A_S \qquad (3.23)$$

$$k_2 n_{XS} A_b = k_3 A_S + k_2 n_{XS} A_S \qquad (3.24)$$

$$A_S = \frac{k_2 n_{XS} A_b}{k_3 + k_2 n_{XS}} \qquad (3.25)$$

$$r_{XS} = k_3 n_H A_S = \frac{k_3 k_2 n_H n_{XS} A_b}{k_3 + k_2 n_{XS}} = \frac{k_3 n_H n_{XS} A_b}{\frac{k_3}{k_2} + n_{XS}} \qquad (3.26)$$

$$OUR = r_{SS} + r_{hydrolysis} = k_1 n_{SS} n_H + \frac{k_3 n_H n_{XS} A_b}{\frac{k_3}{k_2} + n_{XS}}$$

In conventional notation:

$$r_{hydrolysis} = \frac{k_h X_H X_S}{K_h + X_S}$$

$$OUR = r_{SS,total} = k X_H S_S + \frac{k_h X_H X_S}{K_h + X_S}$$

From a mass balance the rate of recycle can be found to be

$$r = \frac{1 - \frac{\theta_d}{\theta_X}}{\frac{n_{Hr}}{n_H} - 1} \qquad (3.27)$$

$$n_{S,total} = n_{SS} + n_{XS} \qquad (3.28)$$

where $n_{S,\mathrm{total}}$ is total number of substrate molecules

The first approach is simple and implicitly assumes that readily degraded substrate is not affected by the available surface area of the cell.

A more reasonable approach is to assume that that readily degraded substrate is also affected by the available surface area. Equation (3.17) above changes to

$$Q n_{SS0} - Q n_{SS} + [f_1 b_H n_H - k_1 n_{SS} n_H (A_b - A_S)] V = 0 \qquad (3.29)$$

$$Qn_{XS0} - Qn_{XS} + [f_2 b_H n_H - k_2 n_{XS} n_H (A_b - A_S)]V = 0 \tag{3.30}$$

$$-Qn_H + [Y(k_1 n_{SS} n_H (A_b - A_S) + k_3 n_H A_S) - b_H n_H]V = 0 \tag{3.31}$$

$$r_{XS} = k_3 n_H A_S \tag{3,32}$$

$$r_{SS,total} = k_1 n_{SS} n_H (A_b - A_S) + k_3 n_H A_S \tag{3.33}$$

$$\frac{dA_S}{dt} = k_2 n_{XS} n_H (A_b - A_S) - k_3 n_H A_S = 0 \tag{3.34}$$

From Eq. (3.34) at steady state:

$$k_2 n_{XS} (A_b - A_S) = k_3 A_S \tag{3.35}$$

$$k_2 n_{XS} A_b - k_2 n_{XS} A_S = k_3 A_S \tag{3.36}$$

$$k_2 n_{XS} A_b = k_3 A_S + k_2 n_{XS} A_S \tag{3.37}$$

$$A_S = \frac{k_2 n_{XS} A_b}{k_3 + k_2 n_{XS}} \tag{3.38}$$

$$r_{XS} = k_3 n_H A_S = \frac{k_3 k_2 n_H n_{XS} A_b}{k_3 + k_2 n_{XS}} = \frac{k_3 n_H n_{XS} A_b}{\frac{k_3}{k_2} + n_{XS}} \tag{3.39}$$

$$r_{SS} = k_1 n_{SS} n_H (A_b - A_S) = k_1 n_{SS} n_H \left(A_b - \frac{k_2 n_{XS} A_b}{k_3 + k_2 n_{XS}} \right) \tag{3.40}$$

$$= k_1 n_{SS} n_H A_b \left(1 - \frac{k_2 n_{XS}}{k_3 + k_2 n_{XS}} \right)$$

$$= k_1 n_{SS} n_H A_b \left(\frac{k_3 + k_2 n_{XS} - k_2 n_{XS}}{k_3 + k_2 n_{XS}} \right)$$

$$= k_1 n_{SS} n_H A_b \left(\frac{k_3}{k_3 + k_2 n_{XS}} \right) = \frac{k_1 n_{SS} n_H A_b}{1 + \frac{k_2}{k_3} n_{XS}} = \frac{\frac{k_1 k_3}{k_2} n_{SS} n_H A_b}{\frac{k_3}{k_2} + n_{XS}} \tag{3.41}$$

$$OUR = k_1 n_{SS} n_H (A_b - A_S) + \frac{k_3 n_H n_{XS} A_b}{\frac{k_3}{k_2} + n_{XS}} = \frac{\frac{k_1 k_3}{k_2} n_{SS} n_H A_b}{\frac{k_3}{k_2} + n_{XS}} + \frac{k_3 n_H n_{XS} A_b}{\frac{k_3}{k_2} + n_{XS}}$$

In conventional notation:

$$r_{hydrolysis} = \frac{k_h X_H X_S}{K_h + X_S}$$

$$r_{SS} = \frac{k_S S_S X_H}{K_h + X_S}$$

$$r_{SS,total} = \frac{k_S S_S X_H}{K_h + X_S} + \frac{k_h X_H X_S}{K_h + X_S} = \frac{k_S S_S + k_h X_S}{K_h + X_S} X_H$$

$$r = \frac{1 - \frac{\theta_d}{\theta_X}}{\frac{n_{Hr}}{n_H} - 1} \tag{3.42}$$

$$n_{S,total} = n_{SS} + n_{XS} \tag{3.43}$$

This approach assumes that both readily and slowly degradable substrate occupy microbe surface area. The approach is somewhat analagous to assuming storage of readily degradable substrate.

where A_{SS} is heterotroph surface area occupied by rapidly degraded substrate

A_{XS} is heterotroph surface area occupied by slowly degraded substrate

$$Qn_{SS0} - Qn_{SS} + [f_1 b_H n_H - k_1 n_{SS} n_H (A_b - A_S)]V = 0 \tag{3.44}$$

$$Qn_{XS0} - Qn_{XS} + [f_2 b_H n_H - k_2 n_{XS} n_H (A_b - A_S)]V = 0 \tag{3.45}$$

$$-Qn_H + \{Y[k_1 n_{SS} n_H (A_b - A_S)\ k_2 n_{XS} n_H (A_b - A_S)] - b_H n_H\}V = 0 \tag{3.46}$$

$$A_S = A_{SS} + A_{XS} \tag{3.47}$$

Rates of metabolism of rapidly and slowly degraded substrate are:

$$r_{SS} = k_4 n_H A_{SS} \tag{3.48}$$

$$r_{XS} = k_3 n_H A_{XS} \tag{3.49}$$

$$\frac{dA_{XS}}{dt} = k_2 n_{XS} n_H (A_b - A_S) - k_3 n_H A_{XS} = 0 \tag{3.50}$$

k_3 should be different from k_2 since one is a mass transport coefficient (affected by mixing in the basin) and the other is a metabolic coefficient.

$$\frac{dA_{SS}}{dt} = k_1 n_{SS} n_H (A_b - A_S) + k_3 n_H A_{XS} - k_4 n_H A_{SS} = 0 \tag{3.51}$$

From (3.50): $k_2 n_{XS} (A_b - A_S) = k_3 A_{XS}$ (3.52)

From Eqs. (3.52) and (3.47):

$$k_2 n_{XS} A_b - k_2 n_{XS} A_{XS} - k_2 n_{XS} A_{SS} = k_3 A_{XS} \tag{3.53}$$

$$k_2 n_{XS} A_b - k_2 n_{XS} A_{SS} = k_3 A_{XS} + k_2 n_{XS} A_{XS} \tag{3.54}$$

$$A_{XS} = \frac{k_2 n_{XS} (A_b - A_{SS})}{k_3 + k_2 n_{XS}} \tag{3.55}$$

From Eq. (3.51):

$$k_1 n_{SS} n_H (A_b - A_S) + k_3 n_H A_{XS} = k_4 n_H A_{SS} \tag{3.56}$$

$$k_1 n_{SS} A_b - k_1 n_{SS} A_{XS} - k_1 n_{SS} A_{SS} + k_3 A_{XS} = k_4 A_{SS} \tag{3.57}$$

$$k_1 n_{SS} A_b - k_1 n_{SS} A_{XS} + k_3 A_{XS} = k_4 A_{SS} + k_1 n_{SS} A_{SS} \tag{3.58}$$

$$A_{SS} = \frac{k_1 n_{SS} (A_b - A_{XS}) + k_3 A_{XS}}{k_4 + k_1 n_{SS}} \tag{3.59}$$

From Eqs. (3.58) and (3.55):

$$k_1 n_{SS} A_b + (k_3 - k_1 n_{SS}) \frac{k_2 n_{XS} (A_b - A_{SS})}{k_3 + k_2 n_{XS}} - k_1 n_{SS} A_{SS} = k_4 A_{SS} \tag{3.60}$$

$$\begin{aligned} k_1 n_{SS} A_b &- \frac{k_1 k_2 n_{SS} n_{XS} A_b}{k_3 + k_2 n_{XS}} + \frac{k_1 k_2 n_{SS} n_{XS} A_{SS}}{k_3 + k_2 n_{XS}} + \frac{k_3 k_2 n_{XS} A_b}{k_3 + k_2 n_{XS}} - \frac{k_3 k_2 n_{XS} A_{SS}}{k_3 + k_2 n_{XS}} \\ &- k_1 n_{SS} A_{SS} = k_4 A_{SS} \end{aligned} \tag{3.61}$$

$$k_1 n_{SS} A_b + k_2 n_{XS} A_b \left(\frac{k_3}{k_3 + k_2 n_{XS}} - \frac{k_1 n_{SS}}{k_3 + k_2 n_{XS}} \right) = k_4 A_{SS} + k_1 n_{SS} A_{SS} +$$

$$k_2 n_{XS} A_{SS}\left(\frac{k_3}{k_3+k_2 n_{XS}}-\frac{k_1 n_{SS}}{k_3+k_2 n_{XS}}\right) \tag{3.62}$$

$$A_{SS} = A_b \frac{k_1 n_{SS} + k_2 n_{XS}\left(\frac{k_3}{k_3+k_2 n_{XS}}-\frac{k_1 n_{SS}}{k_3+k_2 n_{XS}}\right)}{k_4 + k_1 n_{SS} + k_2 n_{XS}\left(\frac{k_3}{k_3+k_2 n_{XS}}-\frac{k_1 n_{SS}}{k_3+k_2 n_{XS}}\right)} \tag{3.63}$$

$$= A_b \frac{k_1 n_{SS}(k_3 + k_2 n_{XS}) + k_3 - k_1 n_{SS}}{(k_4 + k_1 n_{SS})(k_3 + k_2 n_{XS}) + k_2 n_{XS}(k_3 - k_1 n_{SS})}$$

$$= A_b \frac{k_1 k_3 n_{SS} + k_1 k_2 n_{SS} n_{XS} + k_3 - k_1 n_{SS}}{k_4 k_3 + k_4 k_2 n_{XS} + k_1 k_3 n_{SS} + k_1 k_2 n_{SS} n_{XS} + k_2 n_{XS}(k_3 - k_1 n_{SS})}$$

This is a logistic type expression where there is a maximum point defined by an intermediate concentration of X_S.

The next conceptualization assumes that readily degradable substrate is able to be directly taken into a cell. Transport of readily degradable into the cell and metabolism are lumped into a single coefficient.

$$Qn_{SSO} - Qn_{SS} + [-k_1 n_{SS} n_H]V = 0 \tag{3.64}$$

$$Qn_{XSO} - Qn_{XS} + [f_2 b_H n_H - k_2 n_{XS} n_H (A_b - A_S)]V = 0 \tag{3.65}$$

The death-regeneration approach of releasing slowly degradable substrate through endogenous decay is used. A_S is area occupied by slowly degraded substrate. Slowly degradable substrate occupies a portion of cell surface area while it is hydrolyzed.

$$-Qn_b + [Y(k_1 n_{SS} n_H + k_3 n_H A_S) - b_H n_H]V = 0 \tag{3.66}$$

Slowly degraded substrate is transformed into readily degraded substrate which is then metabolized.

Uptake of X_S:

$$r_{XS} = -k_1 n_H n_{XS}(A_b - A_S) \tag{3.67}$$

Rate of transformation of X_S to S_S which is immediately metabolized:

$$r_{XSm} = k_2 n_H A_S \tag{3.68}$$

$$\frac{dA_S}{dt} = k_1 n_{XS} n_H (A_b - A_S) - k_2 n_H A_S = 0 \tag{3.69}$$

$$k_1 n_{XS} (A_b - A_S) = k_2 A_S \tag{3.70}$$

$$A_S = \frac{k_1 n_{XS}}{k_2 (A_b - A_S)} \tag{3.71}$$

$$k_1 n_{XS} A_b - k_1 n_{XS} A_S = k_2 A_S \tag{3.72}$$

$$k_1 n_{XS} A_b = k_2 A_S + k_1 n_{XS} A_S \tag{3.73}$$

$$A_S = \frac{k_1 n_{XS} A_b}{k_2 + k_1 n_{XS}} \tag{3.74}$$

$$r_{SS(1)} = k_2 n_H A_S = \frac{k_1 k_2 n_H n_{XS} A_b}{k_2 + k_1 n_{XS}} = \frac{k_2 n_H n_{XS} A_b}{\frac{k_2}{k_1} + n_{XS}} = \frac{k_m X_S X_H}{K_X + X_S} \tag{3.75}$$

Uptake and metabolism of S_S (instantaneous metabolism of S_S as it is captured)

$$r_{SS(2)} = k_3 n_{SS} n_H (A_b - A_S) \tag{3.76}$$

$$r_{SS(2)} = k_3 n_{SS} n_H (A_b - \frac{k_1 n_{XS} A_b}{k_2 + k_1 n_{XS}}) \tag{3.77}$$

$$r_{SS(2)} = k_3 n_{SS} n_H A_b \left(1 - \frac{k_1 n_{Xs}}{k_2 + k_1 n_{XS}} \right)$$

$$= k_3 n_{SS} n_H A_b \left(\frac{k_2 + k_1 n_{XS} - k_1 n_{Xs}}{k_2 + k_1 n_{XS}} \right) = k_3 n_{SS} n_H A_b \left(\frac{k_2}{k_2 + k_1 n_{XS}} \right)$$

$$= k_3 n_{SS} n_H A_b \left(\frac{k_2 / k_1}{\frac{k_2}{k_1} + n_{XS}} \right)$$

$$r_{SS(2)} = \frac{k_3 k_2 n_{SS} n_b A_b / k_1}{\frac{k_2}{k_1} + n_{XS}} = \frac{k_3 K_X S_S X_H A_{b1}}{K_X + n_{XS}}$$

$$r_{SS(2)} = \frac{k_{m2} S_S X_H}{K_X + X_S}$$

$$OUR = \frac{k_m X_S X_H}{K_X + X_S} + \frac{k_{m2} S_S X_H}{K_X + X_S} = \frac{k_m X_S + k_{m2} S_S}{K_X + X_S} X_H \text{ with no endogenous decay}$$

This rate must be less than the maximum substrate processing rate of the sludge.

If max substrate processing rate = $k_4 n_H = k_4 X_H$ then

$$\frac{k_m X_S + k_{m2} S_S}{K_X + X_S} X_H \leq k_4 X_H$$

$$\frac{k_m X_S + k_{m2} S_S}{K_X + X_S} \leq k_4$$

Determine k_4 in the same test as for the true yield factor.

If the mixing-contact factor is the same for both types of substrate, i.e., $k_1 = k_3$:

$$r_{SS(2)} = \frac{k_1 K_X S_S X_H A_{b1}}{K_X + n_{XS}}$$

The mass balance

$$QX_{S0} - (QX_S)_w - \frac{k_m X_S X_H}{K_X + X_S} V = 0$$

$$\frac{X_{S0}}{\theta_d X_S} - \frac{k_m X_H}{K_X + X_S} = \frac{1}{\theta_X}$$

$$X_{S0}(K_X + X_S) - k_m X_H \theta_d X_S = \frac{\theta_d (K_X + X_S) X_S}{\theta_X}$$

$$\frac{\theta_X}{\theta_d} K_X X_{S0} + \frac{\theta_X}{\theta_d} X_{S0} X_S - k_m X_H \theta_X X_S = K_X X_S + X_S^2$$

$$X_S^2 + \left(-\frac{\theta_X}{\theta_d} X_{S0} + k_m X_H \theta_X + K_X \right) X_S - \frac{\theta_X}{\theta_d} K_X X_{S0} = 0$$

$$QS_{S0} - QS_S - \frac{k_{m2} S_S X_H}{K_X + X_S} V = 0$$

$$-(QX_H)_w + (\mu_{HS} + \mu_{HX} - b_H)X_H V = 0$$

$$\frac{1}{\theta_X} = \mu_{HS} + \mu_{HX} - b_H$$

$$\frac{\mu_{HS}}{\mu_{HX}} = \frac{k_{m2} S_S}{k_m X_S}$$

All these models only focus on the main mechanisms and phenomenon that happen or apply to bacteria. A biological process is a very complex system, there are so many activities happens in biomass that an exact series of simple equations cannot be formulated. The above developments all result in different formulations. The objective of this study was to find the most appropriate model to describe how real biomass behaves and determine the most appropriate kinetic expressions for hydrolysis and metabolism of starch and glucose, respectively.

CHAPTER 4

EXPERIMENTAL METHODS

4.1 Introduction

Before discussing the actual experimental conditions some considerations from previous experimental work that has a bearing on the experimental design will be discussed.

The hyperbolic relationship between specific growth rate and substrate concentration empirically proposed by Monod has been widely used for batch microbial cultures. A plot of specific growth rate versus initial substrate concentration was found to fit a Monod type hyperbolic equation (Gaudy et al., 1971); however, the instantaneous relationship between specific growth rate and substrate concentration during growth was not tested. It should be noted that the exponential growth phases (μ is specific growth rate and is constant) were developed at the initial substrate concentration below that required for maximum growth. The half saturation constant K_s is a parameter characteristic of a specific microbial system and substrate. In other words different organisms, substrate or cultural conditions may result in different K_S values. The study also showed that μ for aerobic waste treatment decreased below μ_{HM} (maximum growth rate) only at very low substrate concentrations. However, this may result in an error when based on measuring initial substrate concentration. In the Monod equation, there is an assumption: μ is tightly coupled to S, which means a change in S will result in a corresponding change in μ. Experiments showed that the value of K_s is very small for microbial aerobic waste bacterial growth. Gaudy (1971) stated that growth rate "must be taken from the early part of the growth curve", because substrate concentration may change with time. If a microbial culture is at steady state, it is safe to say that when S concentration is low a small change in S will lead to a significant response for μ. However, if the substrate concentration is high the response of μ is delayed if there is a change in dilution rate or substrate concentration. In many investigations, the K_s value for mixed aerobic culture growth for waste treatment has been often found to be 10 to 20 times greater than those reported by Monod. This may be due to use of biomass with a significant endogenous component.

Liu (2003) demonstrated in her study that the Monod equation could be interpreted in a thermodynamic sense. It was reported that K_s was related to the inverse of the equilibrium constant. If the equilibrium constant is large, K_s will be very small, and the microbial growth process proceeds far towards completion with little residual substrate remaining. In contrast, if the equilibrium constant is small, K_s will be large, concomitantly an extremely small amount of biomass is formed and the position of equilibrium lies far towards the substrate (i.e., high residual substrate remains).

From the literature (Petersena et al., 2003), it has been stated that specific growth rate and doubling time are constants only when all substrates and essential nutrients necessary for growth are present in excess. This is an assumption, which to our knowledge, is usually not taken into account in experimental design. Obviously, this assumption is inconsistent with the data of the Monod equation since substrate concentration is changing during the period when specific growth rate remains constant. There are two assumptions that have been widely accepted without proof. The first is that the Monod equation can describe instantaneous change in substrate concentration during growth at low substrate concentrations below the maximum growth rate. The second assumption is that exponential growth can only occur in a batch culture when specific growth rate equals the maximum specific growth rate. However, recent investigations concerned with the kinetics of continuous culture based on the Monod equation are not adequate for prediction of transient behaviour during periods when a chemostat (i.e., continuous complete mixed bioreactor system) operates with changing substrate concentration.

In view of these problems, it seems important to understand how aerobic microbial waste treatment cultures in the exponential phase behave with rate limiting substrate changes. Exponential growth phases can be maintained by using 100% active biomass (i.e., there is no endogenous decay phase to be accounted for). Using fully active microbial biomass (X_{BH}) the true half saturation coefficient can also be determined in this study.

A chemostat was used in this study to culture active biomass to be used in batch experiments with the different substrates. The usual array of parameters was measured in these experiments. A summary of methodologies (APHA, 1992) for all parameters monitored during the study is shown in Table 4.1.

4.2 Description of Experimental Set-up

4.2.1 Feed Tank Set-up

Feed containing glucose and starch and all essential nutrients in excess was dispensed to a chemostat to grow the active biomass used in these experiments. Table 4.2 (Delfin, 1994) shows the chemical composition of the growth medium which was added to tap water. The medium was put into a 20 L feed tank as the feed solution. The initial volume of the chemostat (seed tank) was 10 L and was operated at a hydraulic retention time (HRT) and solids retention time (SRT) of < 1 day. It was very difficult to reach steady state with such a small reactor. After more than half a month, the TSS/ VSS ratio was still not stable. A 20 L chemostat operated at the same HRT was able to maintain desired conditions. The COD of the feed solution was chosen to provide adequate growth at the HRT/SRT at which the chemostat was operated.

Starch specified by the manufacturer as soluble starch was added to the feed without any chemical pretreatment. Soluble starch is a high molecular weight particle that exists in a colloidal/particulate form but can be solubilised by addition of acid or by boiling. However, if pretreated in either of these manners the procedures will destroy the structure of the starch (the molecule's long chain is being broken down into short chain entities) and hydrolyse it to some extent. As a result, starch in this study was directly added to tap water at room temperature without any pretreatment. Since starch may settle in the tank, the feed tank was stirred with a magnetic stirrer at 200 rpm. The feed was replaced every day. The feed container was cleaned before refilling it with feed solution. Microbial growth in the feed tank was of concern; however, with daily cleaning and fresh feed there was no visible growth in the feed tank over a feeding period. During the entire period of batch experiments, COD of the feed tank was measured (according to Standard Methods, APHA et al. 1998a) at least twice a week. Even though the same feed formulation was used every time, the COD and SCOD still had ±15% differences from the target value, and the COD in the morning was higher than in the afternoon. There was probably some oxidation of organic substances throughout the day.

Table 4.1 Analytical Techniques

Parameter	Method (numbers are cited from APHA (1992))	Specific Apparatus	Sample V (mL)
pH	4500-H^+ B. Electrometric	Orion 4-Star Plus benchtop pH/DO meter and Fisher Scientific Flexa-Mix magnetic stirrer	20-50
DO	4500-OG. Membrane Electrode	Orion 4-Star Plus benchtop pH/DO meter	20-50
Temp	2550 B.2. Laboratory	Mercury- filled Celsius thermometer	20-50
COD	5220C. Titrimetric Closed Reflux	Blue M Electric Company Stable- Therm Gravity 150°C oven, COD digestion tubes	5 or 10
BOD_5	5210 B. 5-day BOD Test and 4500-OG. Membrane Electrode	BOD bottles, VWR Scientific Model 2020 incubator, Orion 4-Star Plus benchtop pH/DO meter	300
TSS	2540 D. Total Suspended Solids Dried at 103-105°C	1.2 µm GFC glass filters, filtration apparatus, aluminium dishes, 105°C Fisher Isotemp drying oven desiccators	10-4000
VSS	2540 E. Fixed and Volatile Solids Ignited at 500°C	Same as TSS except Model 186A 550°C Fisher Isotemp muffle furnace	10-400
Flow Rates	Volumetric Graduation	Graduated cylinders, pump	

An Orion general-purpose pH electrode (Orion No. 910500) was used to measure pH values of the feed tank at least twice a week to maintain it at neutral ($NaHCO_3$ was used as buffer solution to adjust the pH if its value in the feed tank dropped) and its temperature was kept at room temperature (23-25°C). Variation in the feed COD around the target value of 600 mg COD/L was tolerable since the sole purpose of the feed and chemostat operation was to culture active biomass acclimatized to starch and glucose. The COD and pH of the feed tank substrate during the experimental period are shown in Table 4.3.

Table 4.2 Chemical Composition of Growth Medium

Component	Concentration (mg/L)
Glucose (COD)[a]	300
Soluble Starch (COD)[b]	300
$(NH_4)_2SO_4$	250
$MgSO_4 \cdot 7H_2O$	100
$FeCl_3 \cdot 6H_2O$	0.5
$CaCl_2$	7.5
$MnSO_4 \cdot H_2O$	10
KH_2PO_4	50
K_2HPO_4	100
Yeast Extract	10

a Dextrose from Grain Process Enterprises LTD, Scarborough, ON
b $(C_6H_{10}O_5)_n$ from Fisher Scientific

Table 4.3 Feed Tank COD and pH

Day	Feed COD	pH	Day	Feed COD	pH
	mg COD/L			mg COD/L	
March 1	704	7.16	March 12	608	7.80
March 2	704	6.67	March 13	608	7.76
March 4	691	6.58	March 14	570	6.33
March 6	552	7.21	March 16	570	7
March 8	633	6.20	March 17	774	7
March 9	896	6.56	March 18	562	7.20
March 10	528	6.70	Average	646	6.94

4.2.2 Seed Reactor Set-up

The activated sludge mixed culture used for the batch experiments was cultivated in a continuous flow reactor (chemostat) with a volume of 20 L to produce an active culture acclimatized at a short SRT (< 1 day) to a starch-glucose feed supplemented with essential nutrients and minerals. The short SRT was set to produce a biomass that was in exponential growth and assumed to be fully active (i.e., no endogenous decay products or activity). The reactor had no recycle, (HRT = SRT) and HRT was reduced from an initial value of 24 h (days 1-3) to 18 h then maintained at this value (the flow rate was initially set at 0.83 L/h and increased to 1.11 L/h) until steady state was achieved. The initial aerobic seed sludge was obtained from the discharge of the activated sludge basin at Robert O. Pickard Environmental Center (ROPEC) in Ottawa-Carleton that operates at an SRT of about 5 days.

During the entire period of batch experiments, TSS, VSS, and SCOD of the feed tank were measured, according to Standard Methods (SM), every two days until steady state conditions were achieved (it took 7 days to obtain VSS = average VSS ± 15% but the chemostat was operated for 60 days before the runs used to obtain data were performed). The operating conditions of the seed reactor chemostat are shown in Table 4.4. The experimental setup is shown in Fig. 4.1. The reactor was kept at room temperature (23-25°C).

Table 4.4 Seed Reactor Conditions

Day	TSS	VSS	VSS/TSS	Effluent COD	Reactor SCOD	pH
	mg/L	mg/L		mg COD/L	mg COD/L	
March 1	462	368	0.80	32	50	7.3
March 2	370	330	0.89	64	32	6.1
March 4	745	460	0.62	72	115	6.6
March 6	755	730	0.97	86.4	36	6.5
Day	TSS	VSS	VSS/TSS	Effluent COD	Reactor SCOD	pH
	mg/L	mg/L		mg COD/L	mg COD/L	
March 8	400	360	0.90	64.8	29	6.2
March 9	470	435	0.93	128	88	6.5
March 10	535	445	0.83	100	59	6.8
March 12	470	430	0.91	38	38	7.4
March 13	620	565	0.91	30.4	38	7.9
March 14	573	535	0.93	30	37	7.4
March 16	575	520	0.90	44.6	30	7.2
March 17	600	555	0.93	35	14	7.8
March 18	720	667	0.93	38	15	6.7
March 19	640	600	0.94	N/A	N/A	6.5
March 20	470	430	0.91	N/A	N/A	N/A
March 23	520	490	0.94	N/A	N/A	N/A
March 25	725	673	0.93	N/A	N/A	N/A
March 26	630	585	0.93	N/A	N/A	N/A
March 28	565	550	0.97	N/A	N/A	N/A
Average	571	512	0.90	58.7	44.65	6.92

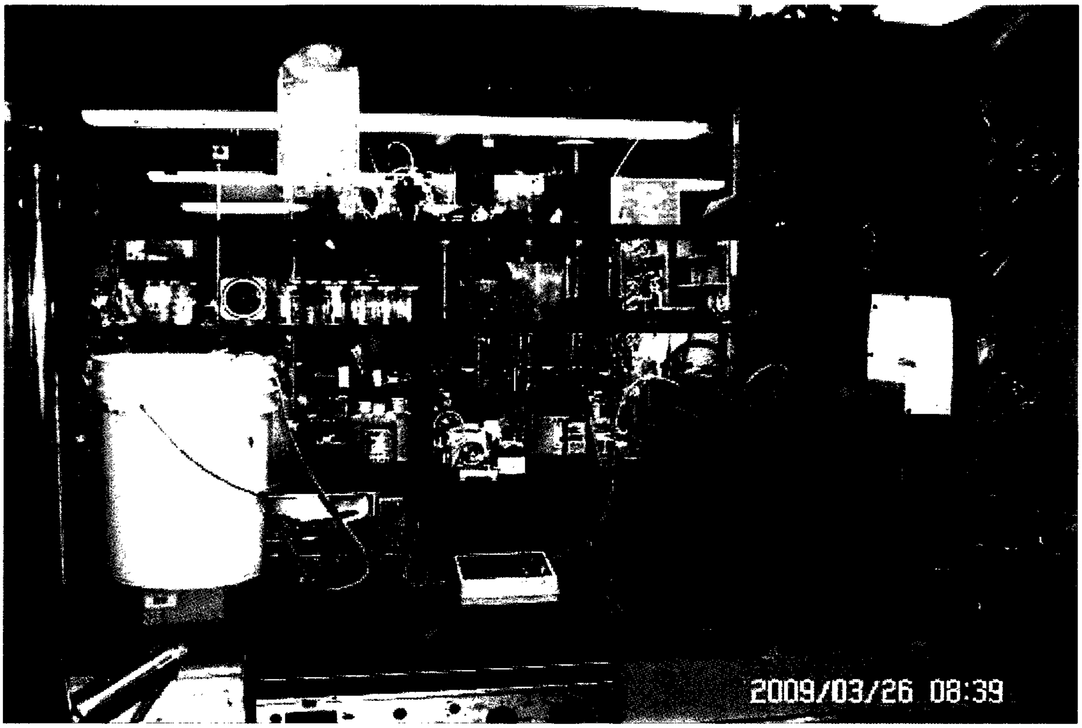


Figure 4.1 Aerobic Seed Reactor Setup

4.3 Batch Experiments

Varying amounts of biomass were taken from the seed reactor and exposed to varying concentrations of glucose and starch in batch experiments. Subsequently the oxygen utilization rate (OUR) was monitored over time in each batch reactor. Two sets of supporting experiments using active biomass (endogenous decay was of minor importance as dictated by the low HRT/SRT of the chemostat as noted above) were studied for model development then calibration and estimation of parameters and model fit. In the first set of experiments only glucose (readily biodegradable substrate) was used as the carbon source and the second set was conducted using only starch (difficult to biodegrade substrate requiring hydrolysis which yields glucose molecules) as the carbon source. The initial values of DO uptake, where substrate and fully active mass concentrations were known, were used to determine rates of readily biodegradable glucose metabolism from the first set of experiments. Results from the starch set along with the readily biodegradable rate model developed from the first set of runs were used to determine the best hydrolysis expression for fully active biomass in the exponential growth phase.

4.3.1 Glucose Metabolism Rate Determination

Young (1999) used respirometry as a tool to identify specific compounds from a batch reactor. This is similar to the resting procedure described in APHA (American Public Health Association, 1992) for the determination of SOUR.

After 60 days acclimation, steady state fully active activated sludge mixed liquor samples were taken from the seed reactor chemostat to determine SOUR. Samples were used as sampled and were not subjected to any pre-treatment, centrifugation or washing. Initial OURs were measured after substrate (glucose or starch) was added to the sludge samples. To start the experiment, different amounts of freshly harvested sludge were collected and transferred into a 300 mL BOD bottle, but only 290 mL of total liquid (mixed liquor sludge and dilution water) were transferred to accommodate the DO probe. The DO meter was calibrated before each use. The relative accuracy for Orion 4-star plus DO meter is ± 0.2 mg/L. The contents of the bottle were magnetically stirred at 400 rpm.

Contents of the BOD bottle were magnetically stirred during both aeration and metabolism phases. However, the bottom of the BOD bottle was not flat, so it was difficult to control the stirrer at a certain rate (periodically the magnetic bar would jump around in the bottle). After realizing the problem, a DO probe with a self-stir mechanism was used instead of the normal DO probe. As a result, the magnetic stirrer was not necessary. All data used for analysis came from bottles stirred with the DO probe stirrer.

In the seed reactor chemostat, microbial biomass may have accumulated some stored substrate or substrate products even though the SRT was very low. In order to consume any potentially stored substrate and residual substrate in liquid phase, the sample was aerated for up to 1 hour depending on how much biomass was present until the DO did not change (and it was assumed that storage of glucose and starch was negligible in the starved condition of the biomass). At this time it was assumed that there was no stored or available residual substrate in solution. After this time, 10 mL of mixed liquor was withdrawn for TSS/VSS measurement used in the calculation of SOUR. Then, 10 mL of glucose solution at the desired concentration was injected into the DO saturated mixed liquor in the BOD bottle (adding it slowly to avoid introducing air bubbles into the system). The initial glucose concentrations in the bottle after addition of the glucose solution resulted in concentrations ranging from 17.1 to 137.9 mg COD/L.

At the time glucose was added, the timer was started. Different concentrations of biomass were also investigated by dilution of the seed reactor mixed liquor aliquot by factors of 0.14, 0.31 and 0.48, respectively. DO response was monitored for the first 15 min after the substrate solution was added to the batch reactor. It was assumed that the increase of VSS over the measurement period was negligible since DO change was only measured for the first 15 min. A noticeable drop in DO did not occur immediately after substrate was added to the BOD bottle, and that may be because either there is a lag phase for biomass to acclimate to substrate before it can grow, or the DO meter had a response time. However, after 5 or 10 seconds, the DO began to drop. OUR and SOUR were calculated based on their definitions [See Eqs (4.1) and (4.2)]. Figure 4.2 shows the experimental set-up of DO measurements.

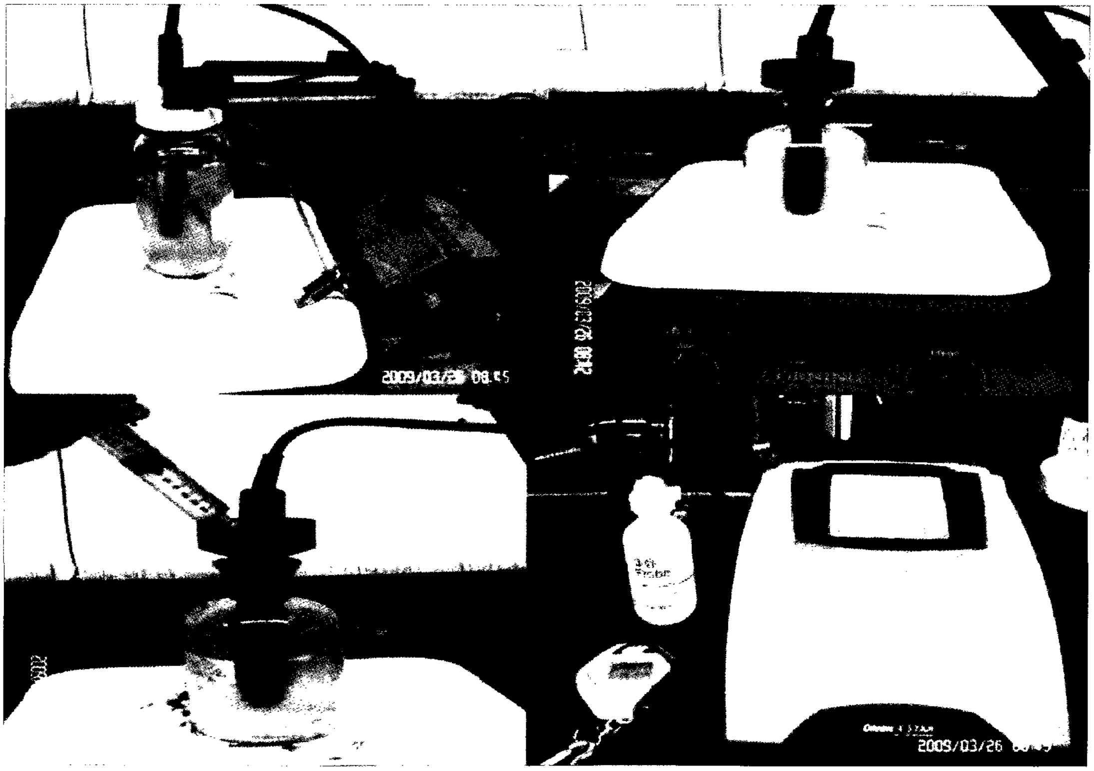


Figure 4.2 OUR Measurement Set-up

Below is an example of OUR and SOUR calculations. Table 4.5 shows the original DO data when glucose concentration, G= 54.5 mg COD/L and X_{BH}= 460 mg VSS/L. All other original data are shown in Appendix B. Since there will still be some glucose and perhaps storage products in the biomass remaining from the mixed liquor withdrawn from the chemostat as discussed above, one hour aeration was necessary before adding substrate. In the starved condition of the biomass at this time, it was assumed that there was no storage of substrate by the biomass when the substrate solution was added to the batch reactor. DO was monitored every minute; OUR was calculated based on the definition:

$$OUR = -\frac{DO_n - DO_{n-1}}{T_n - T_{n-1}} \tag{4.1}$$

where DO_n = DO at time n

DO_{n-1} = DO at time n−1

T = time

$$\text{Average } OUR = \frac{\sum_{time1}^{timei} OUR_i}{i}$$

where i = total time period (15 min)

$$SOUR = \frac{Average\ OUR}{VSS} \tag{4.2}$$

$$OUR = -\frac{DO_1 - DO_0}{T_1 - T_0} = -\frac{5.96mg/L - 6.16mg/L}{1\min - 0\min} = 0.2 \text{ mg/L/min}$$

$$SOUR = \frac{Average\ OUR}{VSS} = \frac{\sum_{0}^{15}(0.2 + 0.23 + 0.27 \cdots + 0.22)/15}{460mgVSS/L} = 5.09\times10^{-4}\ \text{min}^{-1}$$

Table 4.5 DO Over the First 15 min.

March 2, 2009			
Glucose, *G*	54.4	mg COD/L	
Active biomass, X_{BH}	460	mg VSS/L	
Time	DO	OUR	SOUR
(min)	(mg/L)	(mg/L/min)	(min^{-1})
0	6.16		
1	5.96	0.2	4.35E-04
2	5.73	0.23	5E-04
3	5.46	0.27	5.87E-04
4	5.25	0.21	4.57E-04
5	4.99	0.26	5.65E-04
6	4.76	0.23	5E-04
7	4.49	0.27	5.87E-04
8	4.25	0.24	5.22E-04
9	4.04	0.21	4.57E-04
10	3.8	0.24	5.22E-04
11	3.53	0.27	5.87E-04
12	3.33	0.2	4.35E-04
13	3.09	0.24	5.22E-04
14	2.87	0.22	4.78E-04
15	2.65	0.22	4.78E-04
	Average	0.23	5.09E-04

4.3.2 Starch Hydrolysis Rate Determination

Starch hydrolysis rate determination used the same OUR methodology as for glucose using the same DO probe and DO meter. However, since hydrolysis does not consume oxygen, the determination of hydrolysis must be performed indirectly. SOUR (same meaning as μ used above) for both starch hydrolysis and glucose metabolism can be written as follows (glucose metabolism rates are given in Chapter 5):

$$SOUR = \frac{\mu_{HM} \times G}{K_S + G} \tag{4.3}$$

where μ_{HM} = maximum specific growth rate of heterotrophs (min^{-1})

K_S = COD half saturation coefficient (mg COD/L)

G = glucose concentration (mg COD/L)

Glucose concentration changes continuously during the experiment. There are two reasons for the change. Firstly, starch, as slowly biodegradable substrate, was being hydrolysed to readily biodegradable substrate (glucose). This contributes to an increase in glucose concentration. On the other hand, as soon as glucose is produced, it is also being metabolized immediately. This decreases glucose concentration. Again assuming that there was no substrate storage, total glucose concentration at any time can be calculated in the following way:

From Eq. (4.3)

$$SOUR\left(K_S + G\right) = \mu_{HM} \times G$$

$$G_i = \frac{SOUR_i \times K_S}{(\mu_{HM} - SOUR_i)}$$

Because G = glucose concentration = $(S_{i-1} - S_i) - (G_i - G_{i-1})$ (4.4)

where S_{i-1} = starch concentration at time $T-1$

S_i = starch concentration at time T

G_i = glucose concentration at time T

G_{i-1} = glucose concentration at time $T-1$

Substituting Eq. (4.4) into Eq. (4.3), gives the following equation:

$$SOUR = \frac{\mu_{HM} \times \left[(S_{i-1} - S_i) - (G_i - G_{i-1})\right]}{K_S + \left[(S_{i-1} - S_i) - (G_i - G_{i-1})\right]} \tag{4.5}$$

By rearranging Eq. (4.5), Eq. (4.6) can be found as follows:

$$(S_i - S_{i-1}) - (G_i - G_{i-1}) = \frac{SOUR \times K_S}{\mu_{HM} - SOUR} \tag{4.6}$$

Rearranging Eq. (4.6), the starch concentration at any time can be calculated because the initial starch concentration is known.

CHAPTER 5

RESULTS AND DISCUSSION

Introduction: Methodology of Nonlinear Regression

Prism, developed by Graphpad Software Inc., was the statistical package used to analyze the data. It is an easy-to-use software package that allows the user to construct a model and perform the nonlinear regression required to determine model parameters. Datafit developed by Oakdale is curve fitting and data plotting software that was also used in the data analyses. It is a tool that simplifies the tasks of data plotting, regression analysis and statistical analysis. It was also used to run nonlinear regressions. Nonlinear regression was used to fit data to a model that defines Y (SOUR in this case) as a function of X (substrate concentrations, Motulsky, 2003). Y can be any variable such as enzyme activity or microorganism growth rate. With Prism, the X and Y columns are labelled; so entering data into it is very straightforward.

There are built-in models in the programs or the user may enter a new model. It is also necessary to define all the parameters, if any, that have values based on theory or controls. Sometimes it is also necessary to define constraints on the values of the parameters. Before nonlinear regression can begin, initial values for each parameter need to be determined. These two software programs begin fitting models with initial values for each parameter and then adjust these values to improve the fit. If a standard equation was chosen, then the programs will provide the initial values. If not, the initial values need to be specified.

There are several statistical parameters that can be used to evaluate a curve fit to experimental data. The coefficient of multiple determination (R^2) is one of those. If R^2 is high, the curve has a close fit to the data. The 95% confidence interval (CI) or the standard error (SE) values, from which the 95% CIs are calculated, provide information on the precision of the best-fit parameter values. Considering the number and scatter of the data points, the narrower the interval the better. Nonlinear regression finds parameters that make a model fit the data as closely as possible (Motulsky, 2003). However, there is always the possibility that some other models (even simpler models) might fit the experimental data as well or better.

In practice, it is very important to pick a model that is sensible. If the model makes no sense, even if it fits the data well, the reason for best-fit values cannot be interpreted. In some cases, the goal is just to find a smooth curve that covers most of the data points; however, the model must make scientific sense, particularly if the model is to be used in different contexts or for extrapolation.

The goal of nonlinear regression is to find values for the parameters that are most likely to be correct, and this goal cannot be achieved without making an assumption about how the data are scattered around the curve. Most commonly the scatter follows either a Gaussian distribution or the standard deviation of the scatter is the same for all values of X. The best-fit model is the one that has the least sum of squares of the distance between data points and curve.

5.1 Readily Biodegradable Substrate (Glucose) Metabolism Rate

5.1.1 Experimental Data

In the present study, 16 samples were measured. Table 5.1 shows the OURs and SOURs when different fully active biomass concentrations were exposed to different glucose concentrations. Figure 5.1 presents data from Table 5.1 in a figure format. From Fig. 5.1, it can be seen that SOUR increases to approximately 0.0006 min^{-1} when glucose concentration increases until a maximum growth rate occurs.

Table 5.1 Overall Measurement Results for Glucose

Date	Volume	Glucose Concentration	TSS	VSS	OUR	SOUR	F/M Ratio
	Sample+Water mL	mg COD/L	mg/L	mg/L	mg/L/min	min^{-1}	
March 1, 2009	290+0	17.1	420	400	0.203	5.08 E-04	0.04
March 2, 2009	290+0	27.5	410	370	0.215	5.82 E-04	0.07
March 4, 2009	290+0	54.4	520	460	0.234	5.09 E-04	0.12
March 4, 2009	290+0	137.9	520	460	0.245	5.33 E-04	0.30
March 7, 2009	250+40	17.1	600	580	0.241	4.15 E-04	0.03
March 7, 2009	250+40	27.5	570	500	0.275	5.49 E-04	0.06
March 7, 2009	250+40	54.4	640	590	0.289	4.90 E-04	0.09
March 7, 2009	250+40	137.9	570	510	0.296	5.80 E-04	0.27
March 4, 2009	200+90	17.1	450	360	0.079	2.19 E-04	0.05
March 5, 2009	200+90	27.5	440	420	0.109	2.60 E-04	0.07
March 6, 2009	200+90	54.4	400	350	0.204	5.84 E-04	0.16
March 6, 2009	200+90	137.9	330	300	0.169	5.64 E-04	0.46
March 8, 2009	150+140	17.1	380	350	0.170	4.86 E-04	0.05
March 8, 2009	150+140	27.5	360	350	0.153	4.38 E-04	0.08
March 9, 2009	150+140	54.4	390	350	0.174	4.97 E-04	0.16
March 9, 2009	150+140	137.9	380	360	0.179	4.98 E-04	0.38

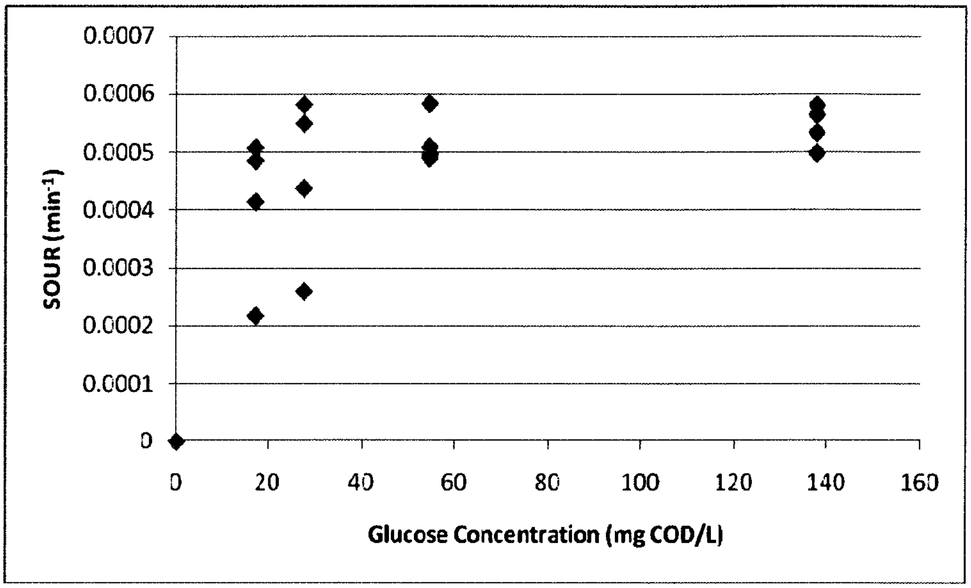


Figure 5.1 SOUR Measurements versus Various Glucose Concentrations

Given the natural variability associated with SOUR measurements at any glucose concentration, it is interesting to examine the best curve if average SOUR is used. Since DO was monitored at four specific glucose concentrations, average SOUR could be calculated at those corresponding concentrations. Table 5.2 and Fig. 5.2 present the average SOUR at different concentrations of glucose.

Table 5.2 Average SOUR at Different Glucose Concentrations

Glucose Concentration (mg COD/L)	Average SOUR (min^{-1})
0	0
17.1	4.07E-04
27.5	4.57E-04
54.4	5.20E-04
137.9	5.44E-04

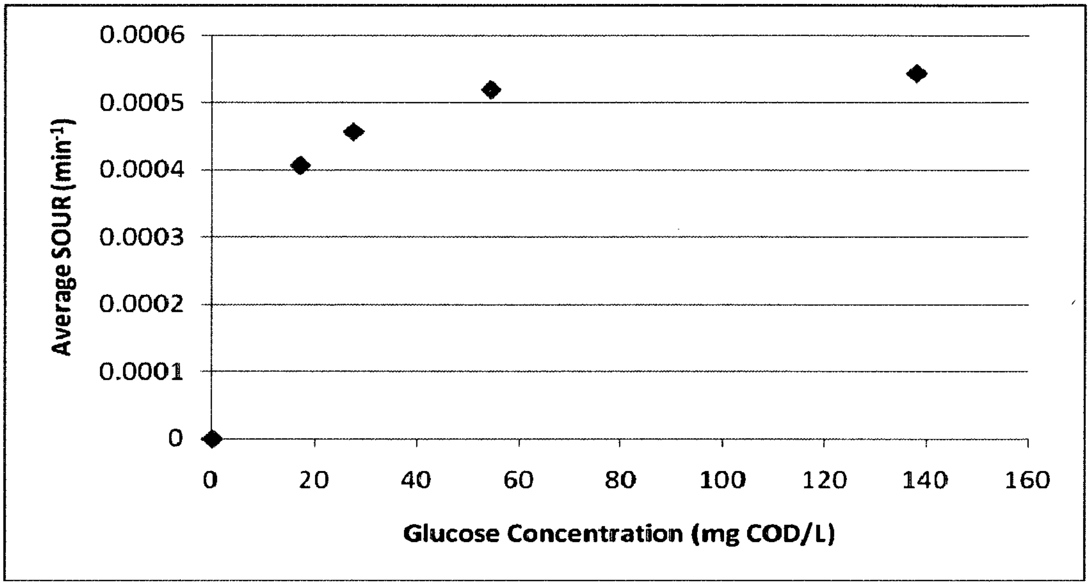
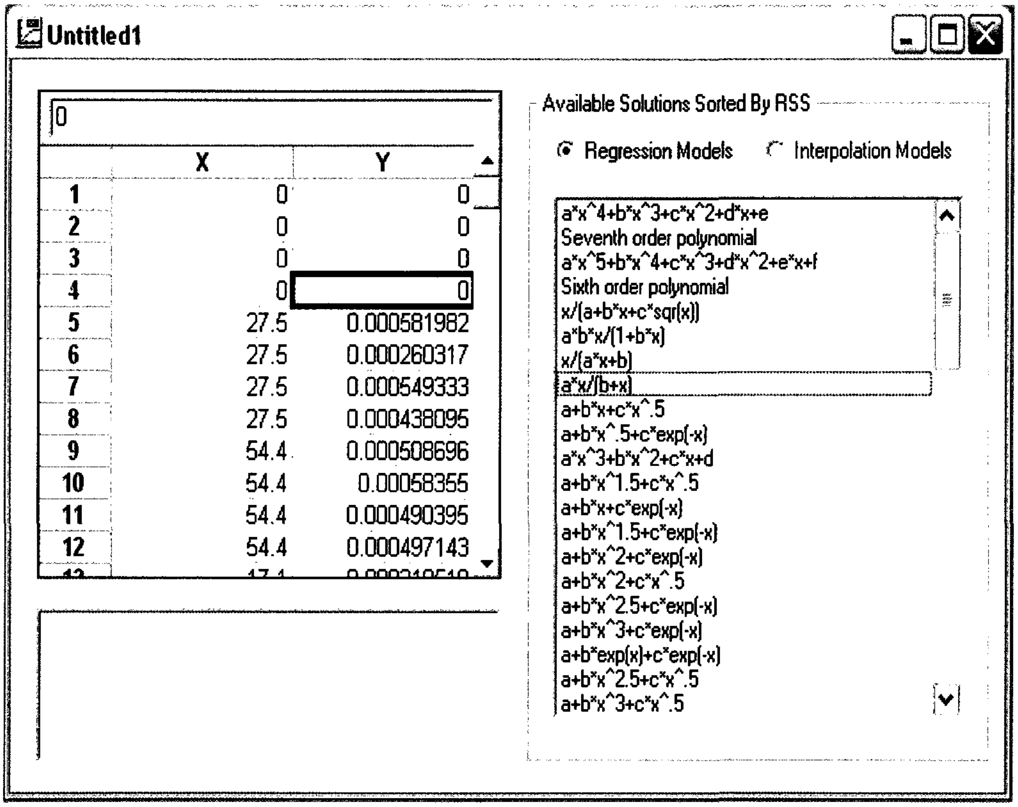


Figure 5.2 Average SOUR at Different Glucose Concentrations

5.1.2 Fitting Models to Biological Data Using Nonlinear Regression

In order to determine which model fits the above experimental data best, Datafit was used as a powerful tool to run nonlinear regressions for the determination of glucose metabolism rate. Figure 5.3 is the interface of Datafit when all experimental data were incorporated. The left hand box is the list of data, while in the right hand box there is a list of model ranking. The highlighted Monod equation placed number 8 of the 294 simple to complex model equations. After executing nonlinear regression on the data in Table 5.1, the results showed that the Monod equation $SOUR = \frac{\mu_{HM} \times G}{K_S + G}$ [Eq. (4.3), where G is the glucose concentration, mg COD/L, the values of variables μ_{HM}, K_S and their 95% CIs are shown in Table 5.4)] was one of the best and the easiest models among all the default models (there are totally 294 models that are built into this software). The top 5 models are too complex and makes no meaning. Even though there are two other models (rank number 6 and 7) that fit the experimental data better (the software lists all 249 models in sequence —best fitting models come first), there were almost no differences among these three models in terms of R^2 (the fit information of the other two models is shown in Appendix C). Figures 5.4 and 5.5 show the curves of SOUR and average SOUR at different glucose concentrations, respectively. In these experiments data scatter was widest at the lower values of SOUR (Fig. 5.4) which is snot expected since measurement errors are magnified at smaller values.

Besides the Monod equation, the dual hyperbolic equation was also checked. However, based on the statistical evaluation, it could not describe the experimental data any better than the Monod equation (for more detailed fit information of dual hyperbolic and other equations refer to Appendix C).



	X	Y
1	0	0
2	0	0
3	0	0
4	0	0
5	27.5	0.000581982
6	27.5	0.000260317
7	27.5	0.000549333
8	27.5	0.000438095
9	54.4	0.000508696
10	54.4	0.00058355
11	54.4	0.000490395
12	54.4	0.000497143

Available Solutions Sorted By RSS

● Regression Models ○ Interpolation Models

a*x^4+b*x^3+c*x^2+d*x+e
Seventh order polynomial
a*x^5+b*x^4+c*x^3+d*x^2+e*x+f
Sixth order polynomial
x/(a+b*x+c*sqr(x))
a*b*x/(1+b*x)
x/(a*x+b)
a*x/(b+x)
a+b*x+c*x^.5
a+b*x^.5+c*exp(-x)
a*x^3+b*x^2+c*x+d
a+b*x^1.5+c*x^.5
a+b*x+c*exp(-x)
a+b*x^1.5+c*exp(-x)
a+b*x^2+c*exp(-x)
a+b*x^2+c*x^.5
a+b*x^2.5+c*exp(-x)
a+b*x^3+c*exp(-x)
a+b*exp(x)+c*exp(-x)
a+b*x^2.5+c*x^.5
a+b*x^3+c*x^.5

Figure 5.3 Interface of Datafit

When analyzing the quality of fit of a model to experimental data, there are often several data points under similar conditions that are far apart from each other. In biosystems this may be caused by normal biological diversity in the sample (i.e., similar individual bacteria may behave differently from others resulting in variation in results). However, in general, variation in data sets from similar biosystems should be statistically random and not follow trends. Additionally, models can incorporate components that do not add significantly to the accuracy of the model. Statistical analysis can be done by

Datafit automatically. When the most appropriate model for a given set is unknown, there are several means to establish which mathematical model fits the given data best. The following are key measurements of the quality of the model fit: sum of squares, residual mean square, residual plots, CIs of the parameters and correlation coefficient.

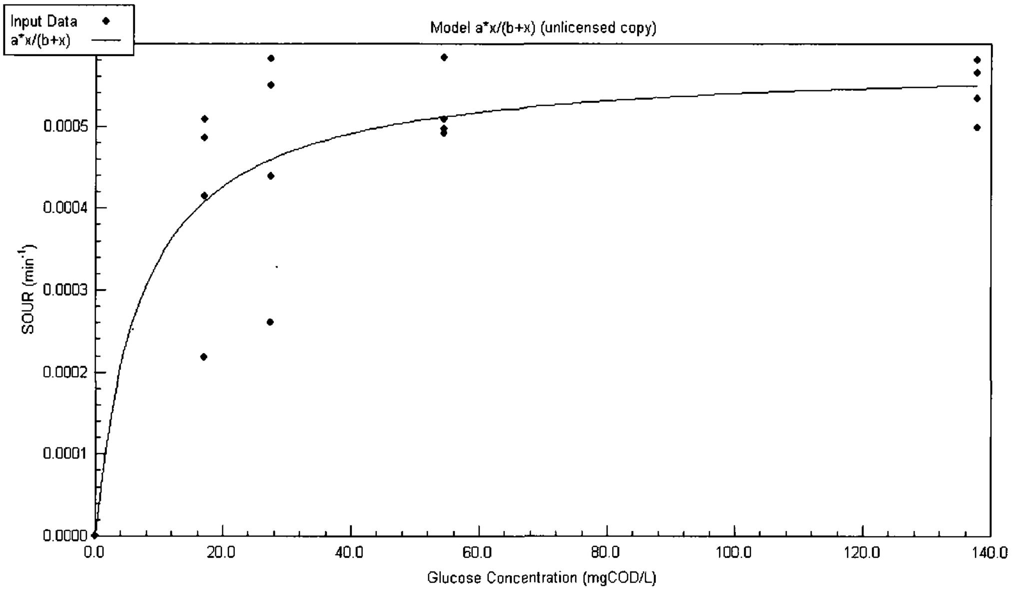


Figure 5.4 SOUR fit to the Monod equation

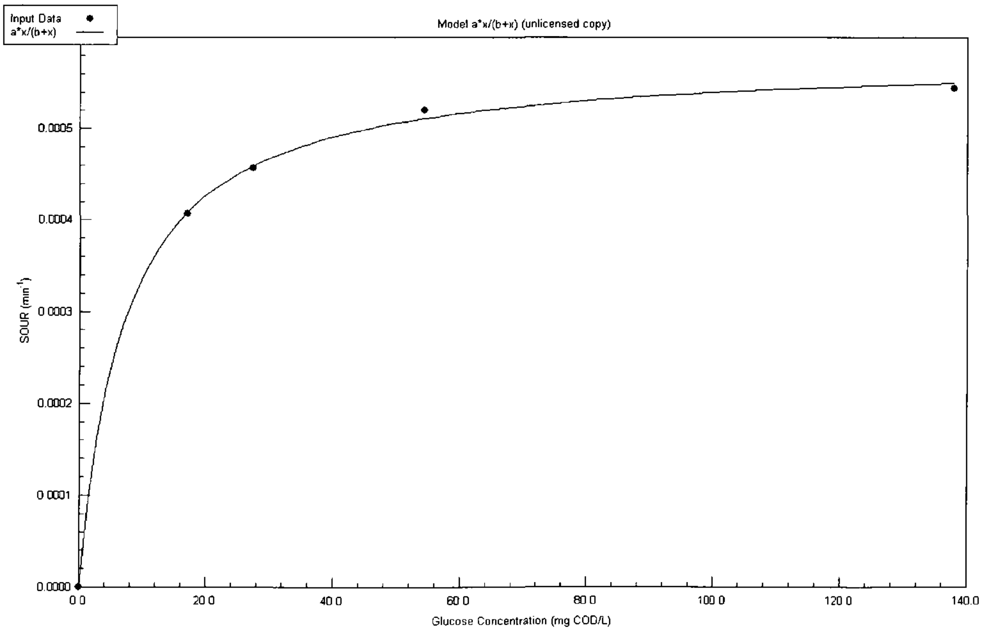


Figure 5.5 Average SOUR fit to the Monod equation

(1) Sum of Squares ($S_r = \sum (Y - f(\theta, X))^2$)

where Y = dependent variable

X = independent variable

Θ = regressed coefficients

As the value decreases, the model is better. Models with more parameters will decrease the sum of squares; however, the simplest (smallest) model that gives adequate results is desired.

(2) Residual Mean Squares (MS)

$$MS = S_{x,y} = (S_r / n - (m+1)^2)$$

where n = number of data points

m = number of parameters (coefficients) within the model

As values become lower the model fit improves. This index includes the number of parameters within the model, so although S_r may continually decreases as the parameters increases $S_{x,y}$ will increase at some point. When this happens, the model is too complex.

(3) Residual Plots

Plot the residuals versus the values of the independent variables (X) and check if the residuals are randomly distributed or if they show a pattern. If they show a pattern then a better model is likely available, so other models need to be evaluated.

(4) CIs of the Parameters (i.e., Θ)

Examine the CIs of the regressed coefficients. If the range includes zero then assume the related part of the model does not really contribute meaningful information, so a model without the term is preferable as it is simpler and the term in question does not improve the prediction much. One should look at adding additional terms or a different mathematical form.

(5) Correlation Coefficient/Coefficient of Determination

$R^2 = (S_t - S_r) / S_t$ = coefficient of determination where S_t = sum of squares around the mean = $\sum (Y - Y_{mean})^2$

S_r = sum of squares of the residuals = $\sum (Y - f(\theta, x))^2$

Y_{mean} = mean value of the observed values of the dependent variable (Y)

As R^2 is closer to one, the model has a better fit. This parameter is not perfect and it is used to the point of abuse. Thus R^2 should not be used as the only measure of quality of fit. In Figs. 5.6 and 5.7, residual errors and residual normal probability plots for this nonlinear regression are presented indicating random distribution of residuals and no trends.

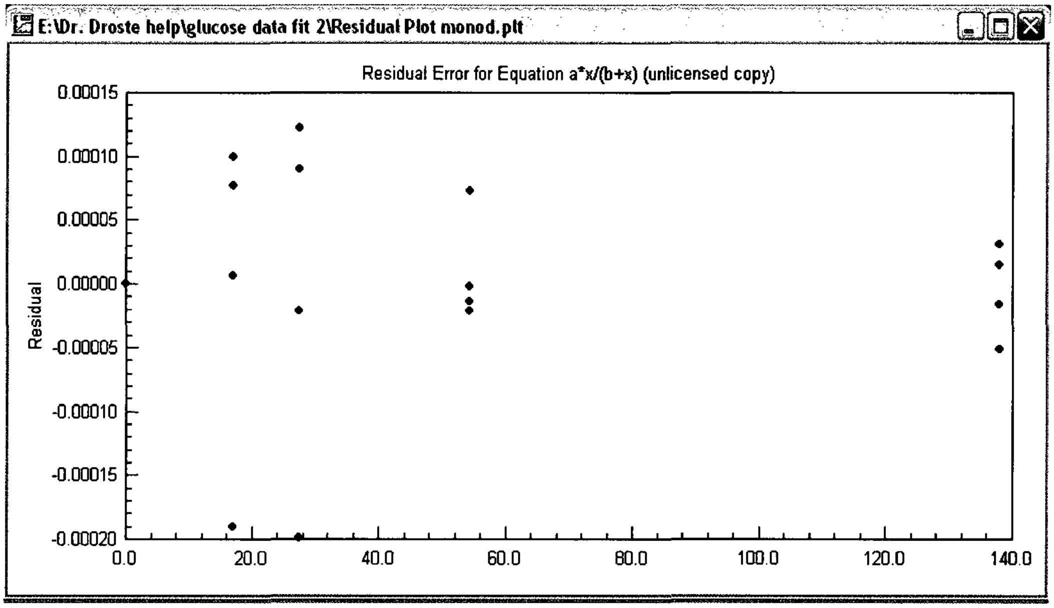


Figure 5.6 Residual Errors for Eq. (4.3)

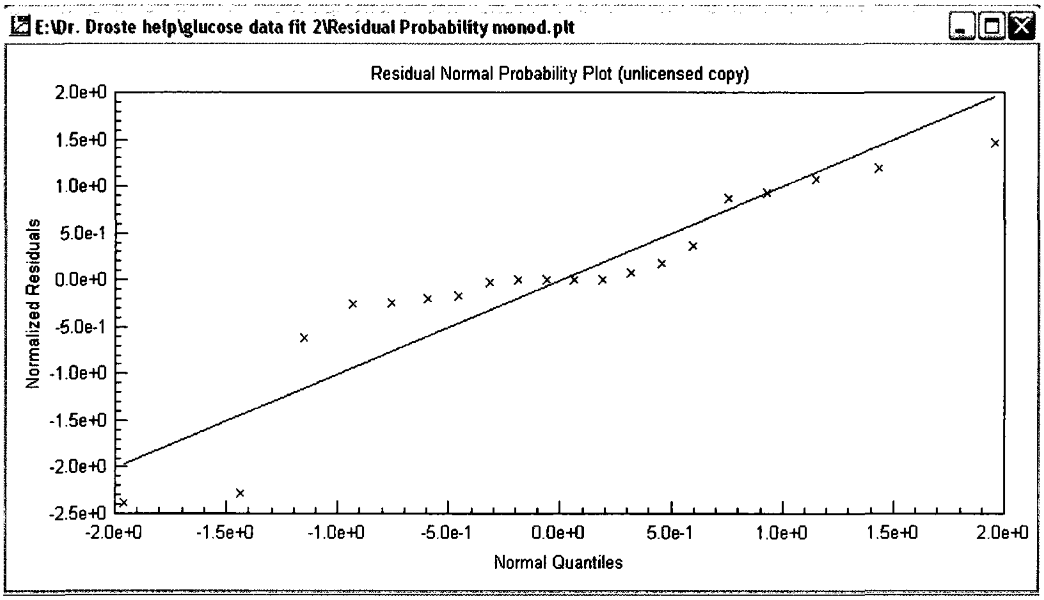


Figure 5.7 Residual Normal Probability Plot for Eq. (4.3)

Table 5.3 Fit information of Eq. (4.3)

Model Definition:

$$SOUR = \frac{\mu_{HM} \times G}{K_S + G}$$

Number of observations = 20
Number of missing observations = 0
Solver type: Nonlinear
Nonlinear iteration limit = 250
Diverging nonlinear iteration limit =10
Number of nonlinear iterations performed = 6
Residual tolerance = 0.0000000001
Sum of Residuals = -4.67E-07
Average Residual = -2.34E-08
Residual Sum of Squares (Absolute) = 1.25E-07
Residual Sum of Squares (Relative) = 1.25E-07
Standard Error of the Estimate = 8.34E-05
Coefficient of Multiple Determination (R^2) = 0.863
Proportion of Variance Explained = 86.3%
Adjusted coefficient of multiple determination (Ra^2) = 0.856
Durbin-Watson statistic = 2.602

G	SOUR	Cal SOUR	Residual	% Error	Abs Residual	Min Residual	Max Residual

mg COD/L	min^{-1}	min^{-1}					
0	0	0	0	0	0	−1.99E-04	1.22E-04
27.5	5.82E-04	4.59E-04	1.23E-04	21.1	1.23E-04		
27.5	2.60E-04	4.59E-04	−2E-04	−76.4	1.99E-04		
27.5	5.49E-04	4.59E-04	9E-05	16.4	9.00E-05		
27.5	4.38E-04	4.59E-04	−2.1E-05	−4.8	2.12E-05		
54.4	5.09E-04	5.11E-04	−2.4E-06	−0.5	2.42E-06		
54.4	5.84E-04	5.11E-04	7.24E-05	12.4	7.24E-05		
54.4	4.90E-04	5.11E-04	−2.1E-05	−4.2	2.07E-05		
54.4	4.97E-04	5.11E-04	−1.4E-05	−2.8	1.39E-05		
17.1	2.19E-04	4.08E-04	−1.9E-04	−86.9	1.90E-04		
17.1	4.15E-04	4.08E-04	6.56E-06	1.6	6.56E-06		
17.1	4.86E-04	4.08E-04	7.73E-05	15.9	7.73E-05		
17.1	5.98E-04	4.08E-04	9.93E-05	19.6	9.93E-05		
137.9	5.33E-04	5.49E-04	−1.6E-05	−3.0	1.62E-05		
137.9	5.64E-04	5.49E-04	1.5E-05	2.6	1.49E-05		
137.9	5.80E-04	5.49E-04	3.09E-05	5.3	3.09E-05		
137.9	4.98E-04	5.49E-04	−5.1E-05	−10.3	5.13E-05		

The R^2 value for the Monod equation is 0.863, which is very close to 1. However, as mentioned above, R^2 cannot be the main criterion to judge whether a model fit to the data is reasonable. A high R^2 indicates that the curve came very close to the data points, but it does not indicate that the fit is sensible in other ways. The best-fit values of the parameters may have values that make no sense or the CIs may be very wide. In this case, all the best- fit values are sensible. Table 5.3 shows some other statistical parameters for Monod equation, like residual of SOUR, average residual, minimum and maximum residuals.

To truly judge parameter estimation one needs to evaluate the precision of the parameter estimates. To do so the 95% CI is generally used. In this case, from Table 5.4, the 95% CI for parameter μ_{HM} which is the maximum specific growth rate is from 4.78×10^{-4} to 6.78×10^{-4}, and for parameter K_S, which is the half saturation coefficient, is from −0.109 to 14.3. There is not a rule of thumb to judge if a 95% CI is wide or narrow. Nevertheless, these CIs are comparatively narrow based on experience.

Table 5.4 95% CIs of Several Parameters for Eq. (4.3)

95% Confidence Intervals					
Variable	Unit	Value	95% (+/–)	Lower Limit	Upper Limit
μ_{HM}	min^{-1}	5.78E-04	1.00E-04	4.78E-04	6.78E-04
K_s	mg COD/L	7.09	7.20	–0.109	14.29

In conclusion, the equation $SOUR = \frac{\mu_{HM} \times G}{K_S + G}$ is simple and the best equation to describe RBCOD (glucose) metabolism rate. During the experiments conducted in this study, substrate concentration is assumed to be unchanged, which means the situation is steady state. The Monod equation can also describe transients. The reason why "growth at a constant value of μ_{HM} was observed at initial concentrations considerably below those required for growth at the maximum rate" is because the value of K_s is very small. Specific growth rate was controlled by substrate concentration but significant variation in growth rate occurs in the low substrate concentration range. Therefore, there can be a significant error due to substrate measurement.

The Monod equation with the above values of its parameters was used in the second phase of study to evaluate true hydrolysis rate account SBCOD.

5.2 Slowly Biodegradable Substrate (Soluble Starch) Hydrolysis Rate

5.2.1 Experimental Data

In ASM1 and ASM2, hydrolysis processes are supposed to be the rate-limiting step in many situations. Determination of kinetic coefficients for hydrolysis processes is an essential part of the model calibration. To date, the measurement of OUR is the best tool for estimation of SBCOD hydrolysis rate. Appendix D shows the values of OUR and SOUR for the batch experiments when different biomass concentrations were exposed to different starch concentrations.

5.2.2 Fitting Models to Biological Data Using Nonlinear Regression

The hydrolysis rates under aerobic conditions were studied by using starch as a model substrate representing SBCOD. From a modeling point of view, slowly biodegradable substrates are those substrates that cannot pass through the cell wall directly and need to be hydrolysed by enzymes. Conversely, readily biodegradable substrate can pass directly through the cell membrane and be utilized directly by biomass. Therefore, SBCOD is the substrate of hydrolysis processes and RBCOD is the product. In this case, starch is being hydrolysed to glucose.

The initial concentration of starch and biomass were selected so that the rate of starch hydrolysis taking place could be monitored. Different starch and biomass combinations were investigated as shown in Table 5.5.

Table 5.5 Starch and Active Biomass Concentration Combinations

	Starch Concentration	Active Biomass Concentration
Run	mg COD/L	mg VSS/L
1	20	250
2	20	380
3	20	370
4	40	310
5	80	430
6	10	250

Batch experiments were conducted to measure the rates of starch hydrolysis by fully active activated sludge. Table 5.6 gives an example on how OUR was monitored and the values of OUR, average OUR and SOUR based on the experimental data (when S = 20 mg COD/L and X_{BH} = 380 mg VSS/L). Similar to the glucose metabolism rate measurements, there is a short initial DO lag phase here as well. However, since the lag phase is only around 15 seconds, it can be assumed to be negligible. Table 5.7 presents an example of starch concentration calculation at different times (when X_{BH} = 430 mg VSS/L, S = 80 mg COD/L). This calculation is based on Eqs. (4.1), (4.2) and (4.3) developed in Chapter 4. Table 5.8 shows the results of all the calculations. Figure 5.8 shows the change in starch concentration over the first 15 minutes for the different initial starch concentrations used in the batch studies (Appendix D shows all experimental data used in determining starch hydrolysis rate).

Table 5.6 Experimental Data and Calculation

March 16, 2009				
Starch	20 mg COD/L		Biomass	380 mg VSS/L
Time	DO	OUR	Average OUR	SOUR
(min)	(mg/L)	(mg/L/min)	(mg/L/min)	(min^{-1})
0	7.59			
1	7.58	0.01		
2	7.54	0.04		
3	7.51	0.03		
4	7.44	0.07		
5	7.44	0	0.03	7.89E-05
6	7.40	0.04	0.036	9.47E-05
7	7.40	0	0.028	7.37E-05
8	7.32	0.08	0.038	1E-04
9	7.29	0.03	0.03	7.89E-05
10	7.28	0.01	0.032	8.42E-05
11	7.24	0.04	0.032	8.42E-05
12	7.22	0.02	0.036	9.47E-05
13	7.24	–0.02	0.016	4.21E-05
14	7.14	0.10	0.03	7.89E-05
15	7.17	–0.03	0.022	5.79E-05

Table 5.7 Calculation of Starch Concentrations at Various Times

		S_i	S_{i-1}	G_i	G_{i-1}	μ_{HM}	K_S	SOUR	$G_i - G_{i-1}$	X_{BH}
Starch Concentration	Time min	(mg COD/L)	(mg COD/L)	(mg COD/L)	(mg COD/L)	(min^{-1})	(mg COD/L)	(min^{-1})	(mg/L)	(mg/L)
80 mg COD/L	0	80				5.78E-04	7.09			430
Biomass Concentration	2.5	79.77	80	0.116	0	5.78E-04	7.09	9.3E-06	1.16E-01	430
430 mg/L	5.5	79.29	79.77	0.297	0.116	5.78E-04	7.09	2.33E-05	1.81E-01	430
	6.5	79.24	79.29	0.176	0.297	5.78E-04	7.09	1.4E-05	–1.22E-01	430
	7.5	78.03	79.24	0.689	0.176	5.78E-04	7.09	5.12E-05	5.14E-01	430
	8.5	79.06	78.03	–0.167	0.689	5.78E-04	7.09	–1.4E-05	–8.56E-01	430
	9.5	78.42	79.06	0.236	–0.167	5.78E-04	7.09	1.86E-05	4.03E-01	430
	10.5	77.93	78.42	0.360	0.236	5.78E-04	7.09	2.79E-05	1.24E-01	430
	11.5	78.52	77.93	–0.112	0.360	5.78E-04	7.09	–9.3E-06	–4.72E-01	430
	12.5	78.81	78.52	0.297	–0.112	5.78E-04	7.09	2.33E-05	4.10E-01	430
	13.5	77.64	78.81	0.236	0.297	5.78E-04	7.09	1.86E-05	–6.15E-02	430
	14.5	78.10	77.64	-0.112	0.236	5.78E-04	7.09	–9.3E-06	–3.48E-01	430

Example of starch concentration calculation at different times.

$$SOUR = \frac{\mu_{HM} \times G}{K_S + G} \tag{4.3}$$

Since SOUR can be obtained from Table 5.4, μ_{HM} and K_S are known, G_i, which is glucose concentration at time i=1, can be determined.

From Eq. (4.3)

$$SOUR\,(K_S + G) = \mu_{HM} \times G$$

$$G_i = \frac{SOUR_i \times K_S}{(\mu_{HM} - SOUR_i)}$$

$$= \frac{9.3E - 06 \min^{-1} \times 7.09 mgCOD\ /\ L}{(5.78E - 04 \min^{-1} - 9.3E - 06\ )} = 0.116 \text{ mg COD/L}$$

Because G = Glucose Concentration = $(S_{i-1} - S_i) - (G_i - G_{i-1})$ *(4.4)*

Substituting Eq. (4.4) into Eq. (4.3), gives the following equation:

$$SOUR = \frac{\mu_{HM} \times [(S_{i-1} - S_i) - (G_i - G_{i-1})]}{K_S + (S_{i-1} - S_i) - (G_i - G_{i-1})} \tag{4.5}$$

By rearranging Eq. (4.5), Eq. (4.6) can be found as follows:

$$(S_i - S_{i-1}) - (G_i - G_{i-1}) = \frac{SOUR \times K_S}{\mu_{HM} - SOUR} \tag{4.6}$$

Rearranging Eq. (4.6), the starch concentration when i=1 can be calculated as follows ($S_{i-1} = S_0 =$ 80 mg COD/L, $G_{i-1} =$ 0 mg COD/L),

$$(S_i - 80 mgCOD\ /\ L) - (0.116 mgCOD\ /\ L - 0 mgCOD\ /\ L) =$$

$$\frac{9.3E - 06 \min^{-1} \times 7.09 mgCOD / L}{5.78E - 04 \min^{-1} - 9.3E - 06 \min^{-1}}$$

So, S_i = 79.77 mg COD/L

Table 5.8 Overall Measurement Results for Starch

Time	Starch Concentration					
(min)	(mg COD/L)					
X_{BH} (mg/L)	380	250	310	370	430	250
0	20	10	40	20	80	20
2.5	1.78E+01	9.60E+00	3.88E+01	1.90E+01	79.8	1.92E+01
5.5	1.61E+01	8.74E+00	3.89E+01	1.85E+01	79.29	1.90E+01
6.5	1.54E+01	6.99E+00	3.82E+01	1.91E+01	79.24	1.93E+01
7.5	1.35E+01	6.61E+00	3.75E+01	1.89E+01	78.03	1.89E+01
8.5	1.27E+01	6.08E+00	3.68E+01	1.76E+01	79.06	1.87E+01
9.5	1.14E+01	4.94E+00	3.65E+01	1.66E+01	78.42	1.89E+01
10.5	1.02E+01	4.20E+00	3.56E+01	1.70E+01	77.93	1.85E+01
11.5	8.64E+00	5.30E+00	3.53E+01	1.79E+01	78.52	1.85E+01
12.5	8.92E+00	4.82E+00	3.50E+01	1.71E+01	77.8	1.86E+01
13.5	7.23E+00	4.01E+00	3.50E+01	1.82E+01	77.64	1.88E+01
14.5	6.77E+00	3.92E+00	3.51E+01	1.88E+01	78.10	1.87E+01

The effects of substrate concentration on the rate of hydrolysis by the activated sludge were investigated in aerobic batch experiments. Again only the first 15 minutes were monitored because biomass concentration and substrate concentration are both changed after a long period of time, and it was assumed that they are not significantly changed during this very short time interval.

From DO measurements experiments and starch concentration calculation, it is not difficult to obtain starch concentration variation during the first 15 min. When starch concentration was plotted over time, it was found that the "one phase decay" curve was the best to describe starch hydrolysis rate (See Fig. 5.8). From Prism, the following "one phase decay" equation formulated was fit.

$$S = (S_0 - Plateau) \times \exp(-K \times T) + Plateau \qquad (5.1)$$

Where S = starch concentration

S_0 = the S value when time is zero

T = time

Plateau = the S value at infinite time

K = the hydrolysis rate constant, expressed in reciprocal of the X axis time units.

The one phase decay model is an exponential decay equation that can model many chemical and biological processes; it is the standard first-order decay equation. From Prism, the best curve that fit the experimental data could be found from nonlinear regression. And the best-fit values for 6 data sets, 95% CIs for all parameters and parameter constraints are shown in Table 5.9, respectively.

From Table 5.9, it can be seen that R^2 for each curve is very high except for one. However, the K values are not identical. When parameter constraints were used to try to keep the K value the same for all runs, R^2 could drop to 0.5. As a result, calibration is needed before this equation can be applied. In order to find the relation between d*S*/d*t* with time (so that the relationship can be built into Datafit to find the most appropriate model), derivation of Eq. (5.1) was needed.

$$S = (S_0 - Plateau) \times \exp(-K \times T) + Plateau \tag{5.1}$$

To make the derivation easier, it is assumed that:

a = S_0- Plateau, b = K, c = Plateau, Y = S, X = T

So, Eq. (5.1) can be written as Eq. (5.2) as follows

$$Y = a * \exp(-b \times X) + c \tag{5.2}$$

Doing the derivation of Eq. (5.2), Eq. (5.3) can be found

$$d\left\lfloor a^{(-b \times X)} + c \right\rfloor / dX = -a^{-b \times X} \times b \times \log(a)$$

$$dS / dt = -(\mathrm{S_0} \text{ - Plateau })^{-K \times t} \times K \times \log(\mathrm{S_0} \text{ - Plateau }) \tag{5.3}$$

Equation (5.3) gives the relationship of *dS/dt* vs time for a given initial substrate and initial biomass concentration. From this equation, since plateau and K values are known (see Table 5.9), *dS/dt* vs time can be plotted at a given initial starch concentration and similarly, *dS/dt* vs initial starch concentration during the first 15 min can also be plotted. Six runs at varying X and S given in Appendix E using Eq. (5.3) can then be used to determine the relationship between starch concentration, biomass and dS/dt with either the Monod or other models that incorporate X and S as parameters. An example of the curve for a single condition S = 20 mg COD/L and X_{BH} = 380 mg VSS/L is given in Fig. 5.9. All the other runs are shown in Appendix E. Similarly Fig. 5.10 shows the relationship between starch concentration changing rate with respect to starch concentration that remains in the mixed liquor for a given X_{BH} and initial S. Similarly all of the 6 runs are summarized in Appendix E.

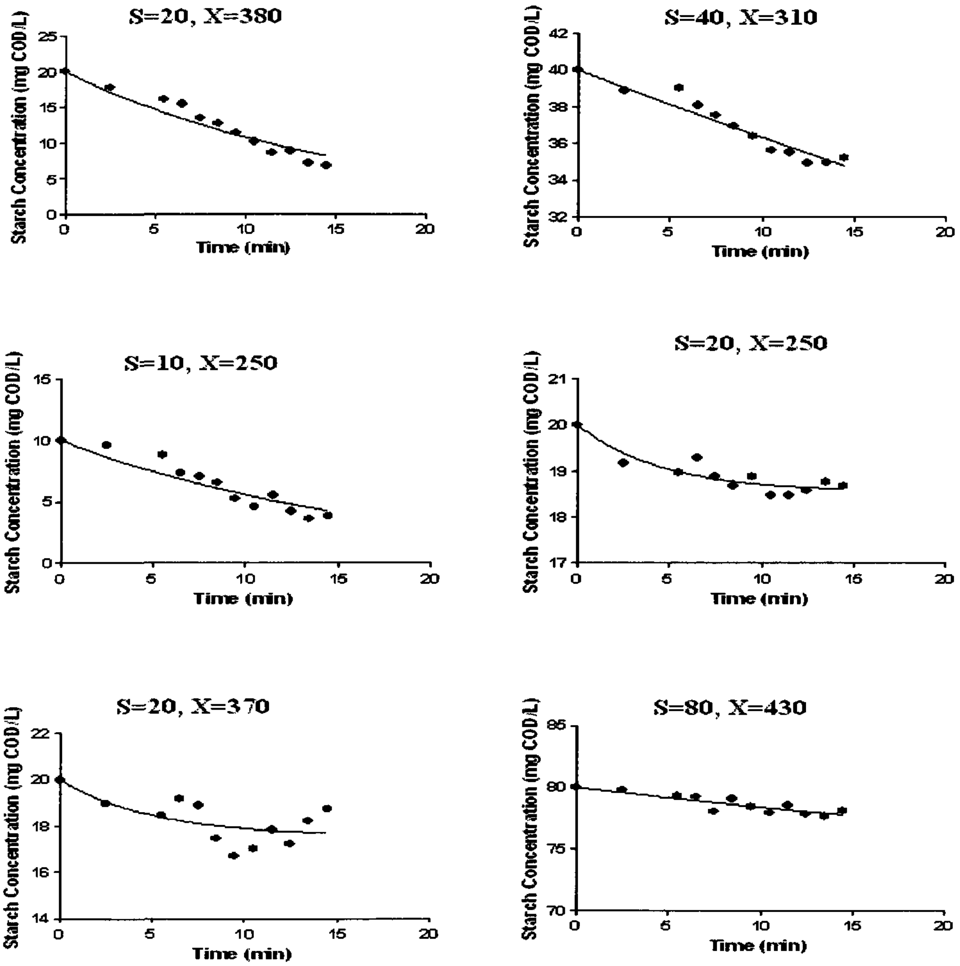


Figure 5.8 Starch Concentration Changes with Time

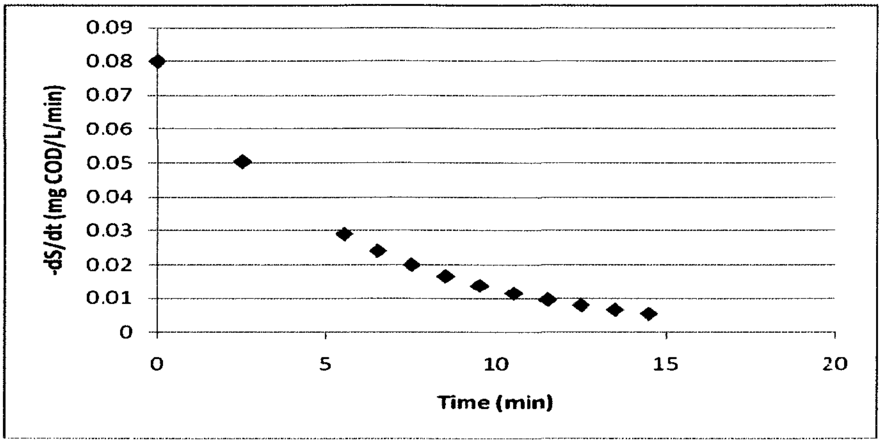


Figure 5.9 Starch Concentration Change Rate with Time (i.e., S = 20 mg COD/L, X_{BH}= 380 mg VSS/L)

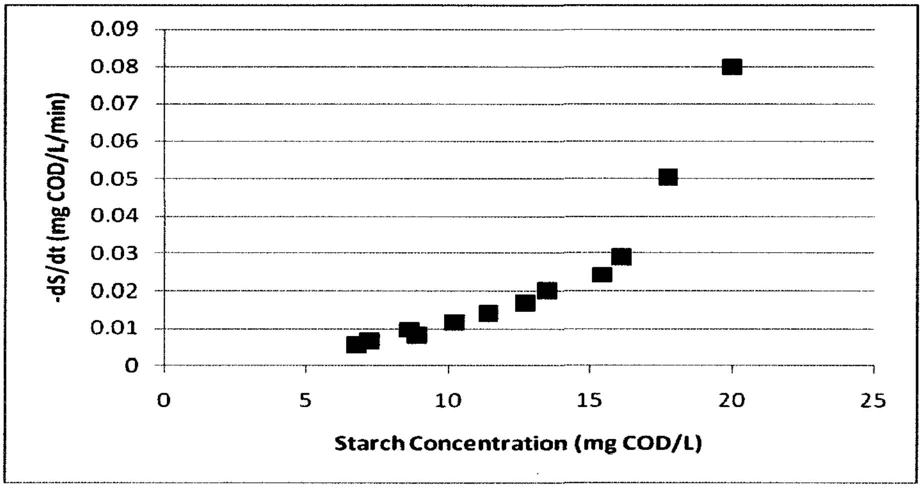


Figure 5.10 Starch Concentration Change Rate with Starch Concentration (i.e., S = 20 mg COD/L, X_{BH} = 380 mg VSS/L)

It can be seen from Fig. 5.9 that starch concentration dropped dramatically during the first 5 min., and then the changing rate slows down. This may be because at the beginning, there are many “sites” for enzymes to adsorb on the surface of starch molecules, and thereafter since there are limited sites available, adsorption rate is slow. Figure 5.10 shows that changing rate increases as starch concentration increases and concomitant reaction sites increases lending support to the enzyme site availability concept. In order to evaluate biomass concentration and starch concentration influence on hydrolysis rate, Table 5.10 shows the calculated starch concentration at different times and the corresponding dS/dt and dS/dt/X_{BH} for S =20 mg COD/L and X_{BH} = 380 mg VSS/L. Again all 6 runs at varying starch and biomass concentrations are summarized in Appendix E.

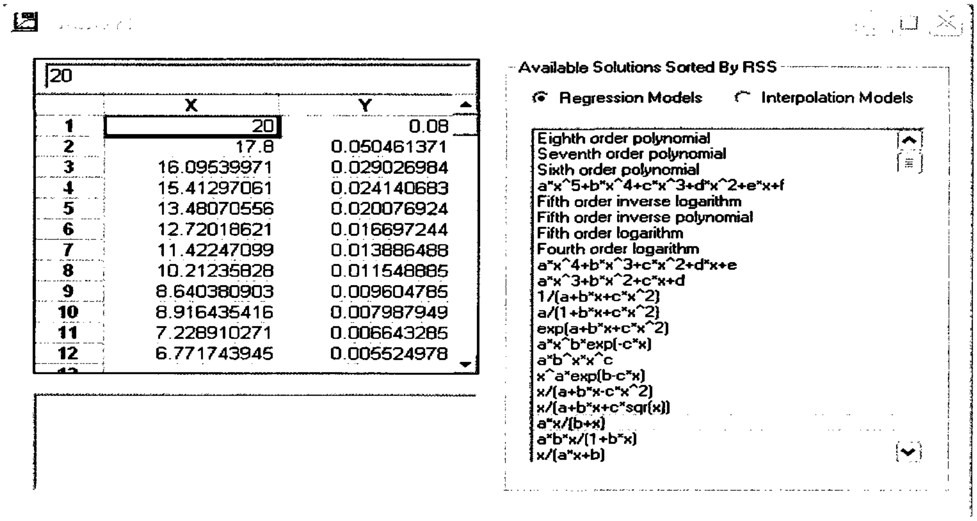
Here is an example of how dS/dt was calculated when S = 20 mg COD/L, X_{BH} = 380 mg VSS/L.

$$d\left[a^{(-b\times X)} + c\right]/\,dX = -a^{-b\times X}\times b\times \log(a) \qquad (5.3)$$

From Table 5.9, we can find a = a_0 – Plateau = 20 – 7.49E-011 ≈ 20 mg COD/L. b = K = 0.062, so

$$dS\;/\;dT = dY\;/\;dX = -a^{-b\times X}\times b\times \log(a) = -20^{-0.062*T}\times 0.062\times \log(20)$$
$$= -0.08\times 20^{-0.062*T}$$

Figures 5.11 and 5.12 present the 2-D and 3-D Datafit interfaces when all experimental data in Table 5.8 and Appendix E which describes the relationship between hydrolysis rate, S and X_{BH}, were entered and then used to evaluate the Monod type or dual hyperbolic type models respectively. As can be seen from Fig. 5.11, a Monod type equation $\frac{dS}{dt} = \frac{\mu_{HM}\times S}{K_1 + S}$ [define this equation as Eq. (5.4)] is one of the best among the 250 models (rank No. 19) in the Datafit model library (not shown). In fact the Monod was the 19th best model while the 8th-order model was best but would be rated as too complicated and of little scientific sensibility (righthand screen of Fig. 5.11). Similarly the dual hyperbolic was the 56th best model (see Fig. 5.16) but because it had a good scientific basis and was fairly simple and readily usable it was also selected for evaluation. Statistical analysis can be done by Datafit automatically. Figure 5.13 shows the model plot when the Monod type equation was applied. From Fig. 5.14, residual normal probability plot for this nonlinear regression is presented and indicates a random distribution with minimal trending indicating a high goodness of fit.



20

	X	Y
1	20	0.08
2	17.8	0.050461371
3	16.09539971	0.029026984
4	15.41297061	0.024140683
5	13.48070556	0.020076924
6	12.72018621	0.016697244
7	11.42247099	0.013886488
8	10.21235828	0.011548885
9	8.640380903	0.009604785
10	8.916435416	0.007987949
11	7.228910271	0.006643285
12	6.771743945	0.005524978

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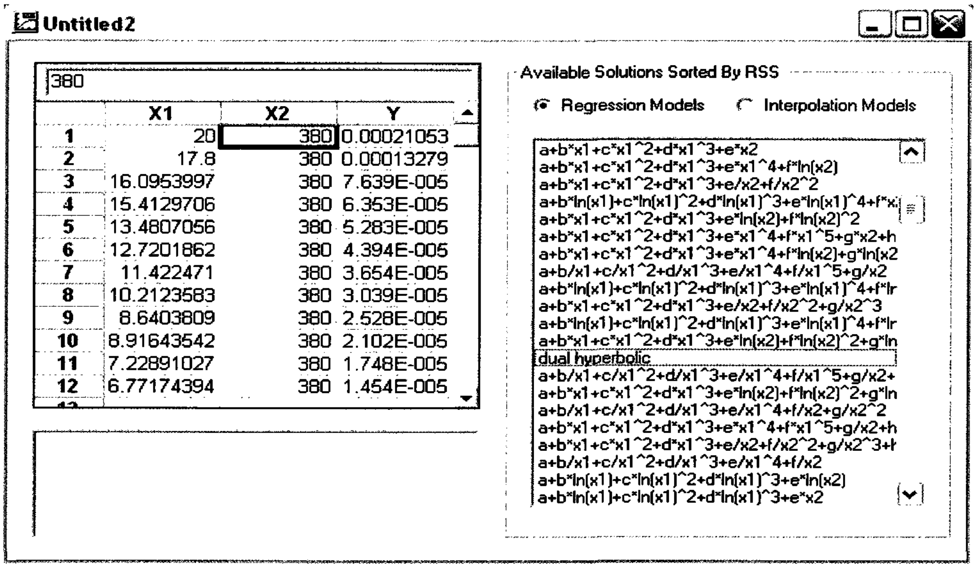
● Regression Models ○ Interpolation Models

```
Eighth order polynomial
Seventh order polynomial
Sixth order polynomial
a*x^5+b*x^4+c*x^3+d*x^2+e*x+f
Fifth order inverse logarithm
Fifth order inverse polynomial
Fifth order logarithm
Fourth order logarithm
a*x^4+b*x^3+c*x^2+d*x+e
a*x^3+b*x^2+c*x+d
1/(a+b*x+c*x^2)
a/(1+b*x+c*x^2)
exp(a+b*x+c*x^2)
a*x^b*exp(-c*x)
a*b^x*x^c
x^a*exp(b-c*x)
x/(a+b*x-c*x^2)
x/(a+b*x+c*sqr(x))
a*x/(b+x)
a*b*x/(1+b*x)
x/(a*x+b)
```

Figure 5.11 2-D-Datafit Interface



Untitled2

380

	X1	X2	Y
1	20	380	0.00021053
2	17.8	380	0.00013279
3	16.0953997	380	7.639E-005
4	15.4129706	380	6.353E-005
5	13.4807056	380	5.283E-005
6	12.7201862	380	4.394E-005
7	11.422471	380	3.654E-005
8	10.2123583	380	3.039E-005
9	8.6403809	380	2.528E-005
10	8.91643542	380	2.102E-005
11	7.22891027	380	1.748E-005
12	6.77174394	380	1.454E-005

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● Regression Models ○ Interpolation Models

```
a+b*x1+c*x1^2+d*x1^3+e*x2
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*ln(x2)
a+b*x1+c*x1^2+d*x1^3+e/x2+f/x2^2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f*x
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)+f*ln(x2)^2
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*x1^5+g*x2+h
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*ln(x2)+g*ln(x2
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x1^5+g/x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f*lr
a+b*x1+c*x1^2+d*x1^3+e/x2+f/x2^2+g/x2^3
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f*lr
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)+f*ln(x2)^2+g*ln
dual hyperbolic
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x1^5+g/x2+
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)+f*ln(x2)^2+g*ln
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x2+g/x2^2
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*x1^5+g/x2+h
a+b*x1+c*x1^2+d*x1^3+e/x2+f/x2^2+g/x2^3+l
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x2)
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*x2
```

Figure 5.12 3-D-Datafit Interface

Table 5.9 Best- fit Values for Starch Hydrolysis Rate

One Phase Decay Best-fit values						
Y_0	= 20.00	= 40.00	= 10.00	= 20.00	= 20.00	= 80.00
Final value						
K	0.06153	0.009671	0.0588	0.2170	0.2004	0.01494
Plateau	7.490E-011	0	6.152E-012	18.54	17.56	68.44
Std. Error						
Plateau	1.204	N/A	6.37	0.1598	0.7285	40.50
K	0.0077	0.00040	0.052	0.075	0.17	0.057
95% Confidence Intervals						
Plateau	0.0 to 2.683	N/A	0.0 to 14.19	18.19 to 18.90	15.94 to 19.18	0.0 to 158.7
K	0.044 to 0.079	0.0088 to 0.0106	0.0 to 0.18	0.050 to 0.38	0.0 to 0.59	0.0 to 0.142
Goodness of Fit						
Degrees of Freedom	10	11	10	10	10	10
R^2	0.924	0.928	0.878	0.832	0.481	0.798
Absolute Sum of Squares	15.3	2.44	6.74	0.34	5.67	1.42
Sy.x	1.24	0.471	0.821	0.185	0.753	0.376
Constraints						
Initial value	$Y_0 = 40.00$	$Y_0 = 80.00$	$Y_0 = 20.00$	$Y_0 = 20.00$	$Y_0 = 20.00$	$Y_0 = 80.00$
Final value	> 0.0	> 0.0	> 0.0	> 0.0	> 0.0	> 0.0
K	$K > 0.0$	$K > 0.0$	$K > 0.0$	$K > 0.0$	$K > 0.0$	$K > 0.0$
Number of points						
Analyzed	12	12	12	12	12	12

Table 5.10 Starch Concentration Change Rate

Starch Concentration	Time	dS/dt	−dS/dt	Starch Conc	−dS/dt/X_{BH}
S =20 mg COD/L	(min)	(mg COD/L/min)	(mg COD/L/min)	(mg COD/L)	(min^{-1})
Biomass Concentration	0	−0.08	0.08	20	2.11E-04
X_{BH} = 380 mg VSS/L	2.5	−0.0505	0.0505	17.8	1.33E-04
	5.5	−0.0290	0.0290	16.09	7.64E-05
	6.5	−0.0241	0.0241	15.41	6.35E-05
	7.5	−0.0201	0.0201	13.48	5.28E-05
	8.5	−0.017	0.0167	12.72	4.39E-05
	9.5	−0.0139	0.0139	11.42	3.65E-05
	10.5	−0.0116	0.0116	10.21	3.04E-05
	11.5	−0.010	0.0096	8.64	2.53E-05
	12.5	−0.0080	0.0080	8.92	2.1E-05
	13.5	−0.0066	0.0066	7.23	1.75E-05
	14.5	−0.0055	0.0055	6.77	1.45E-05

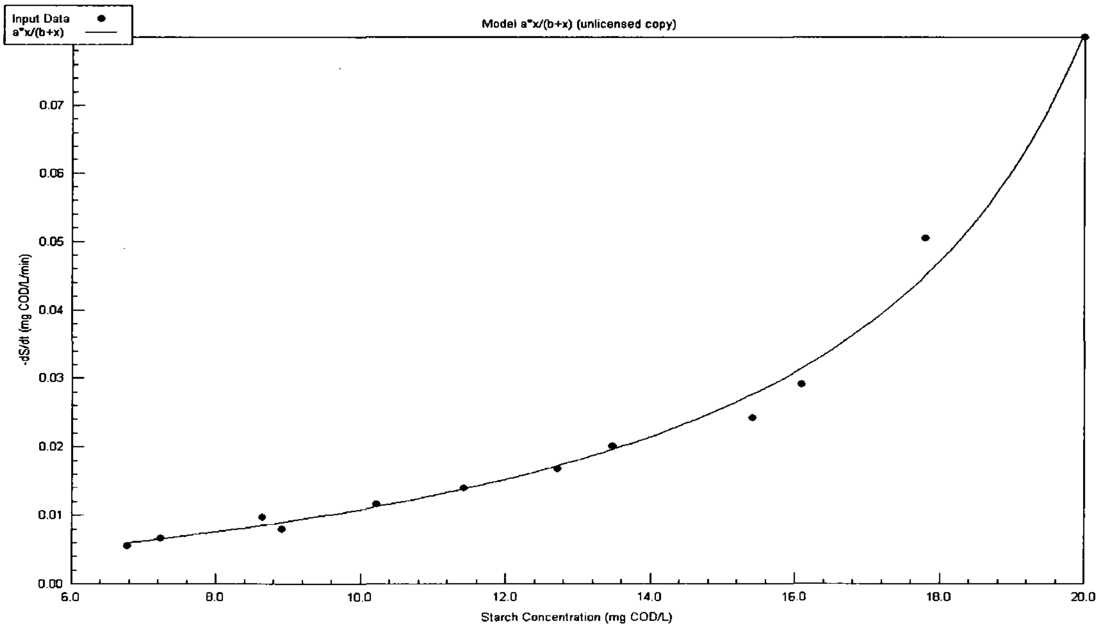

Figure 5.13 Model Plot for Eq. (5.4)

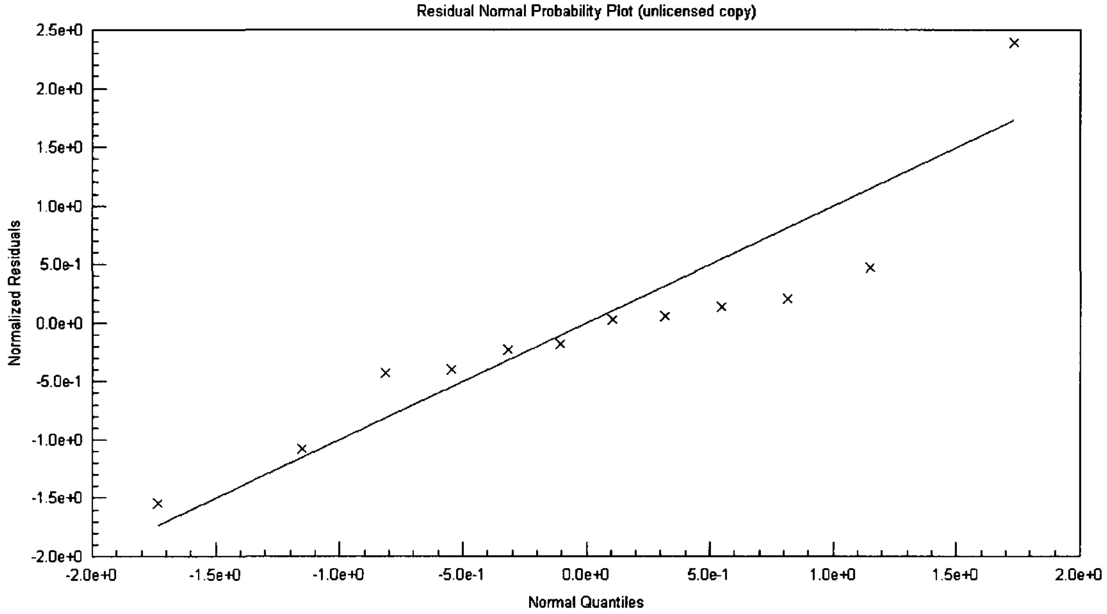

Figure 5.14 Residual Normal Probability Plot for Eq. (5.4)

From Table 5.11, 95% CIs of both parameters in Eq. (5.4) can be seen all indicating very good goodness of fit and accuracy to the data. Table 5.12 (a) and (b) show other fit information and some statistical parameters when Eq. (5.4) was used.

Compared with Monod equation, dual hyperbolic equation $\frac{dS}{dt \times X_{BH}} = \frac{\mu_{HM} \times S}{K_2 \times X_{BH} + c \times S}$ [Eq. (5.5)] was also checked to find out if there is a good relation between hydrolysis rate and SBCOD concentration and biomass concentration (3-D). From Fig. 5.12, it is safe to say that the dual hyperbolic equation is the best simple 3-D model that can describe the hydrolysis data (Appendix F shows all the other fit information when different starch and biomass concentration applied). However, the parameters in Eq. (5.5) are not identical in all cases, which means calibration is needed before this equation can be applied. A single model where the coefficients remained the same for all different initial starch concentrations was also evaluated. However, the results (shown in Fig. 5.15) showed that biomass concentration and starch concentration both have inverse relation with starch hydrolysis rate (From Fig. 5.15, biomass concentration and starch concentration increase both lead to d*S*/d*t* decrease). This phenomenon could not be explained. Also, since all combinations ran at different days, it was difficult to keep biomass identical. As a result, separate condition was used each time and calibration was needed when the fitted equation applied.

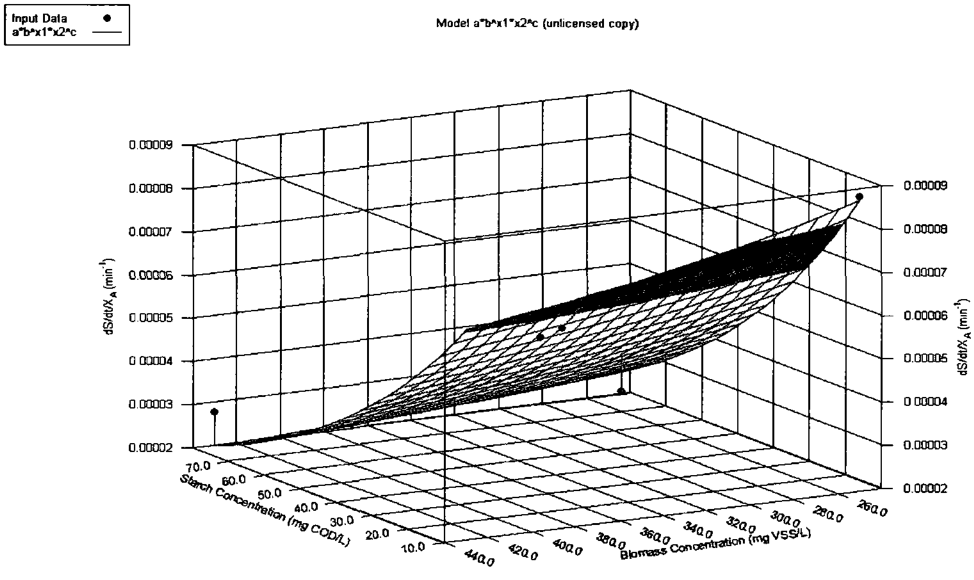

Figure 5.15 Model Plot for all Combinations

From Table 5.13, 95% CIs of several parameters in Eq. (5.5) can be seen (when S = 20 mg COD/L, X_{BH} = 380 mg VSS/L, all the following tables and figures are using the same biomass and substrate concentrations as examples). Table 5.14 (a) and (b) show the fit information and some statistical parameters when Eq. (5.5) was used. Figure 5.16 shows the model plot when Eq. (5.5) was applied. From Fig. 5.17, the residual normal probability plot for this nonlinear regression is presented.

Equation (3.42) developed in Chapter 3 was also tested ($r_{\mathrm{SS,total}} = \dfrac{k_S S_S + k_h X_S}{K_h + X_S} X_H$). However, the model did not fit as well as the Monod and dual hyperbolic model, and R^2 for all runs were comparatively low. The value ranges from 0.11 to 0.49 for the most of runs at different biomass and starch concentration. Only at low starch concentration (2. mg COD/L) and biomass concentration of 380 mg VSS/L, the model fit fairly well with R^2 of 0.9. Table 5.15 shows the R^2 values for all starch and biomass combinations.

In conclusion, starch hydrolysis rate can be described by using either a Monod type equation or a dual hyperbolic equation that is calibrated to environmental conditions.

Table 5.11 95% CIs of Parameters for Eq. (5.4)

95% Confidence Intervals					
Variable	Unit	Value	95% (+/–)	Lower Limit	Upper Limit
μ_{HM}	min^{-1}	1.48E-02	2.19E-03	–1.7E-02	0.013
K_1	mg COD/L	–23.66	0.656	–24.32	–23.0

Table 5.12 (a) Fit information of Eq. (5.4)

Model Definition:

$$\frac{dS}{dt} = \frac{\mu_{HM} \times S}{K_2 + S}$$

Number of observations = 12
Number of missing observations = 0
Solver type: Nonlinear
Nonlinear iteration limit = 250
Diverging nonlinear iteration limit =10
Number of nonlinear iterations performed = 4
Residual tolerance = 0.0000000001
Sum of Residuals = -1.30E-03
Average Residual = -1.086E-04
Residual Sum of Squares (Absolute) = 5.24E-05
Residual Sum of Squares (Relative) = 5.24E-05
Standard Error of the Estimate = 2.29E-03
Coefficient of Multiple Determination (R^2) = 0.99
Proportion of Variance Explained = 99.01%
Adjusted coefficient of multiple determination (Ra^2) = 0.989
Durbin-Watson statistic = 2.463

Table 5.12 (b) Fit information of Eq. (5.4)

S	d*S*/d*t*	Cal d*S*/d*t*	Residual	% Error	Abs Residual	Min Residual	Max Residual
mg COD/L	mg COD/L/ min	mg COD/L/min	mg COD/L/min		mg COD/L/min	mg COD/L/min	mg COD/L/ min
20	0.08	0.0809	−0.00091	−1.14	0.00091	-0.00354	0.00548
17.8	0.0505	0.0450	0.00548	10.86	0.0055		
16.1	0.0290	0.0315	−0.00248	−8.55	0.0025		
15.4	0.0241	0.0277	−0.00353	−14.64	0.0035		
13.5	0.0201	0.0196	0.000466	2.32	0.00047		
12.7	0.0167	0.0172	−0.00052	−3.12	0.00052		
11.4	0.0139	0.0138	0.00006	0.46	0.00006		
10.2	0.0116	0.0113	0.000303	2.62	0.00030		
8.6	0.0096	0.0085	0.00109	11.30	0.0011		
8.9	0.00799	0.0090	−0.00097	−12.11	0.00097		
7.2	0.00664	0.0065	0.000128	1.93	0.00013		
6.8	0.00552	0.0059	−0.00041	−7.47	0.00041		

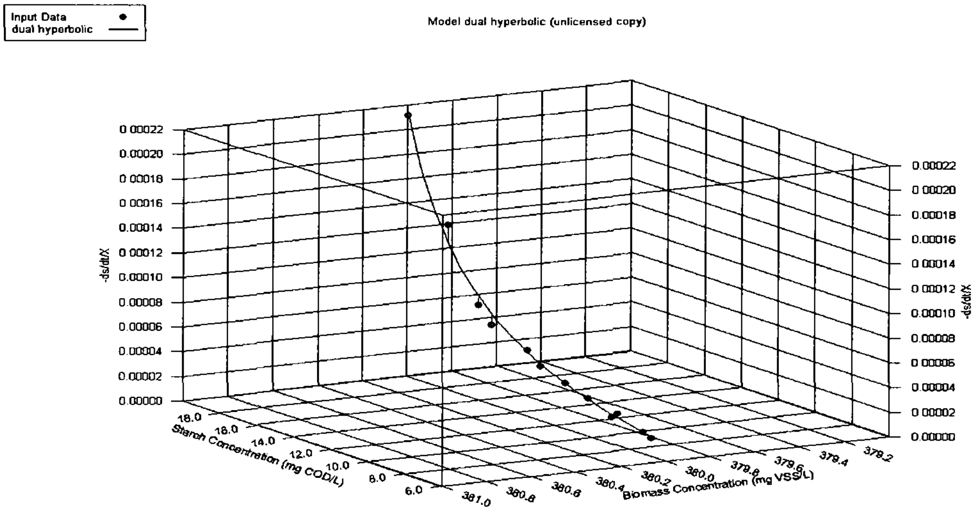

Figure 5.16 Model Plot for Eq. (5.5)

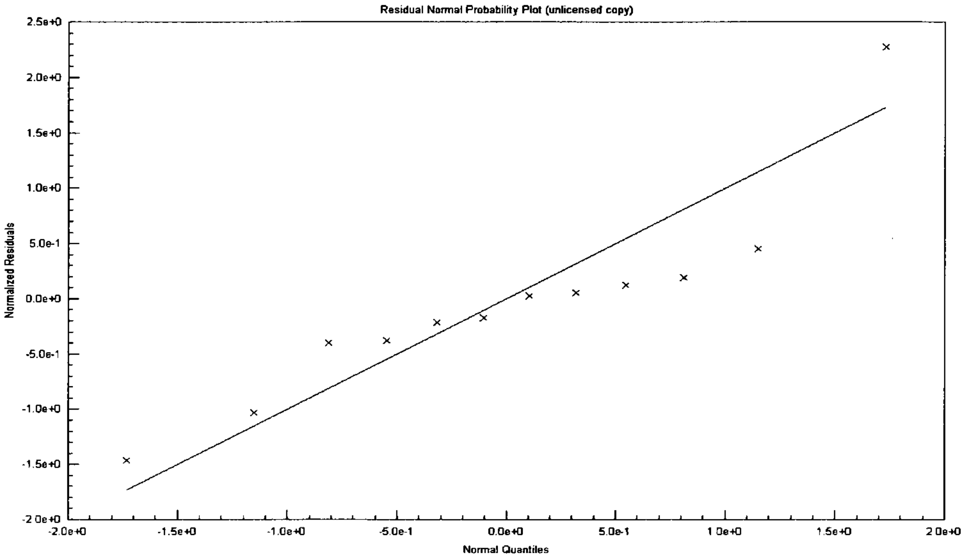

Figure 5.17 Residual Normal Probability Plot for Eq. (5.5)

Table 5.13 95% CIs of Several Parameters for Eq. (5.5)

95% Confidence Intervals					
Variable	Unit	Value	95% (+/–)	Lower Limit	Upper Limit
μ_{HM}	min^{-1}	6.58E-03	4381.3	–4381.3	4381.3
K_2	mg COD/L	10.5	7E+06	–7E+06	7E+06
C	N/A	–169	1.13E+08	–1.13E+08	1.13E+08

Table 5.14 (a) Fit information of Eq. (5.5)

Model Definition:

$$\frac{dS}{dt \times X_{BH}} = \frac{\mu_{HM} \times S}{K_2 \times X_{BH} + c \times S}$$

Number of observations = 12
Number of missing observations = 0
Solver type: Nonlinear
Nonlinear iteration limit = 250
Diverging nonlinear iteration limit =10
Number of nonlinear iterations performed = 22
Residual tolerance = 0.0000000001
Sum of Residuals = −3.43E-06
Average Residual = −2.86E-07
Residual Sum of Squares (Absolute) = 3.6E-10
Residual Sum of Squares (Relative) = 3.6E-10
Standard Error of the Estimate = 6.35E-06
Coefficient of Multiple Determination (R^2) = 0.99
Proportion of Variance Explained = 99.01%
Adjusted coefficient of multiple determination (Ra^2) = 0.988
Durbin-Watson statistic = 2.46

Table 5.14 (b) Fit information of Eq. (5.5)

S	X	dS/dt/X	Cal dS/dt/X	Residual	% Error	Abs Residual	Min Residual	Max Residual
mg COD/L	mg VSS/L	min^{-1}	min^{-1}	min^{-1}		min^{-1}	min^{-1}	min^{-1}
20	380	2.11E-04	0.000213	−2.4E-06	−1.14	2.41E-06	−9.30E-06	1.44E-05
17.8	380	1.33E-04	0.000118	1.44E-05	10.86	1.44E-05		
16.1	380	7.64E-05	8.29E-05	−6.5E-06	−8.55	6.53E-06		
15.4	380	6.35E-05	7.28E-05	−9.3E-06	−14.6	9.30E-06		
13.5	380	5.28E-05	5.16E-05	1.23E-06	2.32	1.23E-06		
12.7	380	4.39E-05	4.53E-05	−1.4E-06	−3.12	1.37E-06		
11.4	380	3.65E-05	3.64E-05	1.69E-07	0.463	1.69E-07		
10.2	380	3.04E-05	2.96E-05	7.97E-07	2.62	7.97E-07		
8.6	380	2.53E-05	2.24E-05	2.86E-06	11.31	2.86E-06		
8.9	380	2.10E-05	2.36E-05	−2.5E-06	−12.12	2.55E-06		
7.2	380	1.75E-05	1.71E-05	3.37E-07	1.93	3.37E-07		
6.8	380	1.45E-05	1.56E-05	−1.1E-06	−7.47	1.09E-06		

Table 5.15 R^2 values for all runs when Eq. (3.42) was applied

Starch Conc (mg COD/L)	20	40	10	20	20	80
Biomass Conc (mg VSS/L)	380	310	250	250	370	430
R^2	0.90	0.49	0.77	0.24	0.12	0.11

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Various theoretical formulation for RBCOD and SBCOD metabolism were developed. A justification for the Monod formulation was given. Other formulations based on the Monod rate formulation and first-order kinetics were developed. Diffusion and storage were considered in the development of the models. A justification for the dual hyperbolic formulation was developed.

A fully active acclimated aerobic culture acclimatized to RBCOD (glucose) and SBCOD (starch) was developed in the laboratory. This culture together with respirometry measurement was used to determine the best model when the culture was exposed to simple RBCOD in batch experiments. Many batch studies with varying concentrations of glucose and active mass were monitored to evaluate over 249 models. The Monod model gave a good fit to the data.

Another series of batch tests were conducted using only starch as a substrate. Again substrate concentration and active mass concentrations were varied in the batch tests. Using the results of the RBCOD assays and respirometry for these experiments the relationship between SBCOD and active biomass accounting for RBCOD metabolism was evaluated using a one-phase decay model. However the one-phase or first-order model where SBCOD was the dependent variable had a different rate constant for each experimental condition. An additional conclusion from this evaluation was support for the enzyme reaction site theory that increase in starch concentration results in increased rates of reaction due to a greater number of reaction sites.

The results from the above evaluation were used to determine the adequacy of starch hydrolysis models based on a Monod type relationship and a dual hyperbolic relation that allowed for determining the true reaction constants accounting for starch concentration, biomass concentration and taking into account degradation of RBCOD.

Both of these models are fairly simple in terms of concept as well as determination of parameters and have scientific sensibility in describing hydrolysis of SBCOD. But again the model parameters for both models changed with each experimental condition.

The values of all model parameters for a fully active microbial culture degrading RBCOD and SBCOD have been presented and are accurate at 95% CIs with a good random distribution of residuals indicating a very good goodness of fit.

6.2 Recommendations for Future Research

- In order to get more accurate respirometry data, a large-scale respirometer is recommended to measure DO instead of a bench scale DO meter. It has several advantages:
- Respirometry depends on a "what goes in must come out" principle. It is easier to control because it is completely sealed.
- DO can be monitored and recorded automatically.
- A starch- glucose substrate combination can be used to double check hydrolysis rate.
- Measurement of starch concentration would be useful to further verify results.

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Appendix A

Nomenclature

Glossary

A_b	total surface area of a bioparticle is A_b
b_A	endogenous decay (lysis) coeff. of autotrophs. (h^{-1})
b_{ANO}	anoxic endogenous respiration coeff. of autotroph. (h^{-1})
b_H	endogenous decay (lysis) coeff. of heterotrophs (h^{-1})
CI	confidence interval
f_P	fraction of inert biomass generated in decay (-)
f_{SI}	fraction of S_I released in hydrolysis (-)
G	glucose concentration (mg COD/L)
f_{XI}	fraction of nonbiodegradable particulate COD released in biomass decay (heterotroph) (-)
η_g	anoxic growth factor (-)
η_h	anoxic hydrolysis factor (-)
i_{NBM}	N content of biomass (g N/g COD, g N/g TSS)
i_{PBM}	P content of biomass (g P/g COD, g P/g TSS)
i_{NSI}	N content of inert sol. COD, S_I [g N /g COD]
i_{NSS}	N content of deg. substrates, S_S [g N /g COD]
i_{NXI}	N content of inert part. COD, X_I [g N /g COD]
i_{NXS}	N content of slowly biodegradable substrate, X_S [g N /g COD]
i_{TSSBM}	TSS to COD ratio for biomass (g TSS/g COD)
i_{TSSXI}	TSS to COD ratio for X_I (g TSS/g COD)
i_{TSSXS}	TSS to COD ratio for X_S (g TSS/g COD)
i_{XB}	N content of biomass [g N /g COD]
i_{XP}	N content of endogenous biomass [g N /g COD]
k_a	ammonification rate constant
K_1, K_2, K_3, c	model parameters
K_{ALKA}, K_{ALKH},	half-velocity coeff. for alkalinity for autotrophs/hetererotrophs (mmol/L)
k_h	hydrolysis rate coefficient (h^{-1}, g BOD/gTSS/h)
K_{NH}	heterotroph. half-velocity coefficient for ammonia (mg N/L)
K_{NO}	half-velocity coefficient for nitrate (mg N/L)
K_{NOX}	half-velocity coefficient for NO_x (mg N/L)
K_{OA}, K_{OH}	autotrophs/hetererotrophs half-velocity coefficient for O_2 (mg O_2/L)

K_s	COD half saturation coefficient (COD/L)
k_{STO}	COD storage rate (h^1)
K_{STO}	half-velocity coefficient for stored COD (mg/L)
K_X	half-velocity coefficient for hydrolysis (mg/L)
K_{HS}	slowly hydrolysis substrate rate constant
K_{HR}	readily hydrolysis substrate rate constant
μ_A/μ, μ_H	specific growth rate of autotrophs/heterotrophs (h^{-1})
μ_{Am}, μ_{Hm}	maximum specific growth rate of autotrophs/heterotrophs(h^{-1})
μ_{Hm}	maximum specific growth rate of heterotrophs (h^{-1})
n_H	bioparticles (no./L)
ν	stoichiometric coefficient (-)
n_S	primary particles (substrate molecules) (no./L)
ρ_7	rate constant for hydrolysis of organic N(mg N/L/h)
θ_d	HRT (h)
θ_X	SRT (d)
S_I	nbd (inert) soluble COD (mg/L)
S_{Ie}	effluent nbd (inert) soluble COD (mg/L)
S_{N2}	N_2 (mg N/L)
S_{NH}	$NH_3 + NH_4^+$ (mg N/L)
S_{No}	degradable soluble organic N (mg N/L)
S_r	sum of squares
S_{NOX}	$NO_3^- + NO_2^-$ (mg N/L)
SE	standard error
S_O,	DO/DO saturation concentration (mg O_2/L)
S_S	degradable soluble COD (mg/L)
S_{Se}	effluent degradable soluble COD (mg/L)
S_{So}	influent degradable soluble COD (mg/L)
V	volume
T	time
Ra^2	adjusted coefficient of multiple determination
R^2	coefficient of multiple determination
S	general substrate concentration (mg/L) or starch concentration (mg COD/L)
X_A	autotrophs (mg/L)
X_{AH}	active autotrophic biomass (mg/L)

X_{BH}	active heterotrophic biomass (mg/L)
X_H	heterotrophs (mg/L)
X_I	nbd particulate COD (mg/L)
X_{ie}	effluent nonbiodegradable (inert) particulate COD (mg/L)
X_{ND}	entrapped organic nitrogen (mg N/L)
X_P	particulate products arising from biomass decay (mg/L)
X_S	degradable particulate organics (mg COD/L, mg BOD/L)
X_{Se}	effluent nbd degradable particulate COD (mg/L)
X_{STO}	stored COD (mg/L)
X_{TSS}	TSS (mg/L)
Y_A	yield of autotrophic biomass per NO_3^--N (g COD/g N, g TSS/g N)
Y_H	aerobic yield coefficient of heterotrophs (-, g TSS/g BOD)
Y_H, Y_{HNO}	aerobic/anoxic yield coefficient of heterotrophs (-, g TSS/g BOD)
Y_{STONO}, Y_{STOO2}	anoxic/aerobic yield coefficient of stored COD (-)

Abbreviations

ASM	Activated sludge model
COD	Chemical oxygen demand
$CBOD_5$	5-day carbonaceous biochemical oxygen demand
MOE	Ministry of Environment
CSTR	Continuous stirred- tank reactor
DO	Dissolved oxygen
HRT	Hydraulic retention time
IAWQ	International Association on Water Quality
IWA	International Water Association
MLSS	Mixed liquor suspended solids
nbd	Nonbiodegradable
OUR	Oxygen uptake rate
SOUR	Specific oxygen uptake rate
SCOD	Soluble COD
SIC	Starch- iodine complex

RBCOD	Readily biodegradable COD
SBCOD	Slowly biodegradable COD
SRT	Solids retention time
SBR	Sequencing batch reactors
ROPEC	Robert O Pickard Environmental Centre
UCT	University of Cape Town
TSS	Total suspended solids
VSS	Volatile suspended solids
VFA	Volatile fatty acid
APAH	American Public Health Association
SM	Standard Methods
MS	Residual Mean Squares

Appendix B
DO Measurements When Glucose Applied as Substrate

March 1 2009

G	17.1	mgCOD/L
X	400	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	7.37	
1	7.1	0.27
2	6.8	0.3
3	6.59	0.21
4	6.38	0.21
5	6.13	0.25
6		
7	5.73	0.2
8	5.53	0.2
9	5.31	0.22
10	5.16	0.15
11	4.97	0.19
12	4.81	0.16
13	4.65	0.16
14	4.53	0.12
	Average OUR	0.203

March 2 2009

G	54.4	mgCOD/L
X	460	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	6.16	
1	5.96	0.2
2	5.73	0.23
3	5.46	0.27
4	5.25	0.21
5	4.99	0.26
6	4.76	0.23
7	4.49	0.27
8	4.25	0.24
9	4.04	0.21
10	3.8	0.24
11	3.53	0.27
12	3.33	0.2
13	3.09	0.24
14	2.87	0.22
15	2.65	0.22
	Average OUR	0.234

March 2 2009

G	27.5	mgCOD/L
X	370	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	7.06	
1	6.86	0.2
2	6.56	0.3
3	6.35	0.21
4	6.16	0.19
5	5.89	0.27
6	5.61	0.28
7	5.41	0.2
8	5.26	0.15
9	5.07	0.19
10	4.89	0.18
11	4.69	0.2
12	4.48	0.21
13	4.25	0.23
14	4.06	0.19
15	3.83	0.23
	Average OUR	0.215

March 4 2009

G	137.9	mgCOD/L
X	460	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	7.61	
1	7.35	0.26
2	7.1	0.25
3	6.87	0.23
4	6.6	0.27
5	6.28	0.32
6	6.13	0.15
7	5.86	0.27
8	5.6	0.26
9	5.39	0.21
10	5.14	0.25
11	4.91	0.23
12	4.68	0.23
13	4.44	0.24
14	4.18	0.26
15	3.93	0.25
	Average OUR	0.245

Appendix B
DO Measurements When Glucose Applied as Substrate

March 7 2009

G	17.1	mgCOD/L
X	580	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	7.1	
1	6.91	0.19
2	6.66	0.25
3	6.36	0.3
4	6.06	0.3
5	5.78	0.28
6	5.49	0.29
7	5.27	0.22
8	5.03	0.24
9	4.78	0.25
10	4.53	0.25
11	4.31	0.22
12	4.1	0.21
13	3.88	0.22
14	3.69	0.19
15	3.49	0.2
	Average OUR	0.241

March 7 2009

G	27.5	mgCOD/L
X	500	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	6.8	
1	6.59	0.21
2	6.34	0.25
3	6.05	0.29
4	5.73	0.32
5	5.45	0.28
6	5.14	0.31
7	4.83	0.31
8	4.54	0.29
9	4.28	0.26
10	4.01	0.27
11	3.74	0.27
12	3.48	0.26
13	3.24	0.24
14	2.97	0.27
15	2.68	0.29
	Average OUR	0.275

March 8 2009

G	17.1	mgCOD/L
X	350	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	6.94	
1	6.79	0.15
2	6.61	0.18
3	6.42	0.19
4	6.28	0.14
5	6.14	0.14
6	5.95	0.19
7	5.79	0.16
8	5.63	0.16
9	5.46	0.17
10	5.27	0.19
11	5.09	0.18
12	4.83	0.26
13	4.65	0.18
14	4.55	0.1
15	4.39	0.16
	Average OUR	0.17

March 8 2009

G	27.5	mgCOD/L
X	350	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	7.01	
1	6.88	0.13
2	6.73	0.15
3	6.61	0.12
4	6.49	0.12
5	6.3	0.19
6	6.16	0.14
7	6	0.16
8	5.84	0.16
9	5.71	0.13
10	5.53	0.18
11	5.35	0.18
12	5.23	0.12
13	5.02	0.21
14	4.87	0.15
15	4.71	0.16
	Average OUR	0.153

Appendix B
DO Measurements When Glucose Applied as Substrate

March 6 2009

G	17.1	mgCOD/L
X	360	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	8.39	
1	8.32	0.07
2	8.28	0.04
3	8.23	0.05
4	8.18	0.05
5	8.09	0.09
6	8.03	0.06
7	7.96	0.07
8	7.89	0.07
9	7.79	0.1
10	7.71	0.08
11	7.62	0.09
12	7.53	0.09
13	7.41	0.12
14	7.31	0.1
15	7.21	0.1
	Average OUR	0.079

March 5 2009

G	54.4	mgCOD/L
X	350	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	6.87	
1	6.76	0.11
2	6.6	0.16
3	6.36	0.24
4		
5	6.04	0.16
6		
7		
8	5.33	0.23666667
9	5.1	0.23
10	4.9	0.2
11		
12	4.4	0.25
13	4.18	0.22
14	3.95	0.23
15	3.74	0.21
	Average OUR	0.204

March 6 2009

G	27.5	mgCOD/L
X	420	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	7.74	
1	7.68	0.06
2	7.62	0.06
3	7.55	0.07
4	7.47	0.08
5	7.38	0.09
6	7.25	0.13
7	7.15	0.1
8	7.05	0.1
9	6.93	0.12
10	6.81	0.12
11	6.68	0.13
12	6.55	0.13
13	6.39	0.16
14	6.24	0.15
15	6.1	0.14
	Average OUR	0.109

March 6 2009

G	27.5	mgCOD/L
X	420	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	7.43	
1	7.25	0.18
2	7.1	0.15
3	6.89	0.21
4	6.7	0.19
5	6.43	0.27
6	6.28	0.15
7	6.18	0.1
8	6.06	0.12
9	5.91	0.15
10	5.72	0.19
11	5.59	0.13
12	5.41	0.18
13	5.22	0.19
14	5.08	0.14
15	4.89	0.19
	Average OUR	0.169

Appendix B
DO Measurements When Glucose Applied as Substrate

March 7 2009

G	54.4	mgCOD/L
X	590	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	6.57	
1	6.21	0.36
2	6.02	0.19
3	5.72	0.3
4	5.42	0.64
5	5.08	0.25
6	4.83	0.27
7	4.56	0.26
8	4.3	0.23
9	4.07	0.28
10	3.79	0.26
11	3.53	0.28
12	3.25	0.25
13	3	0.29
14	2.71	0.24
15	2.47	0.24
	Average OUR	0.289

March 7 2009

G	137.9	mgCOD/L
X	510	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	6.38	
1	6.12	0.26
2	5.82	0.3
3	5.54	0.28
4	5.18	0.58
5	4.96	0.33
6	4.63	0.29
7	4.34	0.3
8	4.04	0.27
9	3.77	0.28
10	3.49	0.24
11	3.25	0.26
12	2.99	0.27
13	2.72	0.26
14	2.46	0.26
15	2.2	0.26
	Average OUR	0.296

March 9 2009

G	54.5	mgCOD/L
X	350	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	6.85	
1	6.71	0.14
2	6.57	0.14
3	6.4	0.17
4	6.19	0.21
5	6.03	0.16
6	5.82	0.21
7	5.64	0.18
8	5.46	0.18
9	5.26	0.2
10	5.09	0.17
11	4.93	0.16
12	4.75	0.18
13	4.6	0.15
14	4.42	0.18
15	4.24	0.18
	Average OUR	0.174

March 9 2009

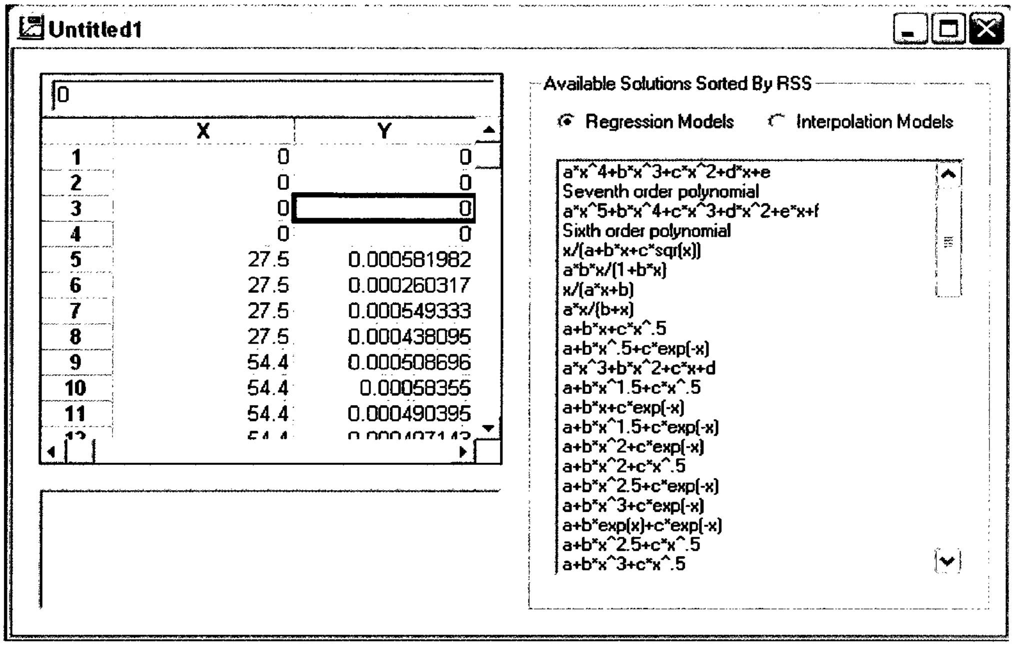
G	137.9	mgCOD/L
X	360	mgVSS/L
Time (min)	DO (mg/L)	OUR (mg/L/min)
0	6.84	
1	6.77	0.07
2	6.57	0.2
3	6.31	0.26
4	6.19	0.12
5	5.96	0.23
6	5.75	0.21
7	5.58	0.17
8	5.38	0.2
9	5.22	0.16
10	5.03	0.19
11	4.87	0.16
12	4.68	0.19
13	4.51	0.17
14	4.35	0.16
15	4.15	0.2
	Average OUR	0.179

Appendix C

Fitting Models to Biological Data Using Nonlinear Regression (Glucose)

1 Interface of Datafit

Run 1 All Experimental Data



0

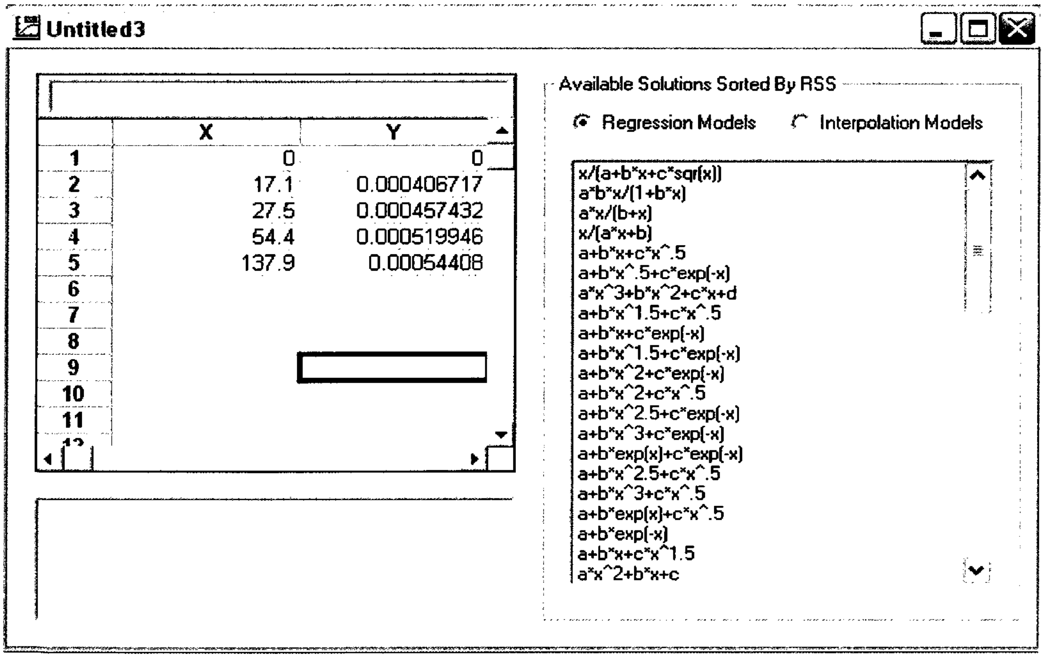
	X	Y
1	0	0
2	0	0
3	0	0
4	0	0
5	27.5	0.000581982
6	27.5	0.000260317
7	27.5	0.000549333
8	27.5	0.000438095
9	54.4	0.000508696
10	54.4	0.00058355
11	54.4	0.000490395

Available Solutions Sorted By RSS

● Regression Models ○ Interpolation Models

```
a*x^4+b*x^3+c*x^2+d*x+e
Seventh order polynomial
a*x^5+b*x^4+c*x^3+d*x^2+e*x+f
Sixth order polynomial
x/(a+b*x+c*sqr(x))
a*b*x/(1+b*x)
x/(a*x+b)
a*x/(b+x)
a+b*x+c*x^.5
a+b*x^.5+c*exp(-x)
a*x^3+b*x^2+c*x+d
a+b*x^1.5+c*x^.5
a+b*x+c*exp(-x)
a+b*x^1.5+c*exp(-x)
a+b*x^2+c*exp(-x)
a+b*x^2+c*x^.5
a+b*x^2.5+c*exp(-x)
a+b*x^3+c*exp(-x)
a+b*exp(x)+c*exp(-x)
a+b*x^2.5+c*x^.5
a+b*x^3+c*x^.5
```

Run 2 Average Glucose Concentration (determined in Chapter 5) Applied



	X	Y
1	0	0
2	17.1	0.000406717
3	27.5	0.000457432
4	54.4	0.000519946
5	137.9	0.00054408
6		
7		
8		
9		
10		
11		

Available Solutions Sorted By RSS

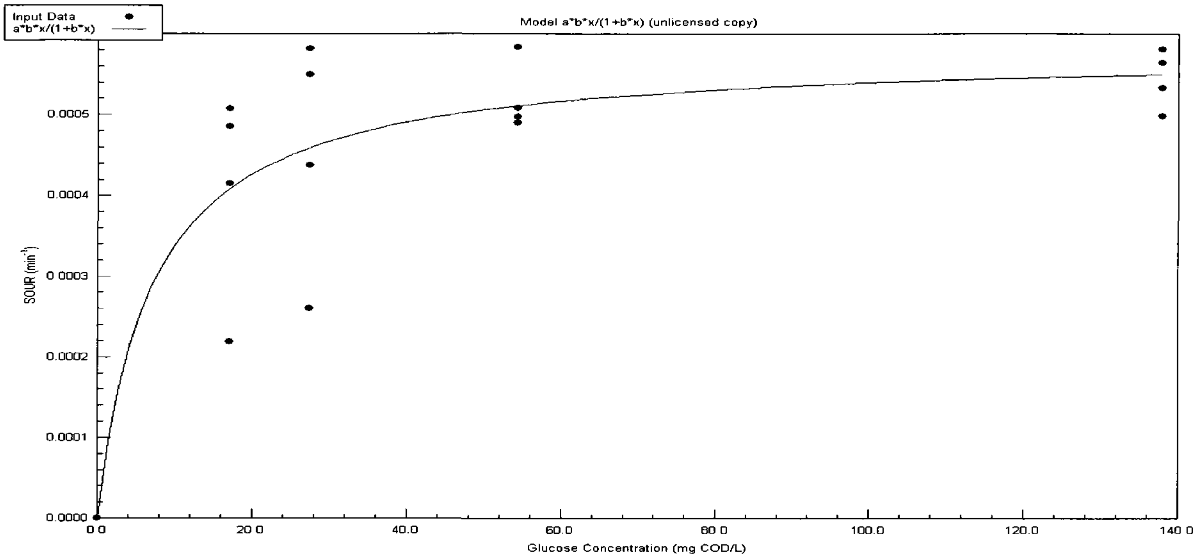
● Regression Models ○ Interpolation Models

```
x/(a+b*x+c*sqr(x))
a*b*x/(1+b*x)
a*x/(b+x)
x/(a*x+b)
a+b*x+c*x^.5
a+b*x^.5+c*exp(-x)
a*x^3+b*x^2+c*x+d
a+b*x^1.5+c*x^.5
a+b*x+c*exp(-x)
a+b*x^1.5+c*exp(-x)
a+b*x^2+c*exp(-x)
a+b*x^2+c*x^.5
a+b*x^2.5+c*exp(-x)
a+b*x^3+c*exp(-x)
a+b*exp(x)+c*exp(-x)
a+b*x^2.5+c*x^.5
a+b*x^3+c*x^.5
a+b*exp(x)+c*x^.5
a+b*exp(-x)
a+b*x+c*x^1.5
a*x^2+b*x+c
```

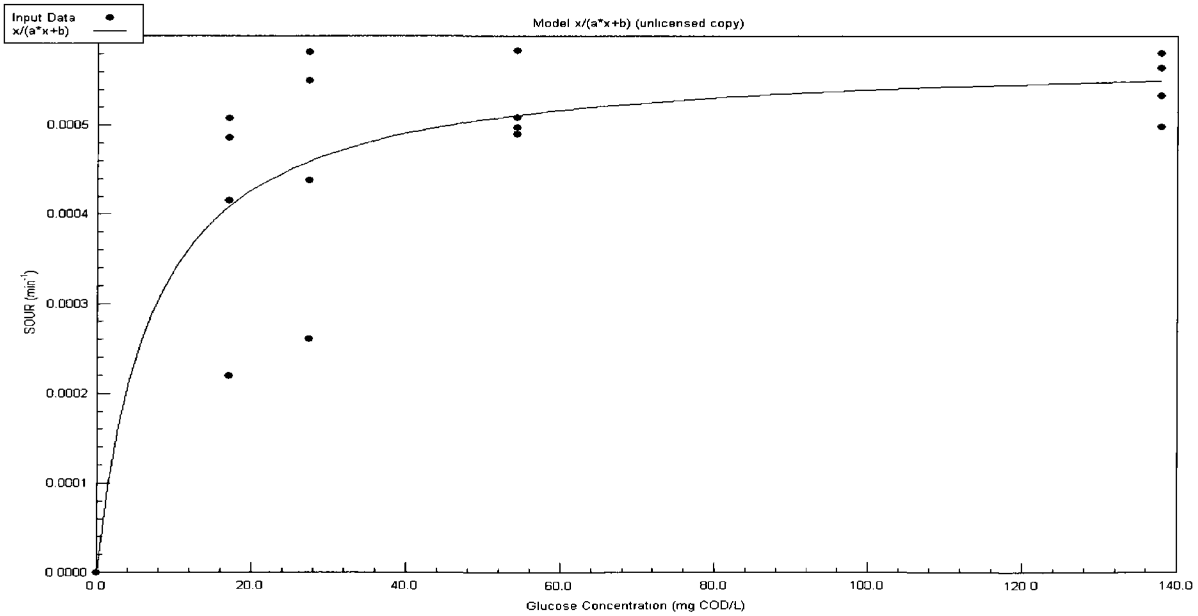
2 Model Plot

Y is specific oxygen uptake rate (min^{-1}), X (X_1) is glucose concentration (mg VSS/L), X_2 is biomass concentration (mg VSS/L)

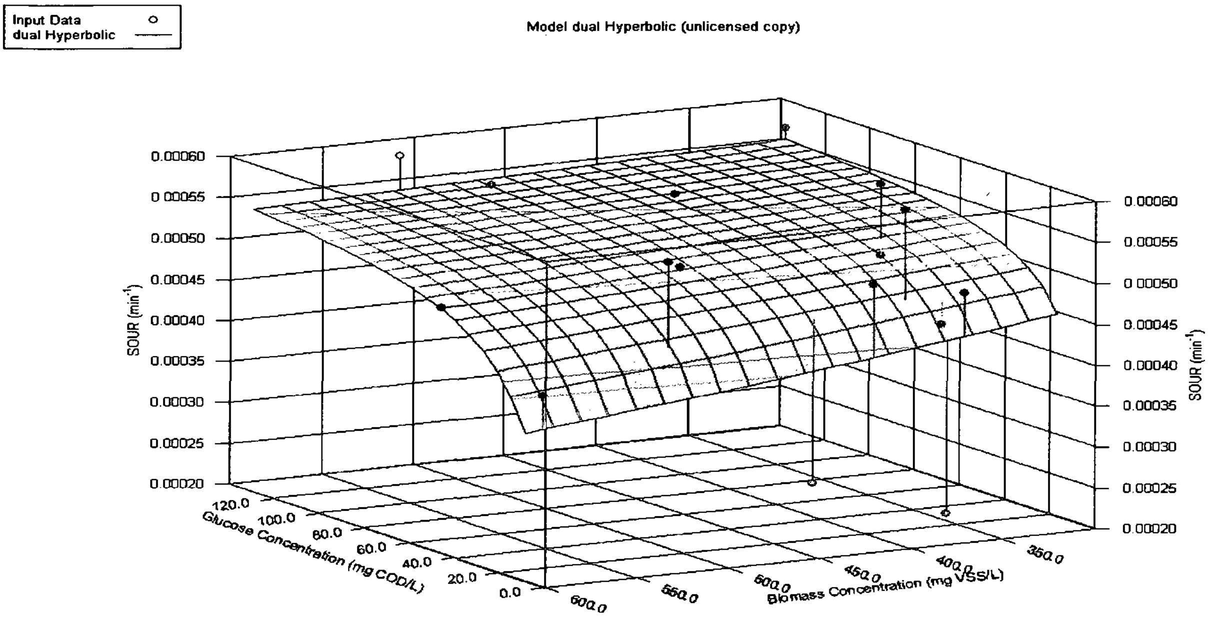
Model 1 $$Y = \frac{a \times b \times X}{1 + b \times X}$$



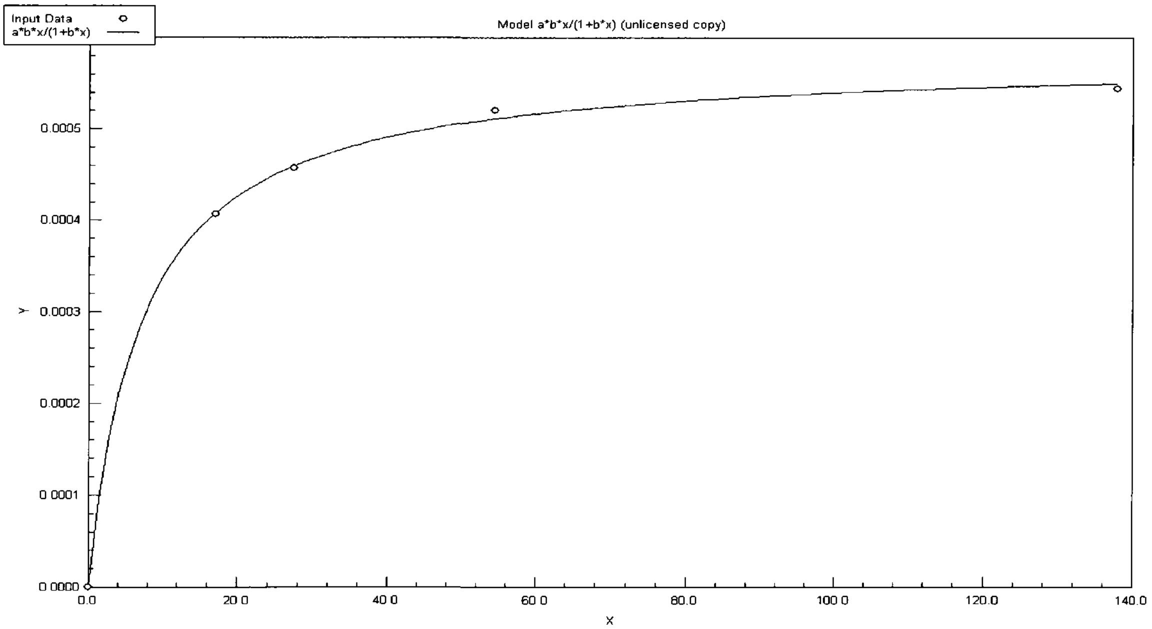
Model 2 $$Y = \frac{X}{a \times X + b}$$



Model 3 $$Y = \frac{a \times X_1}{b \times X_2 + c \times X_1}$$



Model 4 $$Y = \frac{a \times b \times X}{1 + b \times X}$$ with average X



3 Fit Information

Model 1 $$Y = \frac{a \times b \times X}{1 + b \times X}$$

Model Definition:

Y = a*b*x/(1+b*x)

Number of observations = 20

Number of missing observations = 0

Solver type: Nonlinear

Nonlinear iteration limit = 250

Diverging nonlinear iteration limit =10

Number of nonlinear iterations performed = 12

Residual tolerance = 0.0000000001

Sum of Residuals = -4.67E-07

Average Residual = -2.34E-08

Residual Sum of Squares (Absolute) = 1.25E-07

Residual Sum of Squares (Relative) = 1.25E-07

Standard Error of the Estimate = 8.34E-05

Coefficient of Multiple Determination (R^2) = 0.86

Proportion of Variance Explained = 86.324%

Adjusted coefficient of multiple determination (Ra^2) = 0.856

Durbin-Watson statistic = 2.60

95% Confidence Intervals

Variable	**Value**	**95% (+/-)**	**Lower Limit**	**Upper Limit**
a	5.78E-04	1.00E-04	4.78E-04	6.78E-04
b	0.14	0.14	-2.16E-03	0.28

Model 2 $$Y = \frac{X}{a \times X + b}$$

Model Definition:

Y = x/(a*x+b)

Number of observations = 20

Number of missing observations = 0

Solver type: Nonlinear

Nonlinear iteration limit = 250

Diverging nonlinear iteration limit =10

Number of nonlinear iterations performed = 16

Residual tolerance = 0.0000000001

Sum of Residuals = -4.67E-07

Average Residual = -2.34E-08

Residual Sum of Squares (Absolute) = 1.25E-07

Residual Sum of Squares (Relative) = 1.25E-07

Standard Error of the Estimate = 8.34E-05

Coefficient of Multiple Determination (R^2) = 0.863

Proportion of Variance Explained = 86.32%

Adjusted coefficient of multiple determination (Ra^2) = 0.856

Durbin-Watson statistic = 2.60

95% Confidence Intervals

Variable	**Value**	**95% (+/-)**	**Lower Limit**	**Upper Limit**
a	1730.8	299.9	1430.9	2030.7
b	12275.6	10706.0	1569.5	22981.6

Model 3 $Y = \dfrac{a \times X_1}{b \times X_2 + c \times X_1}$

Model Definition:

Y = a*X1/(b*X2+c*X1)

Number of observations = 16

Number of missing observations = 4

Solver type: Nonlinear

Nonlinear iteration limit = 250

Diverging nonlinear iteration limit =10

Number of nonlinear iterations performed = 23

Residual tolerance = 0.0000000001

Sum of Residuals = 2.11E-06

Average Residual = 1.32E-07

Residual Sum of Squares (Absolute) = 1.31E-07

Residual Sum of Squares (Relative) = 1.31E-07

Standard Error of the Estimate = 1.00E-04

Coefficient of Multiple Determination (R^2) = 0.23

Proportion of Variance Explained = 23.30%

Adjusted coefficient of multiple determination (Ra^2) = 0.115

Durbin-Watson statistic = 1.309

95% Confidence Intervals

Variable	**Value**	**95% (+/-)**	**Lower Limit**	**Upper Limit**
a	7.77E-02	1.13E+06	-1.13E+06	1.13E+06
b	2.108	3.06E+07	-3.06E+07	3.06E+07
c	136.40	1.98E+09	-1.98E+09	1.98E+09

Model 4 $Y = \dfrac{a \times b \times X}{1 + b \times X}$ with average X

Model Definition:

Y = a*x/(b+x)

Number of observations = 5

Number of missing observations = 0

Solver type: Nonlinear

Nonlinear iteration limit = 250

Diverging nonlinear iteration limit =10

Number of nonlinear iterations performed = 7

Residual tolerance = 0.0000000001

Sum of Residuals = -1.168E-07

Average Residual = -2.34E-08

Residual Sum of Squares (Absolute) = 1.13E-10

Residual Sum of Squares (Relative) = 1.13E-10

Standard Error of the Estimate = 6.150E-06

Coefficient of Multiple Determination (R^2) = 0.9994				
Proportion of Variance Explained = 99.9%				
Adjusted coefficient of multiple determination (Ra^2) = 0.999				
Durbin-Watson statistic = 2.820				
95% Confidence Intervals				
Variable	**Value**	**95% (+/-)**	**Lower Limit**	**Upper Limit**
a	5.78E-04	2.24E-05	5.55E-04	6.00E-04
b	7.092	1.610	5.483	8.702

4 Data Table

Model 1

$$Y = \frac{a \times b \times X}{1 + b \times X}$$

X value	Y value	Calc Y	Residual	% error	Abs Residual	Min Residual	Max Residual
27.5	0.00058	0.00046	0.000123	21.08	0.00012	-0.0002	0.000123
27.5	0.00026	0.00046	-0.0002	-76.44	0.00020		
27.5	0.00055	0.00046	9.00E-05	16.39	0.00009		
27.5	0.00044	0.00046	-2.10E-05	-4.84	0.00002		
54.4	0.00051	0.00051	-2.40E-06	-0.48	0.00000		
54.4	0.00058	0.00051	7.24E-05	12.41	0.00007		
54.4	0.00049	0.00051	-2.10E-05	-4.23	0.00002		
54.4	0.00050	0.00051	-1.40E-05	-2.81	0.00001		
17.1	0.00022	0.00041	-0.00019	-86.88	0.00019		
17.1	0.00041	0.00041	6.56E-06	1.58	0.00001		
17.1	0.00049	0.00041	7.73E-05	15.92	0.00008		
17.1	0.00051	0.00041	9.93E-05	19.56	0.00010		
137.9	0.00053	0.00055	-1.60E-05	-3.03	0.00002		
137.9	0.00056	0.00055	1.50E-05	2.65	0.00001		
137.9	0.00058	0.00055	3.09E-05	5.32	0.00003		
137.9	0.00050	0.00055	-5.10E-05	-10.31	0.00005		

Model 2

$$Y = \frac{X}{a \times X + b}$$

X value	Y value	Calc Y	Residual	% error	Abs Residual	Min Residual	Max Residual
27.5	0.00058	0.00046	0.000123	21.08	0.00012	-0.0002	0.000123
27.5	0.00026	0.00046	-0.0002	-76.44	0.00020		
27.5	0.00055	0.00046	9.00E-05	16.39	0.00009		
27.5	0.00044	0.00046	-2.10E-05	-4.84	0.00002		
54.4	0.00051	0.00051	-2.40E-06	-0.48	0.00000		
54.4	0.00058	0.00051	7.24E-05	12.41	0.00007		
54.4	0.00049	0.00051	-2.10E-05	-4.23	0.00002		
54.4	0.00050	0.00051	-1.40E-05	-2.81	0.00001		
17.1	0.00022	0.00041	-0.00019	-86.88	0.00019		
17.1	0.00041	0.00041	6.56E-06	1.58	0.00001		
17.1	0.00049	0.00041	7.73E-05	15.92	0.00008		
17.1	0.00051	0.00041	9.93E-05	19.56	0.00010		
137.9	0.00053	0.00055	-1.60E-05	-3.03	0.00002		

137.9	0.00056	0.00055	1.50E-05	2.65	0.00001		
137.9	0.00058	0.00055	3.09E-05	5.32	0.00003		
137.9	0.00050	0.00055	-5.10E-05	-10.31	0.00005		

Model 3 $$Y = \frac{a \times X_1}{b \times X_2 + c \times X_1}$$

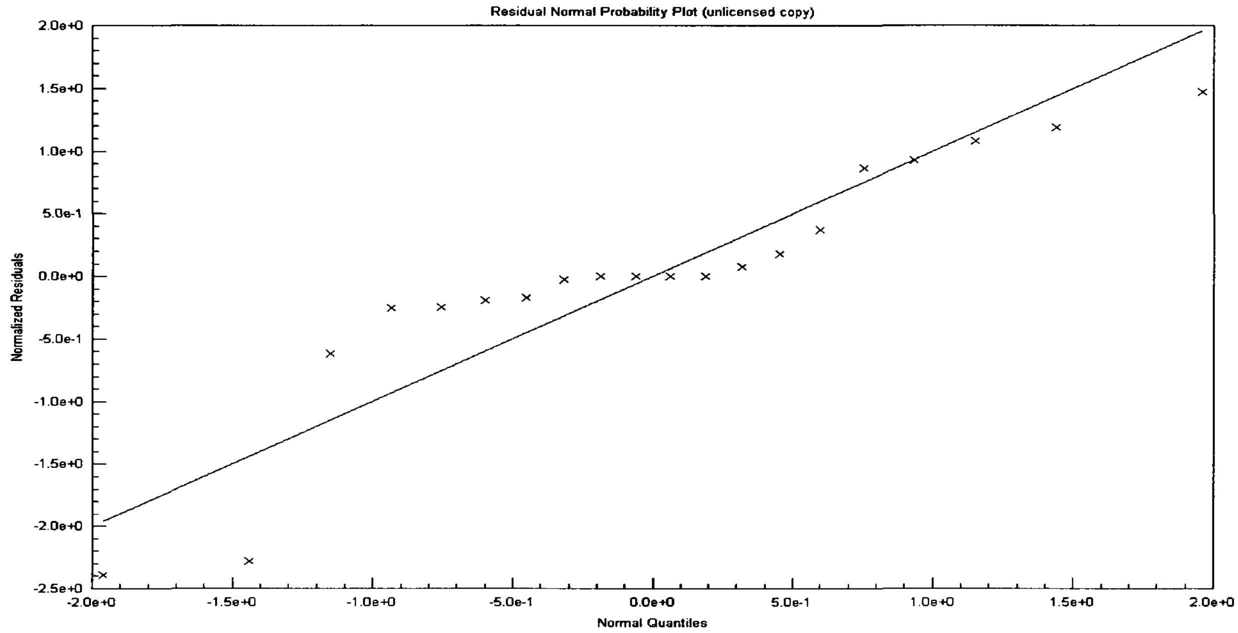
X_1 value	X_2 value	Y value	Calc Y	Residual	% error	Abs Residual	Min Residual	Max Residual
27.5	370	0.00058	0.00047	0.000111	19.02	0.000111	-0.00021	0.000111
54.4	460	0.00051	0.00050	5.18E-06	1.02	5.18E-06		
137.9	460	0.00053	0.00054	-8.10E-06	-1.52	8.08E-06		
17.1	360	0.00022	0.00043	-0.00021	-96.57	0.000211		
27.5	420	0.00026	0.00046	-0.0002	-76.94	0.0002		
54.4	350	0.00058	0.00052	6.57E-05	11.26	6.57E-05		
137.9	300	0.00056	0.00055	1.36E-05	2.42	1.36E-05		
17.1	580	0.00041	0.00037	4.14E-05	9.98	4.14E-05		
27.5	500	0.00055	0.00044	0.000105	19.10	0.000105		
54.4	590	0.00049	0.00049	2.80E-06	0.57	2.80E-06		
137.9	510	0.00058	0.00054	4.18E-05	7.21	4.18E-05		
17.1	350	0.00049	0.00043	5.32E-05	10.96	5.32E-05		
27.5	350	0.00044	0.00048	-3.80E-05	-8.59	3.76E-05		
54.4	350	0.00050	0.00052	-2.10E-05	-4.16	2.07E-05		
137.9	360	0.00050	0.00055	-4.90E-05	-9.86	4.91E-05		
17.1	400	0.00051	0.00042	8.95E-05	17.64	8.95E-05		

Model 4 $Y = \frac{a \times b \times X}{1 + b \times X}$ with average X

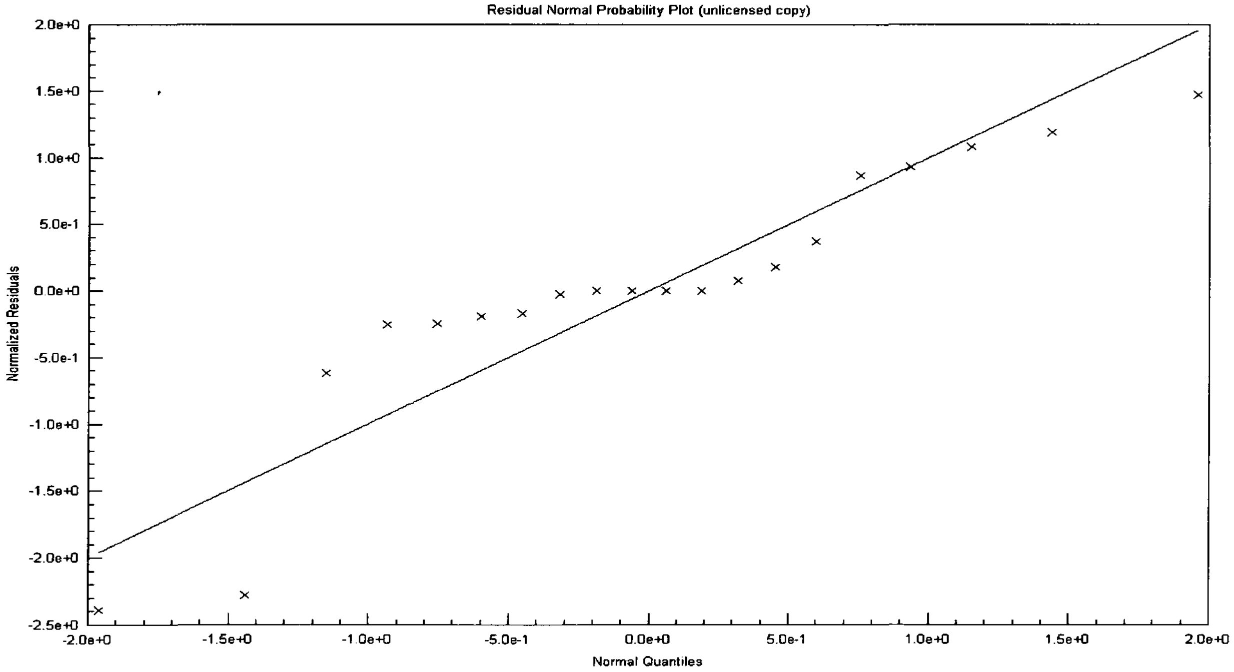
X value	Y value	Calc Y	Residual	% error	Abs Residual	Min Residual	Max Residual
0	0	0	0	0	0	-5.41E-06	8.83E-06
17.1	0.000407	0.000408	-1.70E-06	-0.40844	1.66E-06		
27.5	0.000457	0.000459	-1.90E-06	-0.40848	1.87E-06		
54.4	0.00052	0.000511	8.83E-06	1.697699	8.83E-06		
137.9	0.000544	0.000549	-5.40E-06	-0.99512	5.41E-06		

5 Residual Probability

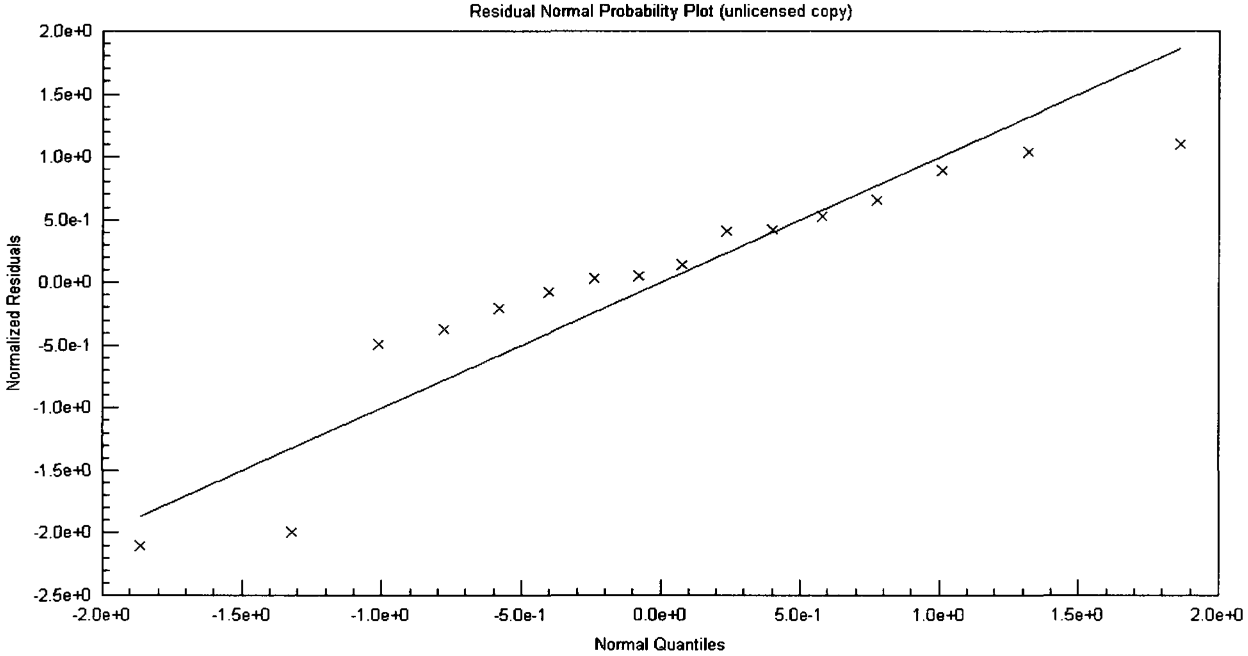
Model 1 $$Y = \frac{a \times b \times X}{1 + b \times X}$$



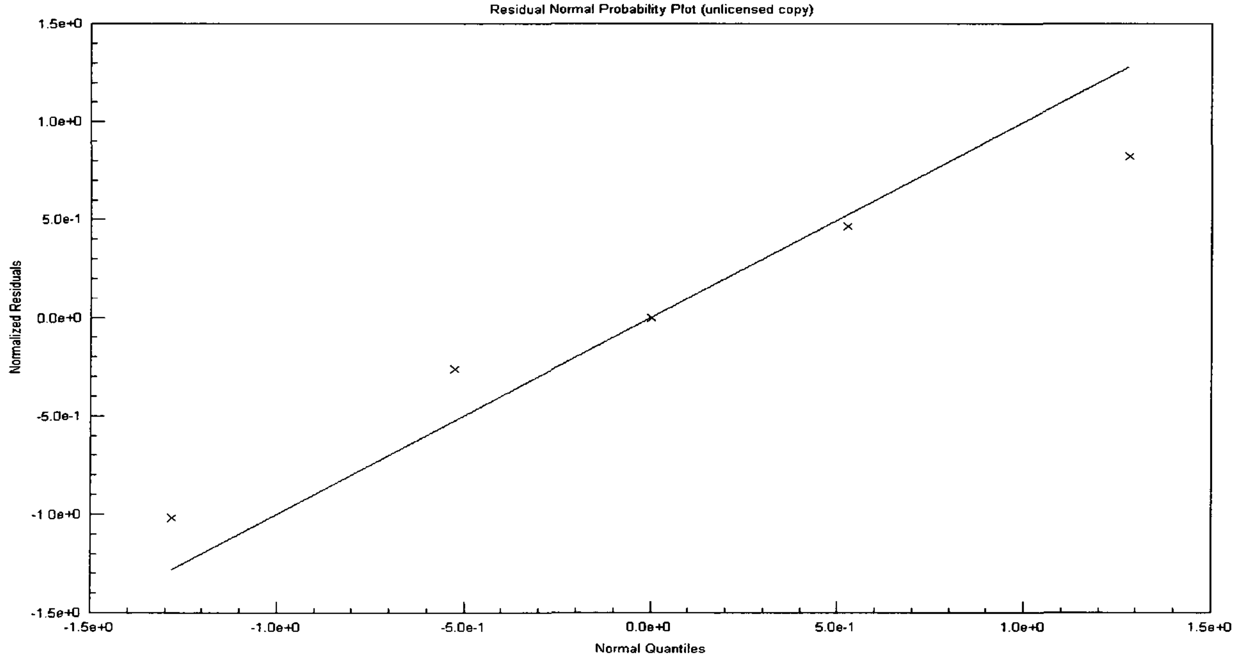
Model 2 $Y = \dfrac{X}{a \times X + b}$



Model 3 $Y = \dfrac{a \times X_1}{b \times X_2 + c \times X_1}$

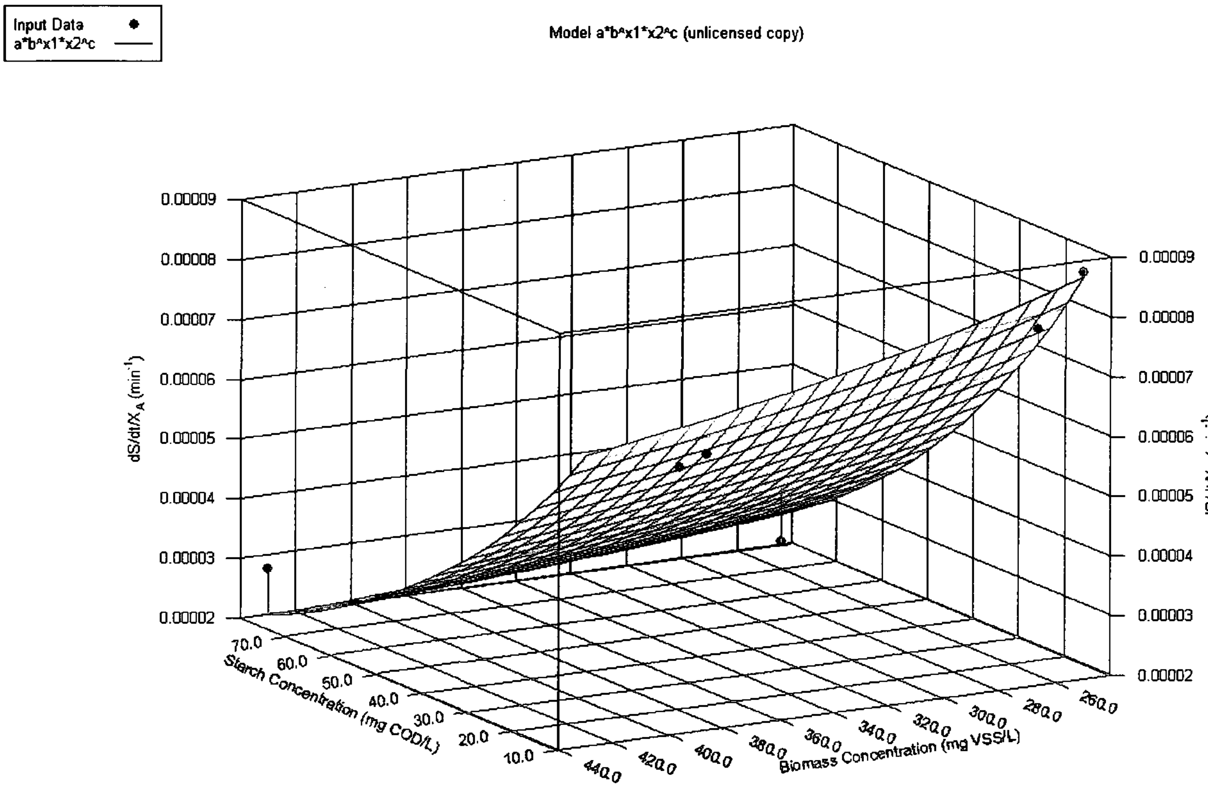


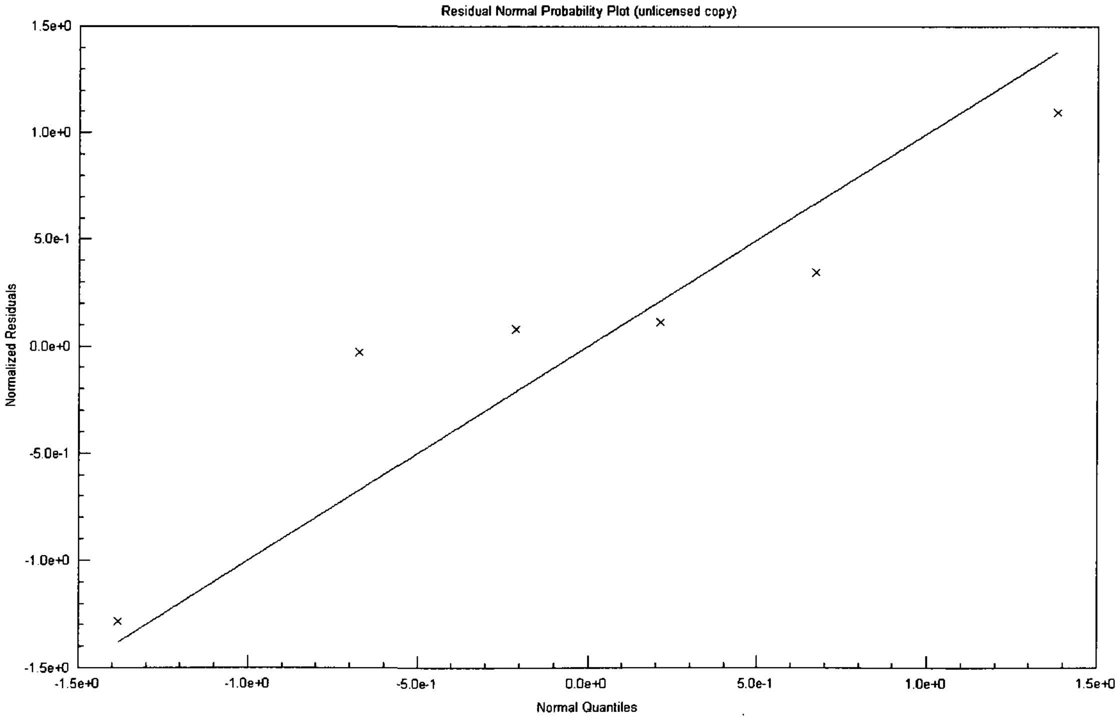
Model 4 $Y = \dfrac{a \times b \times X}{1 + b \times X}$ with average X



*For average ones, all models are the same in terms of data table, model plot, and residual probability.

6 When using all starch and biomass concentration combinations





Model Definition:

$Y = a*b^{x}_{1}*x_{2}^{c}$

Number of observations = 6

Number of missing observations = 0

Solver type: Nonlinear

Nonlinear iteration limit = 250

Diverging nonlinear iteration limit =10

Number of nonlinear iterations performed = 15

Residual tolerance = 0.0000000001

Sum of Residuals = 2.261E-06

Average Residual = 3.768E-07

Residual Sum of Squares (Absolute) = 1.500E-10

Residual Sum of Squares (Relative) = 1.500E-10

Standard Error of the Estimate = 7.07E-06

Coefficient of Multiple Determination (R^2) = 0.94

Proportion of Variance Explained = 94.2%

Adjusted coefficient of multiple determination (Ra^2) = 0.904

Durbin-Watson statistic = 1.82

95% Confidence Intervals

Variable	**Value**	**95% (+/-)**	**Lower Limit**	**Upper Limit**
a	1.20E-03	5.81E-03	-4.62E-03	7.01E-03
b	0.982	1.44E-02	0.968	0.997
c	-0.442	0.875	-1.317	0.433

S	X	dS/dt /X	Cal dS/dt/X	Residual	%Error	Abs residual	Min residual	Max residual
20	380	6.04E-05	6.06E-05	-2.05E-07	-0.34	2.05E-07	-9.10E-06	7.77E-06
10	250	8.81E-05	8.73E-05	8.08E-07	0.92	8.08E-07		
40	310	3.73E-05	4.64E-05	-9.10E-06	-24.39	9.10E-06		
20	370	6.19E-05	6.14E-05	5.54E-07	0.89	5.54E-07		
80	430	2.74E-05	1.96E-05	7.77E-06	28.36	7.77E-06		
20	250	7.54E-05	7.30E-05	2.43E-06	3.22	2.43E-06		

Appendix D
DO Measurements and OUR/SOUR Calculations When Starch Was Applies as Substrate

March 16 2009

Starch	20	mg COD/L
Biomass	380	mgVSS/L

Time (min)	DO (mg/L)	OUR (mg/L/min)	Average OUR (mg/L/min)	SOUR (min-1)
0	7.59			
1	7.58	0.01		
2	7.54	0.04		
3	7.51	0.03		
4	7.44	0.07		
5	7.44	0	0.03	7.89E-05
6	7.4	0.04	0.036	9.47E-05
7	7.4	0	0.028	7.37E-05
8	7.32	0.08	0.038	1E-04
9	7.29	0.03	0.03	7.89E-05
10	7.28	0.01	0.032	8.42E-05
11	7.24	0.04	0.032	8.42E-05
12	7.22	0.02	0.036	9.47E-05
13	7.24	-0.02	0.016	4.21E-05
14	7.14	0.1	0.03	7.89E-05
15	7.17	-0.03	0.022	5.79E-05

March24 2009

Starch	10	mg COD/L
Biomass	250	mgVSS/L

Time (min)	DO (mg/L)	OUR (mg/L/min)	Average OUR (mg/L/min)	SOUR (min-1)
0	7.54			
1	7.55	-0.01		
2	7.54	0.01		
3	7.53	0.01		
4	7.52	0.01		
5	7.52	0	0.004	1.6E-05
6	7.5	0.02	0.01	4E-05
7	7.44	0.06	0.02	8E-05
8	7.46	-0.02	0.014	5.6E-05
9	7.46	0	0.012	4.8E-05
10	7.44	0.02	0.016	6.4E-05
11	7.42	0.02	0.016	6.4E-05
12	7.45	-0.03	-0.002	-8E-06
13	7.44	0.01	0.004	1.6E-05
14	7.42	0.02	0.008	0.000032
15	7.41	0.01	0.006	2.4E-05

Appendix D
DO Measurements and OUR/SOUR Calculations When Starch Was Applies as Substrate

April 2 2009

Starch	40	mg COD/L		
Biomass	310	mgVSS/L		
Time (min)	DO (mg/L)	OUR (mg/L/min)	Average OUR (mg/L/min)	SOUR (min-1)
0	7.18			
1	7.16	0.02		
2	7.16	0		
3	7.15	0.01		
4	7.14	0.01		
5	7.11	0.03	0.014	4.52E-05
6	7.13	-0.02	0.006	1.94E-05
7	7.1	0.03	0.012	3.87E-05
8	7.08	0.02	0.014	4.52E-05
9	7.07	0.01	0.014	4.52E-05
10	7.05	0.02	0.012	3.87E-05
11	7.05	0	0.016	5.16E-05
12	7.05	0	0.01	3.23E-05
13	7.03	0.02	0.01	3.23E-05
14	7.04	-0.01	0.006	1.94E-05
15	7.05	-0.01	0	0

March 24 2009

Starch	20	mg COD/L		
Biomass	250	mgVSS/L		
Time (min)	DO (mg/L)	OUR (mg/L/min)	Average OUR (mg/L/min)	SOUR (min-1)
0	7.47			
1	7.466	0.004		
2	7.442	0.024		
3	7.448	-0.006		
4	7.434	0.014		
5	7.43	0.004	0.008	0.000032
6	7.436	-0.006	0.006	2.4E-05
7	7.442	-0.006	3.55271E-16	1.42E-18
8	7.428	0.014	0.004	1.6E-05
9	7.414	0.014	0.004	1.6E-05
10	7.43	-0.016	0	0
11	7.416	0.014	0.004	1.6E-05
12	7.432	-0.016	0.002	8E-06
13	7.428	0.004	3.55271E-16	1.42E-18
14	7.424	0.004	-0.002	-8E-06
15	7.43	-0.006	1.06581E-15	4.26E-18

Appendix D
DO Measurements and OUR/SOUR Calculations When Starch Was Applies as Substrate

April 2 2009

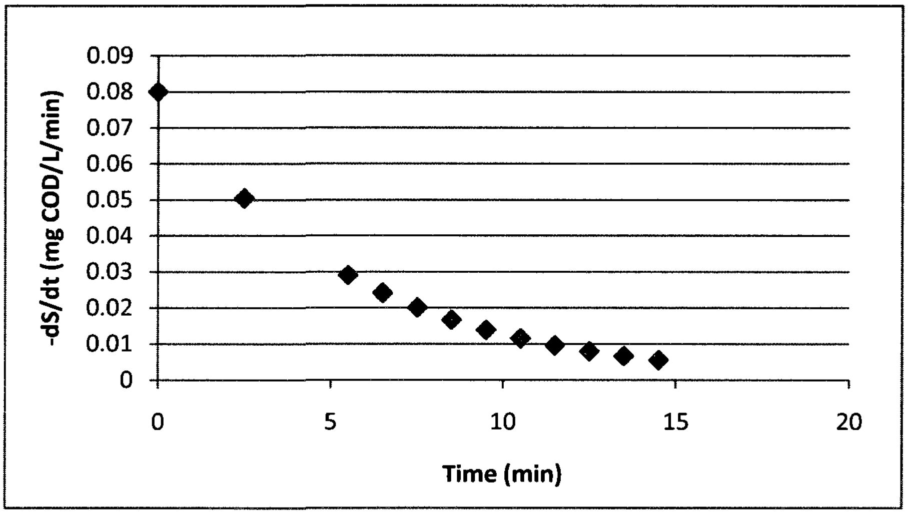
Starch	20	mg COD/L		
Biomass	370	mgVSS/L		
Time (min)	DO (mg/L)	OUR (mg/L/min)	Average OUR (mg/L/min)	SOUR (min-1)
0	7.15			
1	7.12	0.03		
2	7.03	0.09		
3	7.03	0		
4	7.05	-0.02		
5	7.08	-0.03	0.014	3.8E-05
6	7.05	0.03	0.014	3.8E-05
7	7.04	0.01	-0.002	-5.4E-06
8	7.02	0.02	0.002	5.4E-06
9	6.95	0.07	0.02	5.4E-05
10	6.97	-0.02	0.022	5.9E-05
11	7.02	-0.05	0.006	1.6E-05
12	7.09	-0.07	-0.01	-2.7E-05
13	6.99	0.1	0.006	1.6E-05
14	7.02	-0.03	-0.014	-3.8E-05
15	7.05	-0.03	-0.016	-4.3E-05

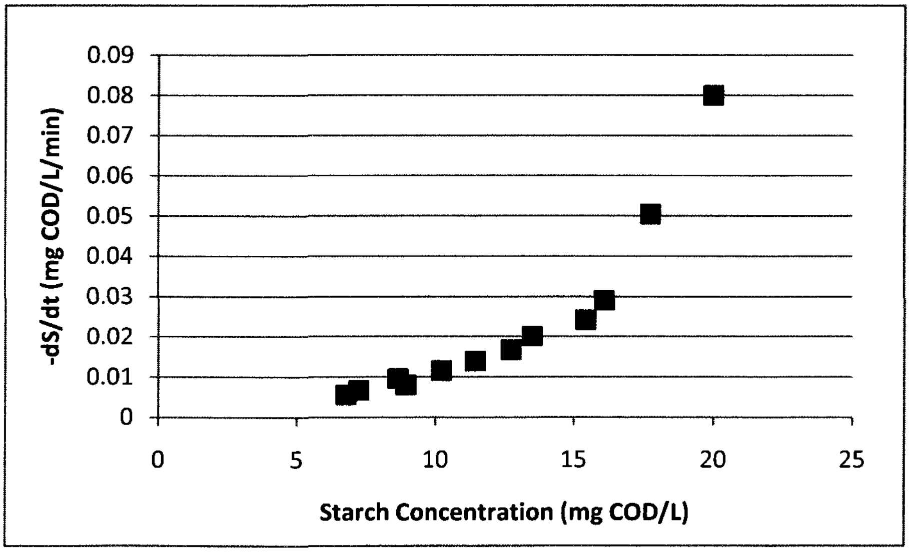
April 1 2009

Starch	80	mg COD/L		
Biomass	430	mgVSS/L		
Time (min)	DO (mg/L)	OUR (mg/L/min)	Average OUR (mg/L/min)	SOUR (min-1)
0	7.41			
1	7.438	-0.028		
2	7.386	0.052		
3	7.484	-0.098		
4	7.352	0.132		
5	7.39	-0.038	0.004	9.3E-06
6	7.388	0.002	0.01	2.3E-05
7	7.356	0.032	0.006	1.4E-05
8	7.374	-0.018	0.022	5.1E-05
9	7.382	-0.008	-0.006	-1.4E-05
10	7.35	0.032	0.008	1.9E-05
11	7.328	0.022	0.012	2.8E-05
12	7.376	-0.048	-0.004	-9.3E-06
13	7.324	0.052	0.01	2.3E-05
14	7.342	-0.018	0.008	1.9E-05
15	7.37	-0.028	-0.004	-9.3E-06

Appendix E
Starch Hydrolysis Rate Change with Time and Starch Concentrations

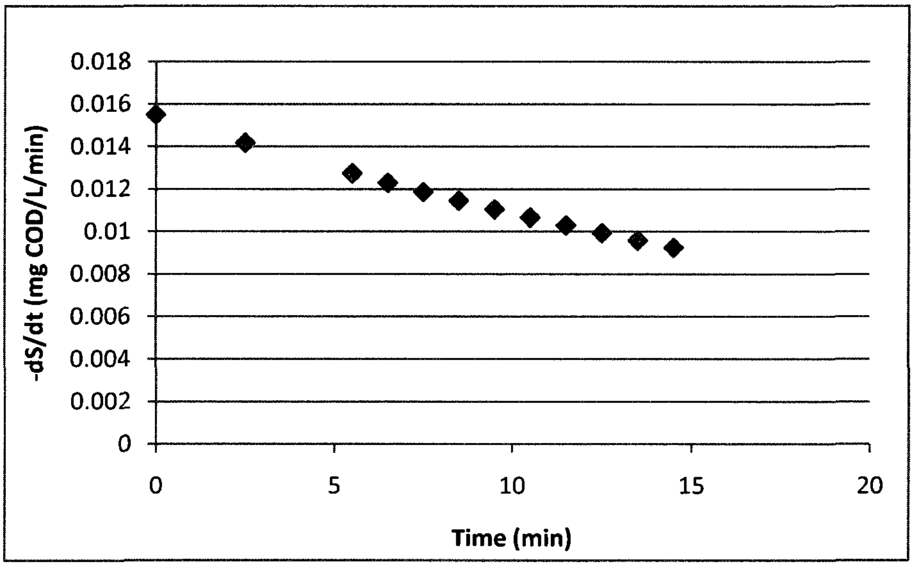
S=20 mg COD/L	t	dS/dt	-dS/dt	S	-dS/dt/X
X=380 mg VSS/L	min	mg COD/L/mi	mg COD/L/mi	mg COD/L	min^{-1}
	0	-0.08	0.08	20	0.000211
	2.5	-0.050	0.050	17.75	0.000133
	5.5	-0.029	0.029	16.10	7.64E-05
	6.5	-0.024	0.024	15.41	6.35E-05
	7.5	-0.020	0.020	13.48	5.28E-05
	8.5	-0.017	0.017	12.72	4.39E-05
	9.5	-0.014	0.014	11.42	3.65E-05
	10.5	-0.012	0.012	10.21	3.04E-05
	11.5	-0.010	0.010	8.64	2.53E-05
	12.5	-0.008	0.008	8.92	2.1E-05
	13.5	-0.007	0.007	7.23	1.75E-05
	14.5	-0.006	0.006	6.77	1.45E-05

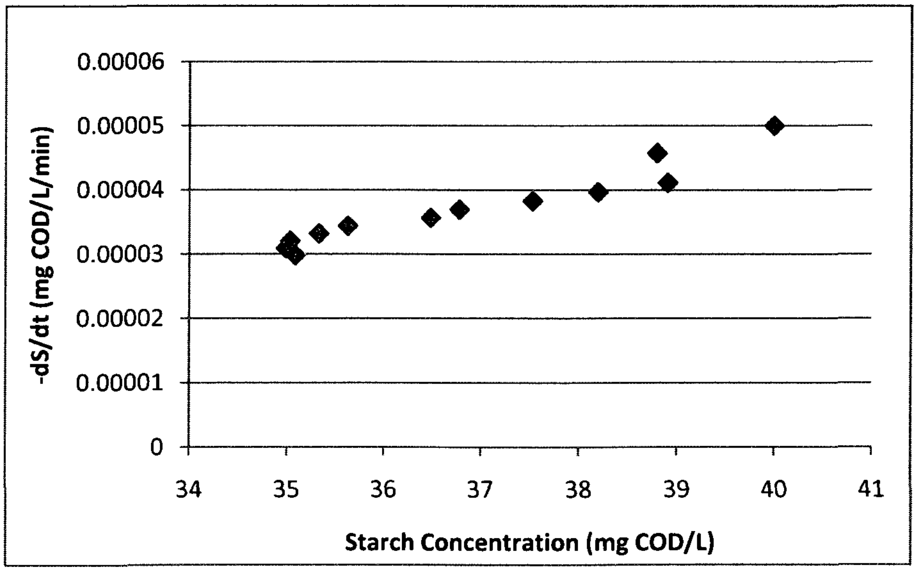




Appendix E
Starch Hydrolysis Rate Change with Time and Starch Concentrations

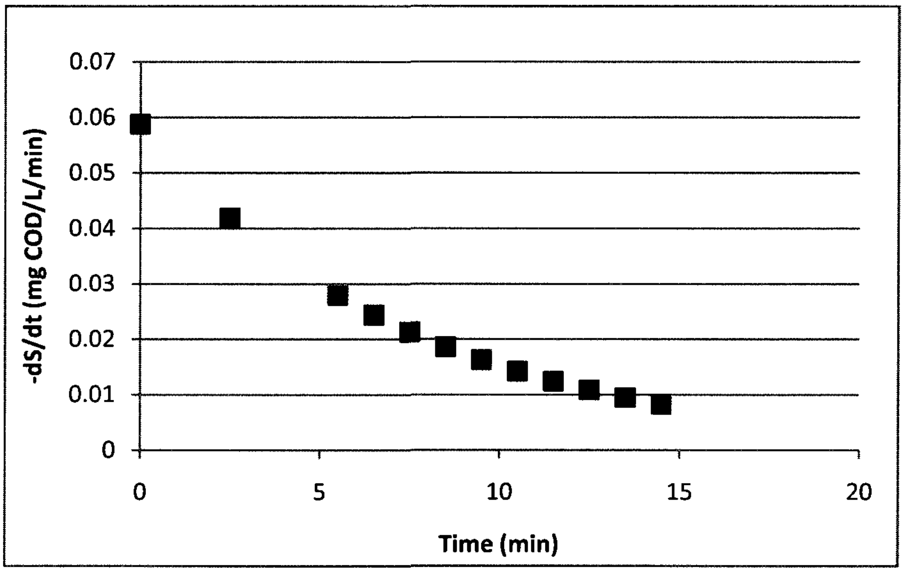
S=40 mg COD/L	t	dS/dt	-dS/dt	S	-dS/dt/X
X=310 mg VSS/L	min	mg COD/L/mi	mg COD/L/mi	mg COD/L	min^{-1}
	0	-0.016	0.016	40	0.00005
	2.5	-0.014	0.014	38.80	4.57E-05
	5.5	-0.013	0.013	38.91	4.11E-05
	6.5	-0.012	0.012	38.20	3.97E-05
	7.5	-0.012	0.012	37.53	3.83E-05
	8.5	-0.011	0.011	36.78	3.69E-05
	9.5	-0.011	0.011	36.48	3.56E-05
	10.5	-0.011	0.011	35.63	3.44E-05
	11.5	-0.010	0.010	35.33	3.32E-05
	12.5	-0.010	0.010	35.04	3.2E-05
	13.5	-0.010	0.010	34.99	3.09E-05
	14.5	-0.009	0.009	35.09	2.98E-05

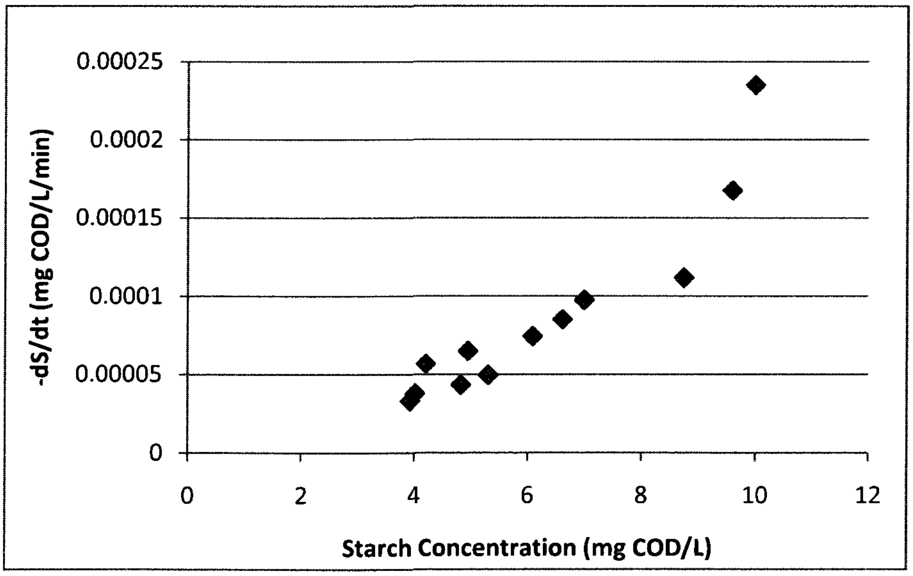




Appendix E
Starch Hydrolysis Rate Change with Time and Starch Concentrations

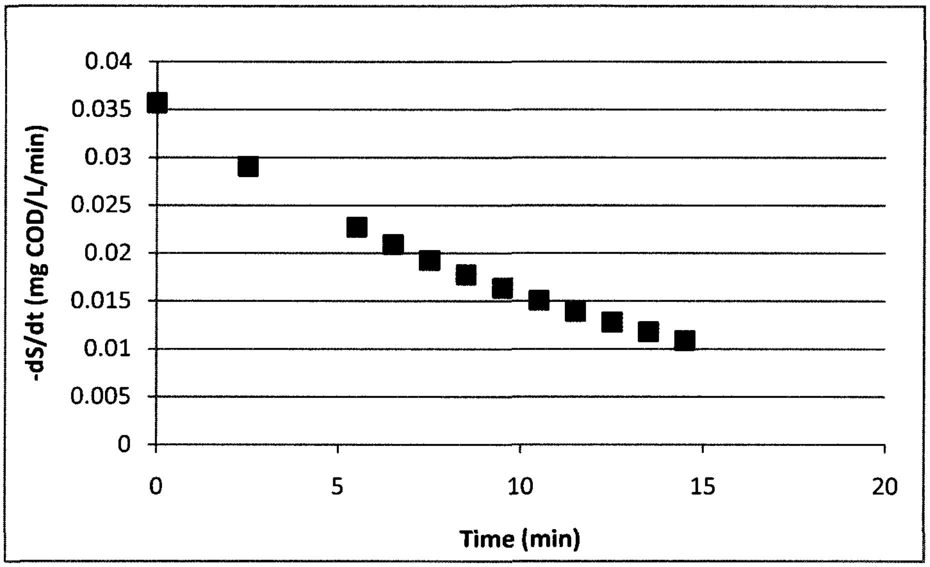
S=10 mg COD/L	t	dS/dt	-dS/dt	S	-dS/dt/X
X=250 mg VSS/L	min	mg COD/L/mi	mg COD/L/mi	mg COD/L	min^{-1}
	0	-0.059	0.059	10	0.000235
	2.5	-0.042	0.042	9.60	0.000168
	5.5	-0.028	0.028	8.74	0.000112
	6.5	-0.024	0.024	6.99	9.75E-05
	7.5	-0.021	0.021	6.61	8.52E-05
	8.5	-0.019	0.019	6.08	7.44E-05
	9.5	-0.016	0.016	4.94	6.5E-05
	10.5	-0.014	0.014	4.20	5.68E-05
	11.5	-0.012	0.012	5.30	4.96E-05
	12.5	-0.011	0.011	4.82	4.33E-05
	13.5	-0.009	0.009	4.01	3.78E-05
	14.5	-0.008	0.008	3.92	3.3E-05

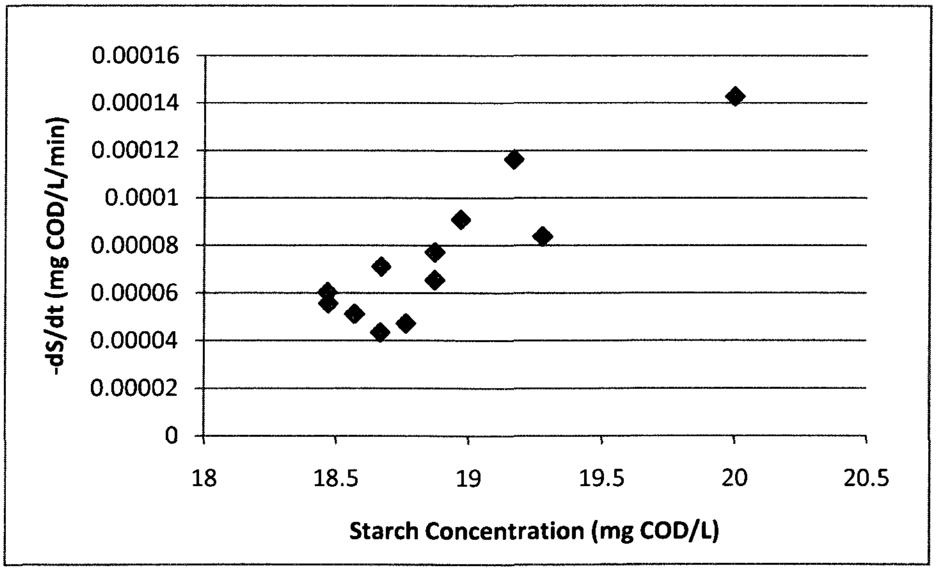




Appendix E
Starch Hydrolysis Rate Change with Time and Starch Concentrations

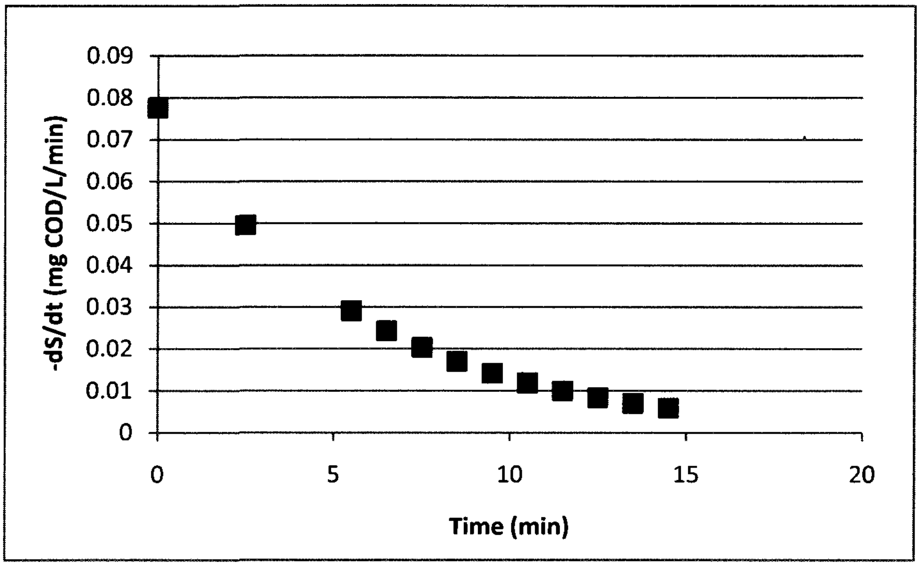
S=20 mg COD/L	t	dS/dt	-dS/dt	S	-dS/dt/X
X=250 mg VSS/L	min	mg COD/L/mi	mg COD/L/mi	mg COD/L	min^{-1}
	0	-0.036	0.036	20	0.000143
	2.5	-0.029	0.029	19.17	0.000116
	5.5	-0.023	0.023	18.97	9.09E-05
	6.5	-0.021	0.021	19.28	8.37E-05
	7.5	-0.019	0.019	18.87	7.71E-05
	8.5	-0.018	0.018	18.67	7.11E-05
	9.5	-0.016	0.016	18.87	6.55E-05
	10.5	-0.015	0.015	18.47	6.03E-05
	11.5	-0.014	0.014	18.47	5.55E-05
	12.5	-0.013	0.013	18.57	5.12E-05
	13.5	-0.012	0.012	18.76	4.71E-05
	14.5	-0.011	0.011	18.67	4.34E-05

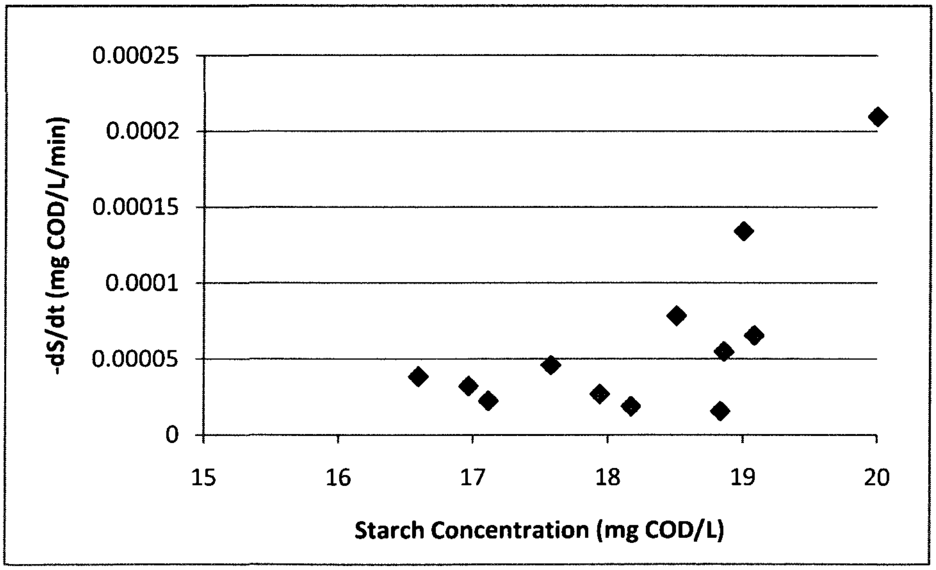




Appendix E
Starch Hydrolysis Rate Change with Time and Starch Concentrations

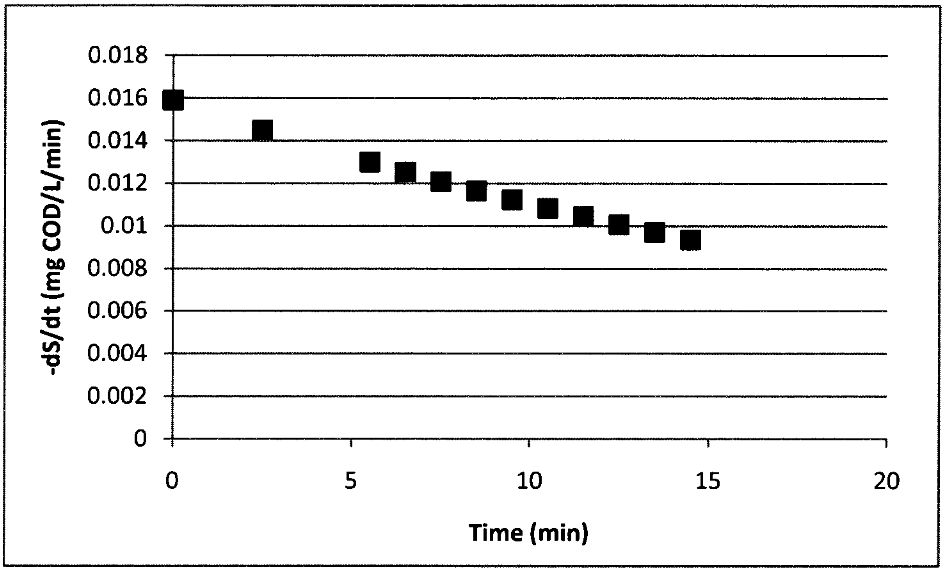
S=20 mg COD/L	t	dS/dt	-dS/dt	S	-dS/dt/X
X=370 mg VSS/L	min	mg COD/L/mi	mg COD/L/mi	mg COD/L	min^{-1}
	0	-0.078	0.078	20	0.00021
	2.5	-0.050	0.050	19.01	0.000134
	5.5	-0.029	0.029	18.51	7.85E-05
	6.5	-0.024	0.024	19.09	6.56E-05
	7.5	-0.020	0.020	18.86	5.49E-05
	8.5	-0.017	0.017	17.58	4.59E-05
	9.5	-0.014	0.014	16.59	3.84E-05
	10.5	-0.012	0.012	16.97	3.21E-05
	11.5	-0.010	0.010	17.94	2.68E-05
	12.5	-0.008	0.008	17.11	2.25E-05
	13.5	-0.007	0.007	18.17	1.88E-05
	14.5	-0.006	0.006	18.84	1.57E-05

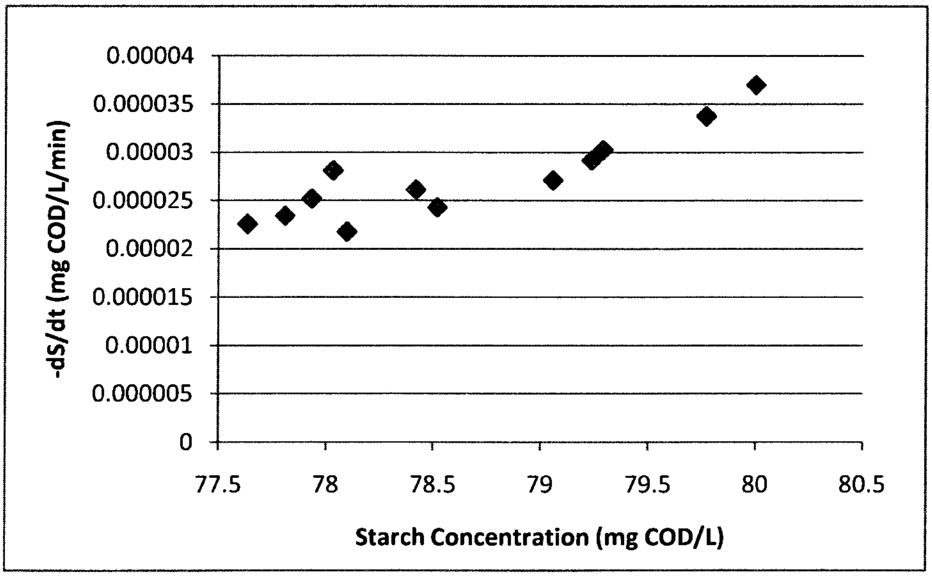




Appendix E
Starch Hydrolysis Rate Change with Time and Starch Concentrations

S=80 mg COD/L	t	dS/dt	-dS/dt	S	-dS/dt/X
X=430 mg VSS/L	min	mg COD/L/mi	mg COD/L/mi	mg COD/L	min^{-1}
	0	-0.0159	0.0159	80	3.7E-05
	2.5	-0.0145	0.0145	79.77	3.37E-05
	5.5	-0.0130	0.0130	79.29	3.02E-05
	6.5	-0.0125	0.0125	79.24	2.92E-05
	7.5	-0.0121	0.0121	78.03	2.81E-05
	8.5	-0.0117	0.0117	79.06	2.71E-05
	9.5	-0.0112	0.0112	78.42	2.61E-05
	10.5	-0.0108	0.0108	77.93	2.52E-05
	11.5	-0.0104	0.0104	78.52	2.43E-05
	12.5	-0.0101	0.0101	77.81	2.34E-05
	13.5	-0.0097	0.0097	77.64	2.26E-05
	14.5	-0.0094	0.0094	78.10	2.18E-05

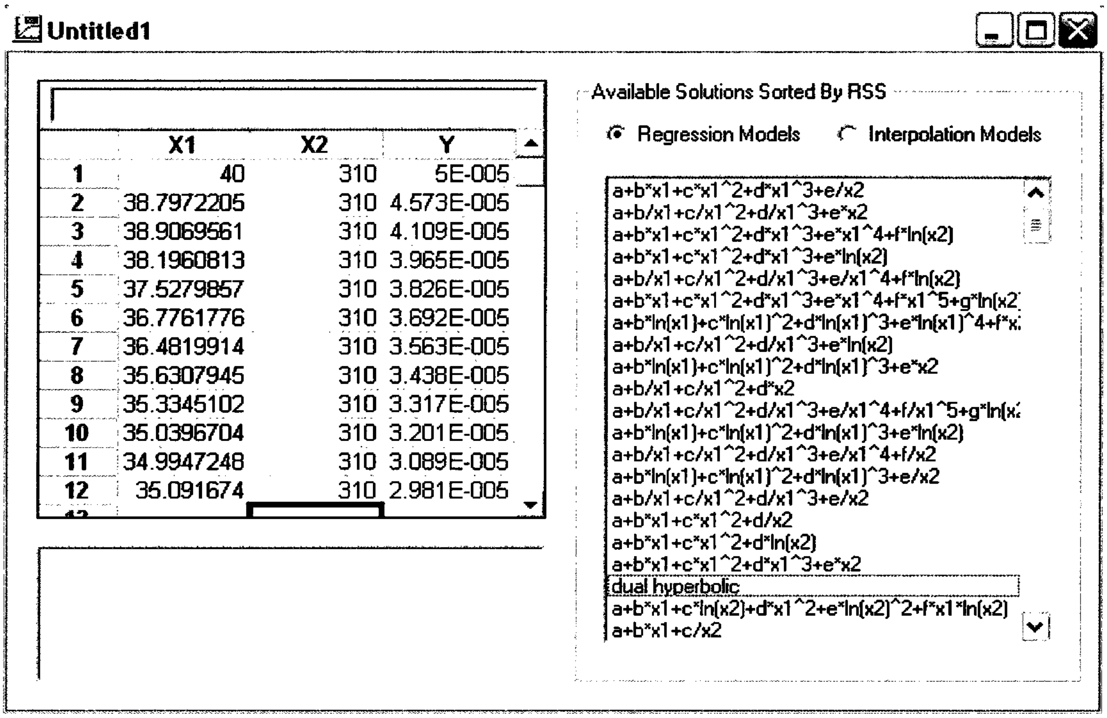




Appendix F

Fitting Dual Hyperbolic Models to Biological Data Using Nonlinear Regression (Starch)

Run 1 S=40 mg COD/L, X=310 mg VSS/L



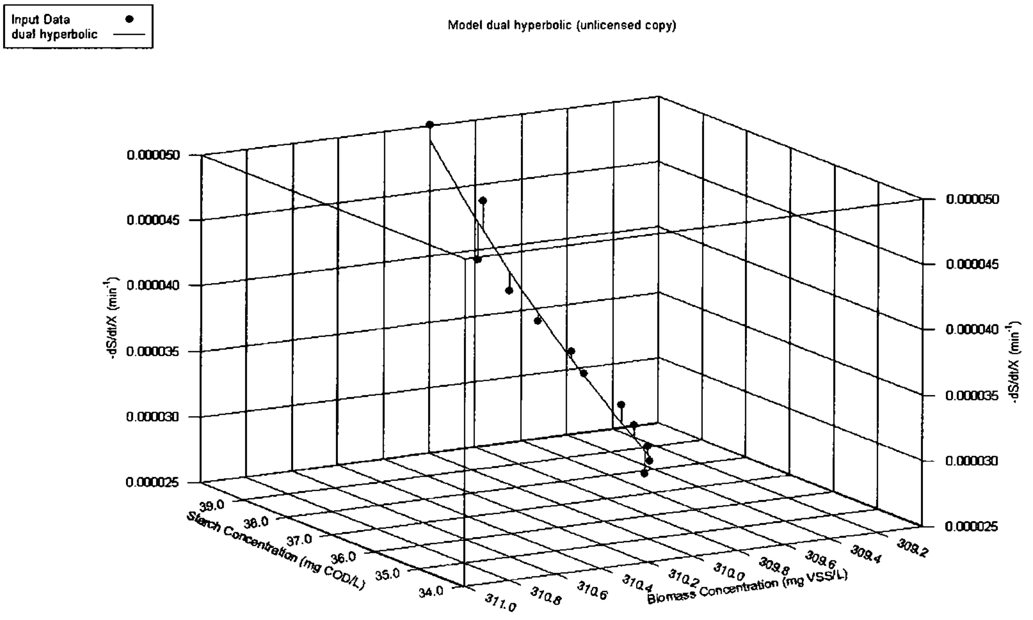
Untitled1

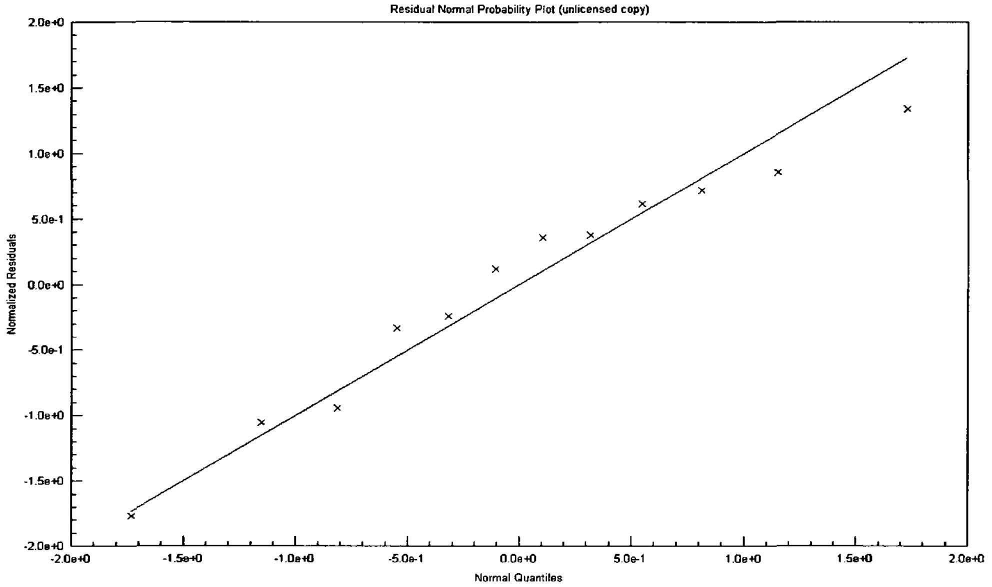
	X1	X2	Y
1	40	310	5E-005
2	38.7972205	310	4.573E-005
3	38.9069561	310	4.109E-005
4	38.1960813	310	3.965E-005
5	37.5279857	310	3.826E-005
6	36.7761776	310	3.692E-005
7	36.4819914	310	3.563E-005
8	35.6307945	310	3.438E-005
9	35.3345102	310	3.317E-005
10	35.0396704	310	3.201E-005
11	34.9947248	310	3.089E-005
12	35.091674	310	2.981E-005

Available Solutions Sorted By RSS

● Regression Models ○ Interpolation Models

```
a+b*x1+c*x1^2+d*x1^3+e/x2
a+b/x1+c/x1^2+d/x1^3+e*x2
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*ln(x2)
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f*ln(x2)
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*x1^5+g*ln(x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f*x
a+b/x1+c/x1^2+d/x1^3+e*ln(x2)
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*x2
a+b/x1+c/x1^2+d*x2
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x1^5+g*ln(x
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x2)
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e/x2
a+b/x1+c/x1^2+d/x1^3+e/x2
a+b*x1+c*x1^2+d/x2
a+b*x1+c*x1^2+d*ln(x2)
a+b*x1+c*x1^2+d*x1^3+e*x2
dual hyperbolic
a+b*x1+c*ln(x2)+d*x1^2+e*ln(x2)^2+f*x1*ln(x2)
a+b*x1+c/x2
```





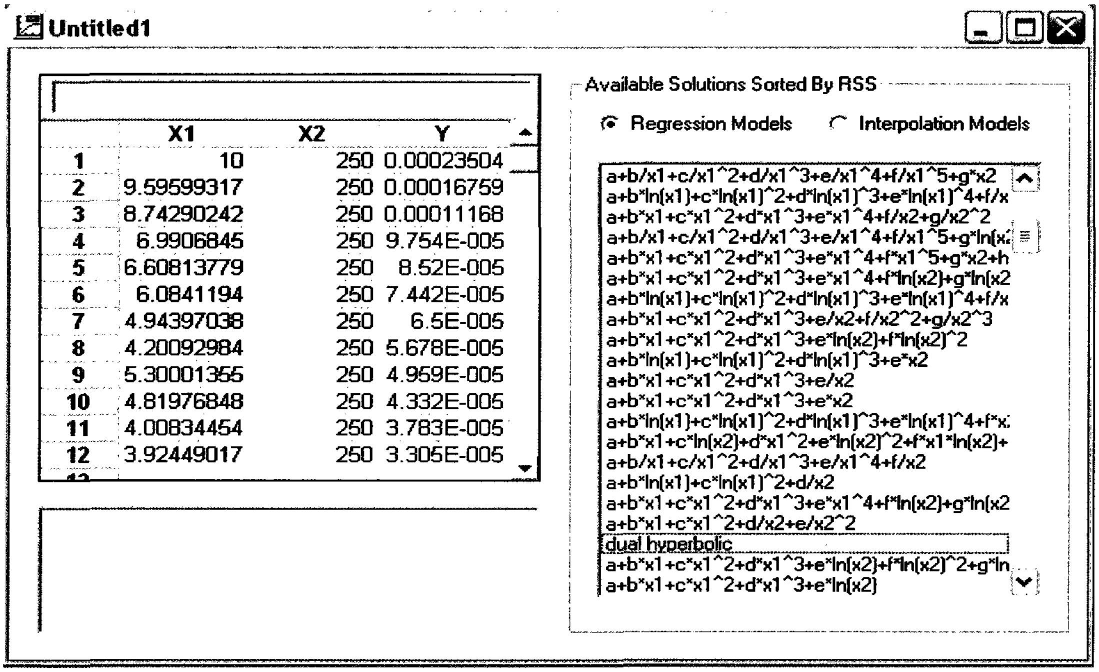
Model Definition:
$Y = a*X_1/(b*X_2+c*X_1)$
Number of observations = 12
Number of missing observations = 0
Solver type: Nonlinear
Nonlinear iteration limit = 250
Diverging nonlinear iteration limit =10
Number of nonlinear iterations performed = 24
Residual tolerance = 0.0000000001
Sum of Residuals = 6.52E–08
Average Residual = 5.43E–09
Residual Sum of Squares (Absolute) = 2.41E–11
Residual Sum of Squares (Relative) = 2.41E–11
Standard Error of the Estimate = 1.64E–06
Coefficient of Multiple Determination (R^2) = 0.94
Proportion of Variance Explained = 94.1%
Adjusted coefficient of multiple determination (Ra^2) = 0.928
Durbin–Watson statistic = 1.482
95% Confidence Intervals

Variable	**Value**	**95% (+/–)**	**Lower Limit**	**Upper Limit**
a	3.41E–03	3228.2	–3.35E+07	3228.2
b	35.3	3.35E+07	–3.35E+07	3.35E+07
c	–203.76	1.93E+08	–1.93E+08	1.93E+08

S	X	dS/dt/X	Cal dS/dt/X	Residual	% Error	Abs Residual	Min Residual	Max Residual
40	310	0.00005	4.88E–05	1.17E–06	2.34	1.17E–06	–2.89E–06	2.20E–06
38.8	310	4.57E–05	4.35E–05	2.20E–06	4.80	2.20E–06		
38.9	310	4.11E–05	4.40E–05	–2.90E–06	–7.04	2.89E–06		
38.2	310	3.97E–05	4.12E–05	–1.50E–06	–3.90	1.55E–06		

37.5	310	3.83E−05	3.88E−05	−5.40E−07	−1.42	5.43E−07		
36.8	310	3.69E−05	3.63E−05	5.83E−07	1.58	5.83E−07		
36.5	310	3.56E−05	3.54E−05	1.96E−07	0.55	1.96E−07		
35.6	310	3.44E−05	3.30E−05	1.41E−06	4.09	1.41E−06		
35.3	310	3.32E−05	3.22E−05	1.00E−06	3.02	1.00E−06		
35.0	310	3.20E−05	3.14E−05	6.13E−07	1.91	6.13E−07		
35.0	310	3.09E−05	3.13E−05	−3.90E−07	−1.27	3.93E−07		
35.1	310	2.98E−05	3.15E−05	−1.70E−06	−5.79	1.73E−06		

Run 2 S=10 mg COD/L, X=250 mg VSS/L



	X1	X2	Y
1	10	250	0.00023504
2	9.59599317	250	0.00016759
3	8.74290242	250	0.00011168
4	6.9906845	250	9.754E-005
5	6.60813779	250	8.52E-005
6	6.0841194	250	7.442E-005
7	4.94397038	250	6.5E-005
8	4.20092984	250	5.678E-005
9	5.30001355	250	4.959E-005
10	4.81976848	250	4.332E-005
11	4.00834454	250	3.783E-005
12	3.92449017	250	3.305E-005

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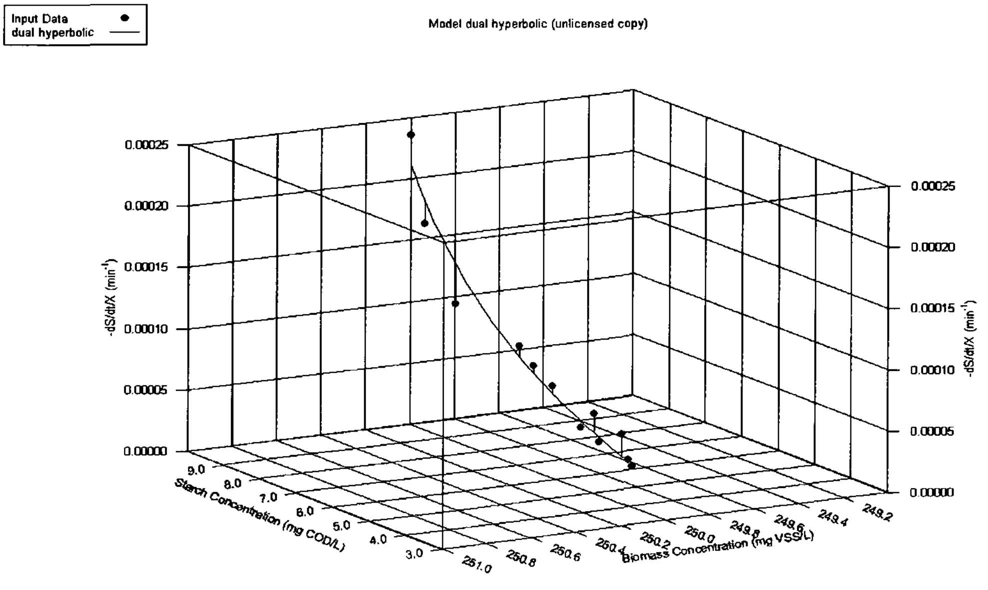
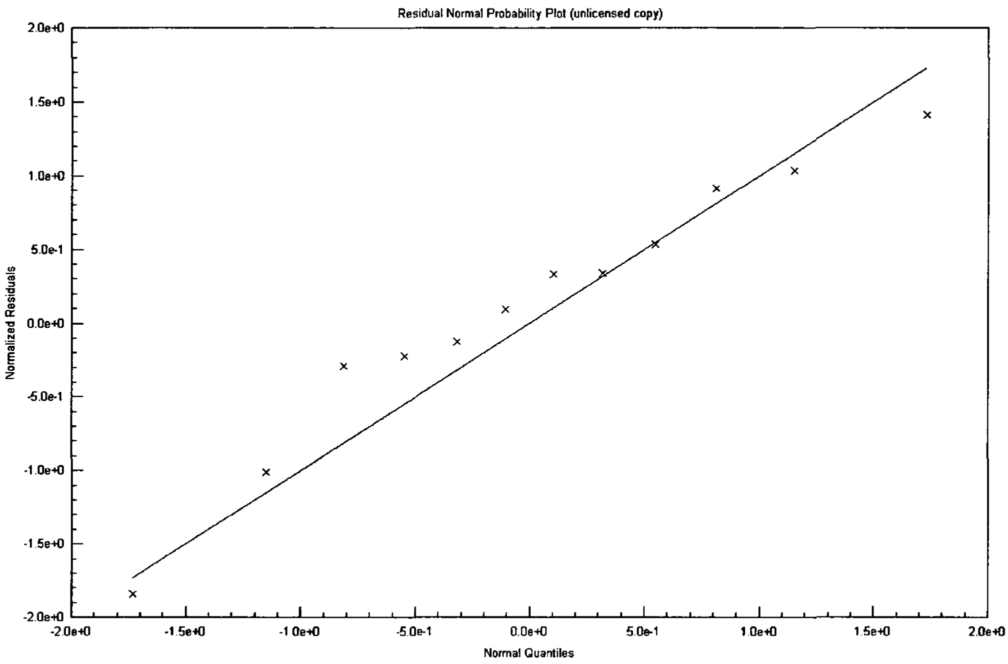
● Regression Models ○ Interpolation Models

```
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x1^5+g*x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f/x
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f/x2+g/x2^2
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x1^5+g*ln(x
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*x1^5+g*x2+h
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*ln(x2)+g*ln(x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f/x
a+b*x1+c*x1^2+d*x1^3+e/x2+f/x2^2+g/x2^3
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)+f*ln(x2)^2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*x2
a+b*x1+c*x1^2+d*x1^3+e/x2
a+b*x1+c*x1^2+d*x1^3+e*x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f*x
a+b*x1+c*ln(x2)+d*x1^2+e*ln(x2)^2+f*x1*ln(x2)+
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x2
a+b*ln(x1)+c*ln(x1)^2+d/x2
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*ln(x2)+g*ln(x2
a+b*x1+c*x1^2+d/x2+e/x2^2
dual hyperbolic
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)+f*ln(x2)^2+g*ln
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)
```

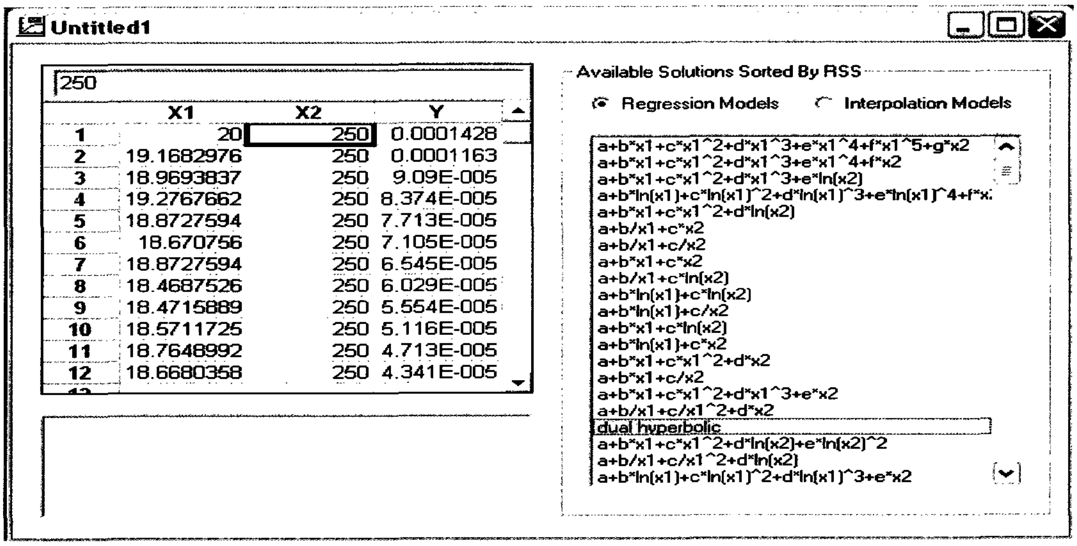




<table>
<tr><td colspan="5">Model Definition:</td></tr>
<tr><td colspan="5">Y = a*X₁/(b*X₂+c*X₁)</td></tr>
<tr><td colspan="5">Number of observations = 12</td></tr>
<tr><td colspan="5">Number of missing observations = 0</td></tr>
<tr><td colspan="5">Solver type: Nonlinear</td></tr>
<tr><td colspan="5">Nonlinear iteration limit = 250</td></tr>
<tr><td colspan="5">Diverging nonlinear iteration limit =10</td></tr>
<tr><td colspan="5">Number of nonlinear iterations performed = 17</td></tr>
<tr><td colspan="5">Residual tolerance = 0.0000000001</td></tr>
<tr><td colspan="5">Sum of Residuals = 2.06E–05</td></tr>
<tr><td colspan="5">Average Residual = 1.72E–06</td></tr>
<tr><td colspan="5">Residual Sum of Squares (Absolute) = 2.74E–09</td></tr>
<tr><td colspan="5">Residual Sum of Squares (Relative) = 2.74E–09</td></tr>
<tr><td colspan="5">Standard Error of the Estimate = 1.75E–05</td></tr>
<tr><td colspan="5">Coefficient of Multiple Determination (R²) = 0.930</td></tr>
<tr><td colspan="5">Proportion of Variance Explained = 93.0%</td></tr>
<tr><td colspan="5">Adjusted coefficient of multiple determination (Ra²) = 0.915</td></tr>
<tr><td colspan="5">Durbin–Watson statistic = 1.61</td></tr>
<tr><td colspan="5">95% Confidence Intervals</td></tr>
<tr><td>Variable</td><td>Value</td><td>95% (+/–)</td><td>Lower Limit</td><td>Upper Limit</td></tr>
<tr><td>a</td><td>6.74</td><td>1.88E+07</td><td>–1.88E+07</td><td>1.88E+07</td></tr>
<tr><td>b</td><td>4122.2</td><td>1.15E+10</td><td>–1.15E+10</td><td>1.15E+10</td></tr>
<tr><td>c</td><td>–71012.1</td><td>1.98E+11</td><td>–1.98E+11</td><td>1.98E+11</td></tr>
</table>

S	X	dS/dt/X	Cal dS/dt/X	Residual	% Error	Abs Residual	Min Residual	Max Residual
10	250	0.000235	0.00021	2.47E−05	10.51	2.47E−05	−3.22E−05	2.47E−05
9.6	250	0.000168	0.000185	−1.80E−05	−10.54	1.77E−05		
8.7	250	0.000112	0.000144	−3.20E−05	−28.79	3.22E−05		
7.0	250	9.75E−05	8.82E−05	9.33E−06	9.57	9.33E−06		
6.6	250	8.52E−05	7.93E−05	5.85E−06	6.87	5.85E−06		
6.1	250	7.44E−05	6.85E−05	5.90E−06	7.93	5.90E−06		
4.9	250	6.50E−05	4.90E−05	1.60E−05	24.55	1.60E−05		
4.2	250	5.68E−05	3.87E−05	1.81E−05	31.89	1.81E−05		
5.3	250	4.96E−05	5.46E−05	−5.00E−06	−10.11	5.01E−06		
4.8	250	4.33E−05	4.72E−05	−3.90E−06	−8.96	3.88E−06		
4.0	250	3.78E−05	3.62E−05	1.62E−06	4.27	1.62E−06		
3.9	250	3.30E−05	3.52E−05	−2.10E−06	−6.46	2.13E−06		

Run 3 S=20 mg COD/L, X=250 mg VSS/L



250

	X1	X2	Y
1	20	250	0.0001428
2	19.1682976	250	0.0001163
3	18.9693837	250	9.09E-005
4	19.2767662	250	8.374E-005
5	18.8727594	250	7.713E-005
6	18.670756	250	7.105E-005
7	18.8727594	250	6.545E-005
8	18.4687526	250	6.029E-005
9	18.4715889	250	5.554E-005
10	18.5711725	250	5.116E-005
11	18.7648992	250	4.713E-005
12	18.6680358	250	4.341E-005

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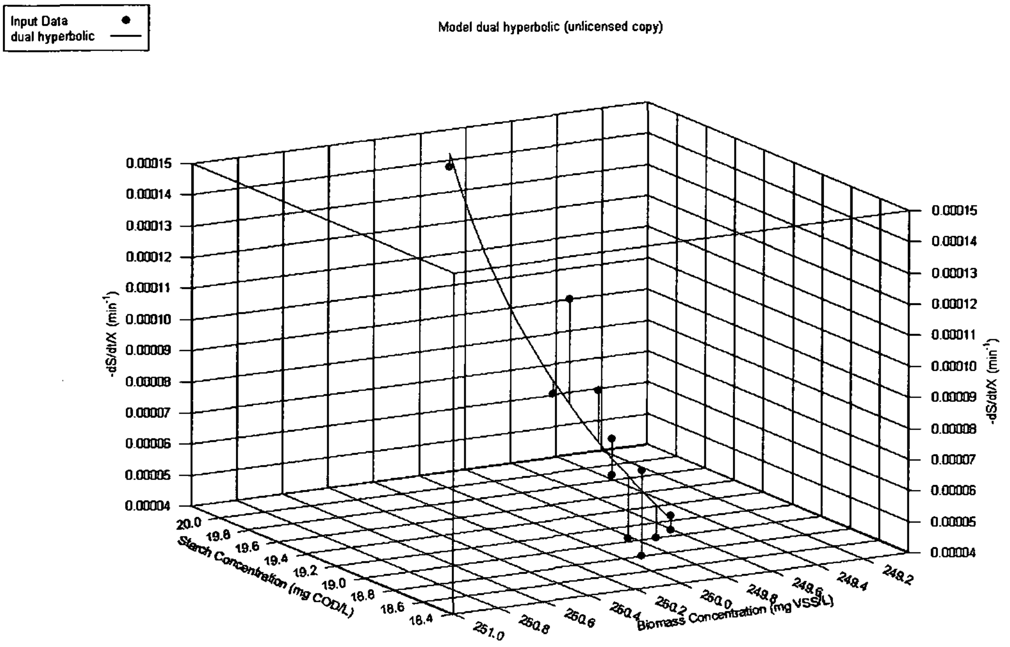
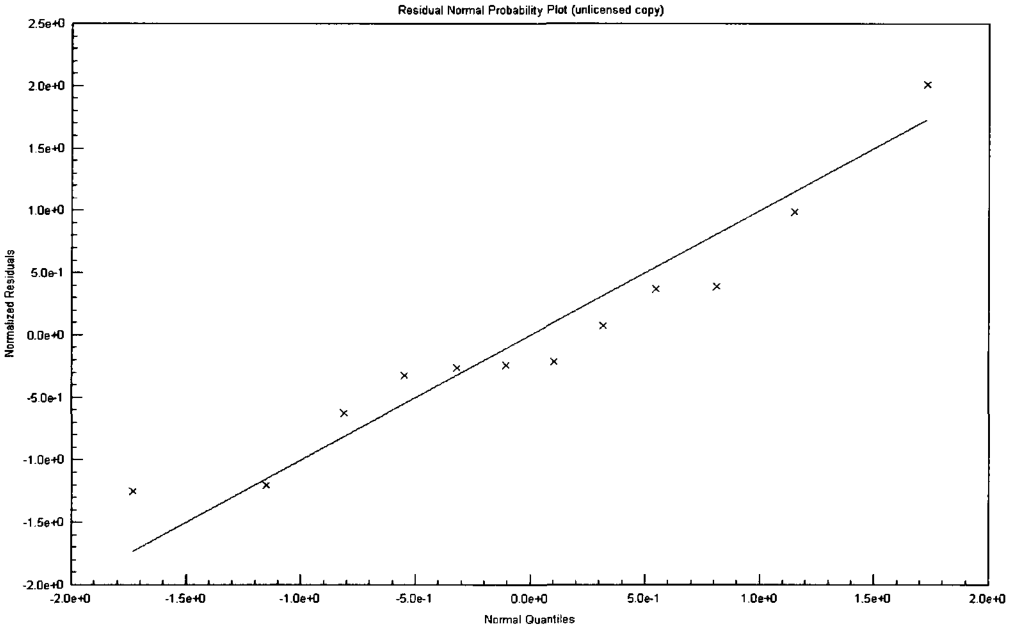
● Regression Models ○ Interpolation Models

```
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*x1^5+g*x2
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*x2
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f*x
a+b*x1+c*x1^2+d*ln(x2)
a+b/x1+c*x2
a+b/x1+c/x2
a+b*x1+c*x2
a+b/x1+c*ln(x2)
a+b*ln(x1)+c*ln(x2)
a+b*ln(x1)+c/x2
a+b*x1+c*ln(x2)
a+b*ln(x1)+c*x2
a+b*x1+c*x1^2+d*x2
a+b*x1+c/x2
a+b*x1+c*x1^2+d*x1^3+e*x2
a+b/x1+c/x1^2+d*x2
dual hyperbolic
a+b*x1+c*x1^2+d*ln(x2)+e*ln(x2)^2
a+b/x1+c/x1^2+d*ln(x2)
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*x2
```





Model Definition:
$Y = a*X_1/(b*X_2+c*X_1)$
Number of observations = 12
Number of missing observations = 0
Solver type: Nonlinear
Nonlinear iteration limit = 250
Diverging nonlinear iteration limit =10
Number of nonlinear iterations performed = 29

Residual tolerance = 0.0000000001

Sum of Residuals = −4.77E−06

Average Residual = −3.97E−07

Residual Sum of Squares (Absolute) = 2.53E−09

Residual Sum of Squares (Relative) = 2.53E−09

Standard Error of the Estimate = 1.68E−05

Coefficient of Multiple Determination (R^2) = 0.74

Proportion of Variance Explained = 73.87%

Adjusted coefficient of multiple determination (Ra^2) = 0.68

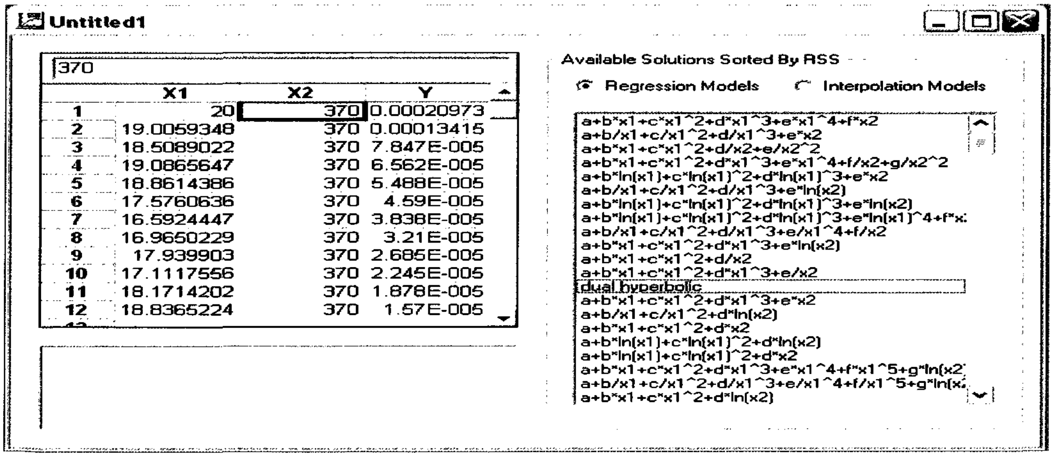
Durbin−Watson statistic = 1.04

95% Confidence Intervals

Variable	**Value**	**95% (+/−)**	**Lower Limit**	**Upper Limit**
a	1.19E−03	870.1	−870.1	870.1
b	11.70	8.52E+06	−8.52E+06	8.52E+06
c	−138.09	1.00E+08	−1.00E+08	1.00E+08

S	X	dS/dt/X	Cal dS/dt/X	Residual	% Error	Abs Residual	Min Residual	Max Residual
20	250	0.000143	0.000147	−4.40E−06	−3.07	4.39E−06	−2.10E−05	3.37E−05
19.2	250	0.000116	8.26E−05	3.37E−05	28.98	3.37E−05		
19.0	250	9.09E−05	7.44E−05	1.65E−05	18.19	1.65E−05		
19.3	250	8.37E−05	8.78E−05	−4.10E−06	−4.87	4.07E−06		
18.9	250	7.71E−05	7.09E−05	6.25E−06	8.10	6.25E−06		
18.7	250	7.11E−05	6.45E−05	6.59E−06	9.27	6.59E−06		
18.9	250	6.55E−05	7.09E−05	−5.40E−06	−8.30	5.43E−06		
18.5	250	6.03E−05	5.90E−05	1.28E−06	2.13	1.28E−06		
18.5	250	5.55E−05	5.91E−05	−3.50E−06	−6.38	3.54E−06		
18.6	250	5.12E−05	6.17E−05	−1.10E−05	−20.55	1.05E−05		
18.8	250	4.71E−05	6.73E−05	−2.00E−05	−42.86	2.02E−05		
18.7	250	4.34E−05	6.44E−05	−2.10E−05	−48.32	2.10E−05		

Run 4 S=20 mg COD/L, X=370 mg VSS/L



370

	X1	X2	Y
1	20	370	0.00020973
2	19.0059348	370	0.00013415
3	18.5089022	370	7.847E-005
4	19.0865647	370	6.562E-005
5	18.8614386	370	5.488E-005
6	17.5760636	370	4.59E-005
7	16.5924447	370	3.838E-005
8	16.9650229	370	3.21E-005
9	17.939903	370	2.685E-005
10	17.1117556	370	2.245E-005
11	18.1714202	370	1.878E-005
12	18.8365224	370	1.57E-005

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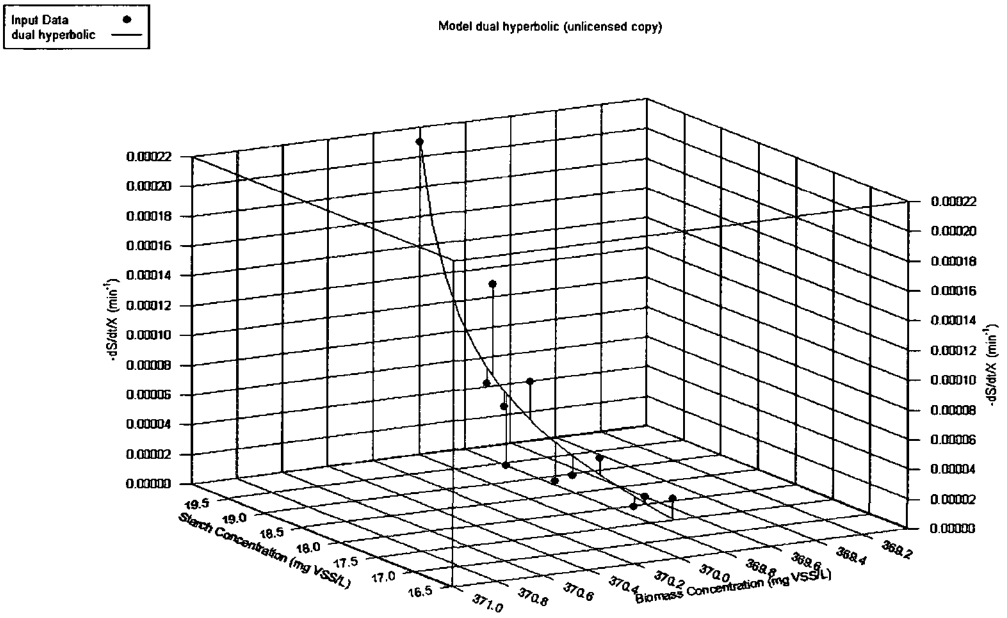
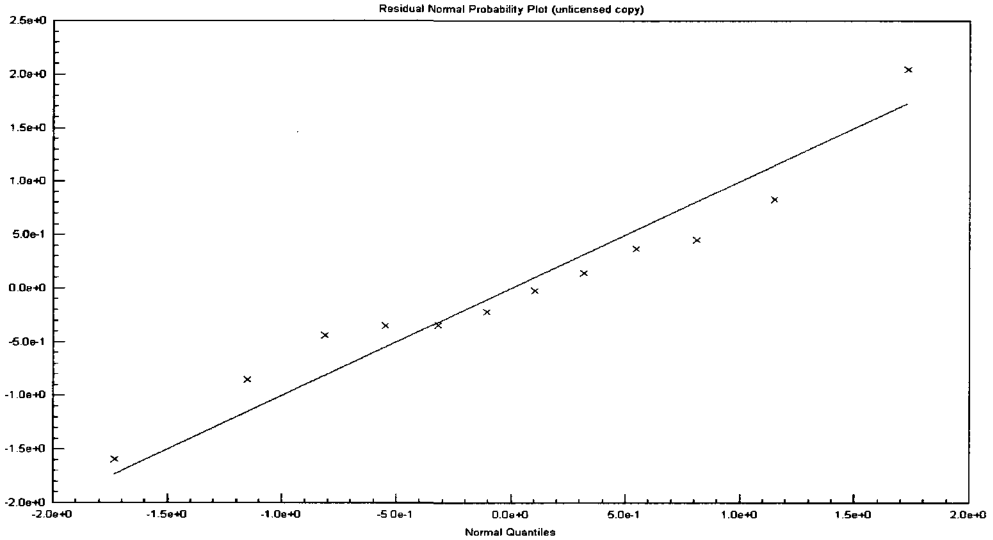
● Regression Models ○ Interpolation Models

```
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*x2
a+b/x1+c/x1^2+d/x1^3+e*x2
a+b*x1+c*x1^2+d/x2+e/x2^2
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f/x2+g/x2^2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*x2
a+b/x1+c/x1^2+d/x1^3+e*ln(x2)
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x2)
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f*x
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x2
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)
a+b*x1+c*x1^2+d/x2
a+b*x1+c*x1^2+d*x1^3+e/x2
dual hyperbolic
a+b*x1+c*x1^2+d*x1^3+e*x2
a+b/x1+c/x1^2+d*ln(x2)
a+b*x1+c*x1^2+d*x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x2)
a+b*ln(x1)+c*ln(x1)^2+d*x2
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*x1^5+g*ln(x2
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x1^5+g*ln(x
a+b*x1+c*x1^2+d*ln(x2)
```



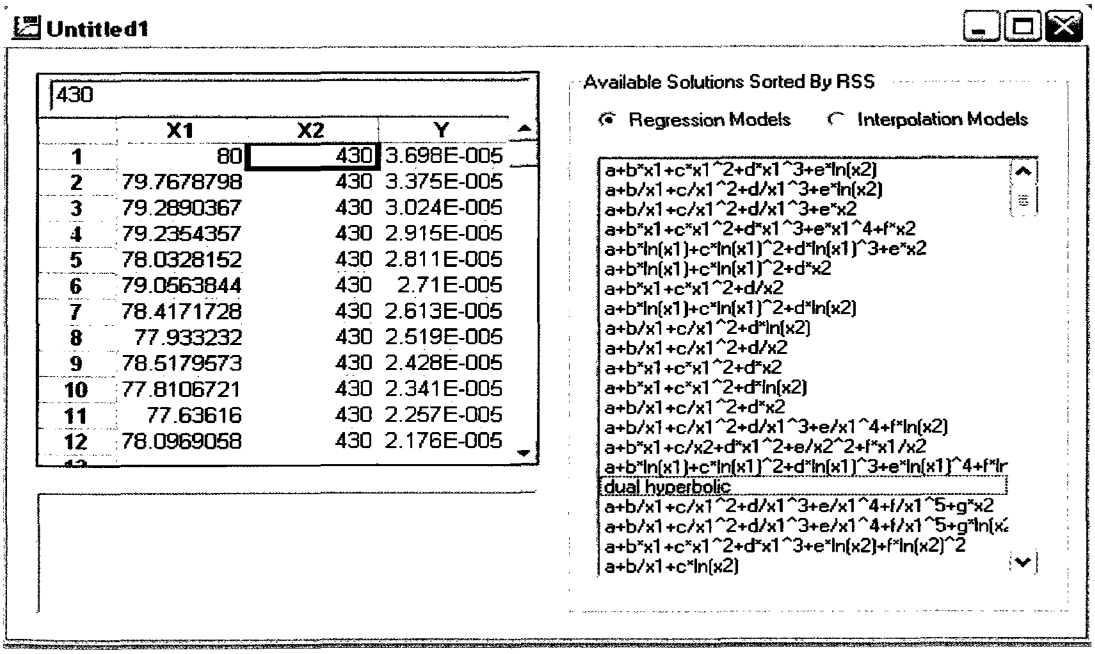


Model Definition:
$Y = a*X_1/(b*X_2+c*X_1)$
Number of observations = 12
Number of missing observations = 0
Solver type: Nonlinear
Nonlinear iteration limit = 250
Diverging nonlinear iteration limit =10
Number of nonlinear iterations performed = 35
Residual tolerance = 0.0000000001
Sum of Residuals = 3.48E–07
Average Residual = 2.90E–08
Residual Sum of Squares (Absolute) = 8.33E–09
Residual Sum of Squares (Relative) = 8.33E–09
Standard Error of the Estimate = 3.04E–05

Coefficient of Multiple Determination (R^2) = 0.768				
Proportion of Variance Explained = 76.78%				
Adjusted coefficient of multiple determination (Ra^2) = 0.716				
Durbin–Watson statistic = 1.010				
95% Confidence Intervals				
Variable	**Value**	**95% (+/–)**	**Lower Limit**	**Upper Limit**
a	0.486	266345.6	–266345.1	266346.1
b	4596.4	2.52E+09	–2.52E+09	2.52E+09
c	–82722.3	4.53E+10	–4.53E+10	4.53E+10

S	X	dS/dt/X	Cal dS/dt/X	Residual	% Error	Abs Residual	Min Residual	Max Residual
20	370	0.00021	0.00021	–7.20E–07	–0.34	7.17E–07	–4.86E–05	6.22E–05
19.0	370	0.000134	7.20E–05	6.22E–05	46.35	6.22E–05		
18.5	370	7.85E–05	5.31E–05	2.54E–05	32.33	2.54E–05		
19.1	370	6.56E–05	7.62E–05	–1.10E–05	–16.17	1.06E–05		
18.9	370	5.49E–05	6.53E–05	–1.00E–05	–19.07	1.05E–05		
17.6	370	4.59E–05	3.47E–05	1.12E–05	24.50	1.12E–05		
16.6	370	3.84E–05	2.46E–05	1.38E–05	35.91	1.38E–05		
17.0	370	3.21E–05	2.78E–05	4.34E–06	13.52	4.34E–06		
17.9	370	2.68E–05	4.03E–05	–1.30E–05	–50.05	1.34E–05		
17.1	370	2.25E–05	2.92E–05	–6.70E–06	–30.02	6.74E–06		
18.2	370	1.88E–05	4.48E–05	–2.60E–05	–138.38	2.60E–05		
18.8	370	1.57E–05	6.43E–05	–4.90E–05	–309.57	4.86E–05		

Run 5 S=80 mg COD/L, X=430 mg VSS/L



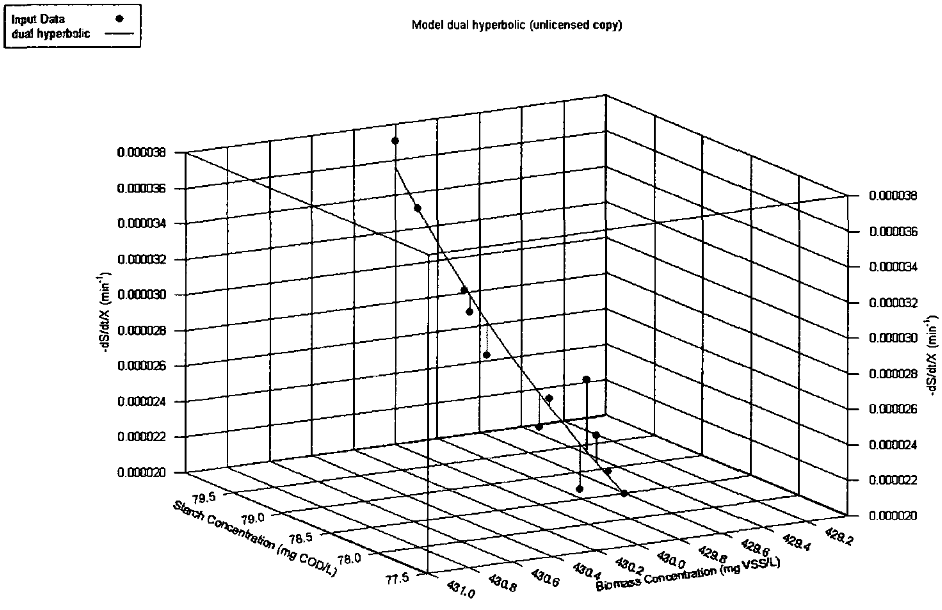
430

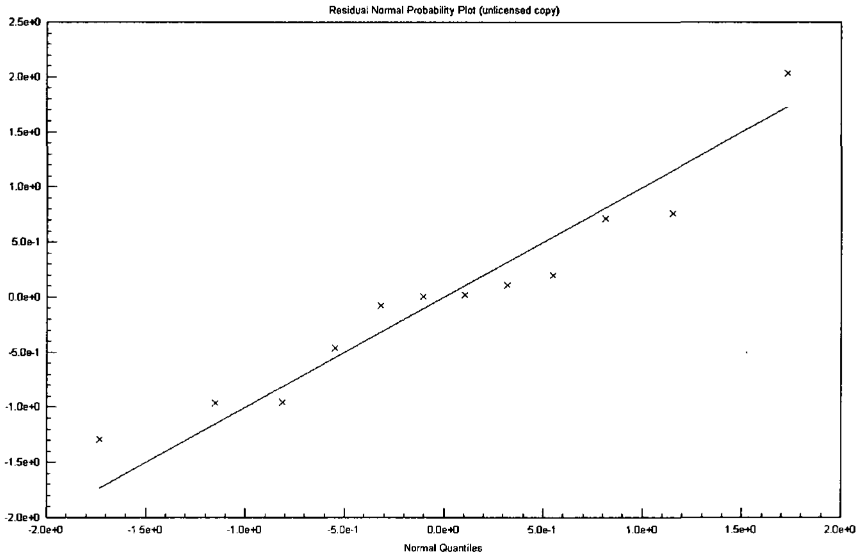
	X1	X2	Y
1	80	430	3.698E-005
2	79.7678798	430	3.375E-005
3	79.2890367	430	3.024E-005
4	79.2354357	430	2.915E-005
5	78.0328152	430	2.811E-005
6	79.0563844	430	2.71E-005
7	78.4171728	430	2.613E-005
8	77.933232	430	2.519E-005
9	78.5179573	430	2.428E-005
10	77.8106721	430	2.341E-005
11	77.63616	430	2.257E-005
12	78.0969058	430	2.176E-005

Available Solutions Sorted By RSS

● Regression Models ○ Interpolation Models

```
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)
a+b/x1+c/x1^2+d/x1^3+e*ln(x2)
a+b/x1+c/x1^2+d/x1^3+e*x2
a+b*x1+c*x1^2+d*x1^3+e*x1^4+f*x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*x2
a+b*ln(x1)+c*ln(x1)^2+d*x2
a+b*x1+c*x1^2+d/x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x2)
a+b/x1+c/x1^2+d*ln(x2)
a+b/x1+c/x1^2+d/x2
a+b*x1+c*x1^2+d*x2
a+b*x1+c*x1^2+d*ln(x2)
a+b/x1+c/x1^2+d*x2
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f*ln(x2)
a+b*x1+c/x2+d*x1^2+e/x2^2+f*x1/x2
a+b*ln(x1)+c*ln(x1)^2+d*ln(x1)^3+e*ln(x1)^4+f*lr
dual hyperbolic
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x1^5+g*x2
a+b/x1+c/x1^2+d/x1^3+e/x1^4+f/x1^5+g*ln(x
a+b*x1+c*x1^2+d*x1^3+e*ln(x2)+f*ln(x2)^2
a+b/x1+c*ln(x2)
```


Input Data
dual hyperbolic
Model dual hyperbolic (unlicensed copy)
-dS/dt/X (min^{-1})
0.000038
0.000036
0.000034
0.000032
0.000030
0.000028
0.000026
0.000024
0.000022
0.000020
Starch Concentration (mg COD/L)
79.5
79.0
78.5
78.0
77.5
Biomass Concentration (mg VSS/L)
431.0
430.8
430.6
430.4
430.2
430.0
429.8
429.6
429.4
429.2


Residual Normal Probability Plot (unlicensed copy)
Normalized Residuals
2.5e+0
2.0e+0
1.5e+0
1.0e+0
5.0e-1
0.0e+0
-5.0e-1
-1.0e+0
-1.5e+0
-2.0e+0
Normal Quantiles
-2.0e+0
-1.5e+0
-1.0e+0
-5.0e-1
0.0e+0
5.0e-1
1.0e+0
1.5e+0
2.0e+0

Model Definition:

$Y = a*X_1/(b*X_2+c*X_1)$

Number of observations = 12

Number of missing observations = 0

Solver type: Nonlinear

Nonlinear iteration limit = 250

Diverging nonlinear iteration limit =10

Number of nonlinear iterations performed = 36

Residual tolerance = 0.0000000001

Sum of Residuals = 1.69E–07

Average Residual = 1.41E–08

Residual Sum of Squares (Absolute) = 3.56E–11

Residual Sum of Squares (Relative) = 3.56E–11

Standard Error of the Estimate = 1.99E–06

Coefficient of Multiple Determination (R^2) = 0.85

Proportion of Variance Explained = 84.60%

Adjusted coefficient of multiple determination (Ra^2) = 0.81

Durbin–Watson statistic = 2.58

95% Confidence Intervals

Variable	**Value**	**95% (+/–)**	**Lower Limit**	**Upper Limit**
a	4.90E–04	115.5	–115.5	115.5
b	48.5	1.14E+07	–1.14E+07	1.14E+07
c	–246.9	5.82E+7	–5.82E+7	5.82E+7

S	X	dS/dt/X	Cal dS/dt/X	Residual	% Error	Abs Residual	Min Residual	Max Residual
80	430	3.70E–05	3.56E–05	1.42E–06	3.83	1.42E–06	–2.57E–06	4.04E–06
79.8	430	3.37E–05	3.37E–05	4.35E–08	0.13	4.35E–08		
79.3	430	3.02E–05	3.04E–05	–1.60E–07	–0.52	1.59E–07		
79.2	430	2.92E–05	3.01E–05	–9.10E–07	–3.13	9.12E–07		
78.0	430	2.81E–05	2.41E–05	4.04E–06	14.36	4.04E–06		
79.1	430	2.71E–05	2.90E–05	–1.90E–06	–7.04	1.91E–06		
78.4	430	2.61E–05	2.57E–05	3.98E–07	1.52	3.98E–07		
77.9	430	2.52E–05	2.37E–05	1.51E–06	6.01	1.51E–06		
78.5	430	2.43E–05	2.62E–05	–1.90E–06	–7.88	1.91E–06		
77.8	430	2.34E–05	2.32E–05	2.11E–07	0.90	2.11E–07		
77.6	430	2.26E–05	2.26E–05	1.41E–08	0.06	1.41E–08		
78.1	430	2.18E–05	2.43E–05	–2.60E–06	–11.82	2.57E–06		