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Kinetics of DCOD Consumption by Bacterial Suspensions

Shirley Poliszuk
University of New Orleans

KINETICS OF DCOD CONSUMPTION BY BACTERIAL SUSPENSIONS

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ABSTRACT

The aim of this investigation was to study the kinetics of readily biodegradable soluble substrate, and the interactions between the bioflocculation process and such kinetics. Several batch test experiments were performed at different soluble-substrate-biomass ratios in order to evaluate its impact on the kinetics and the order of the reaction. Similarly the consumption of dissolved substrate was compared using two different bacterial suspensions: (1) flocculated suspension; and (2) dispersed cells suspensions. In this research flocculated biomass from a complete mixed activated sludge (CMAS) system was tested using sequencing batch reactors (SBR). Results indicate that when the So/Xo ratio is low (below 0.3) the removal of readily biodegradable soluble substrate can be well described by first-order kinetics with an asymptotic non-biodegradable portion for both flocculated and dispersed cells suspensions. However, it was found that the dissolved COD consumption for freely dispersed cells proceeds at a faster rate than for flocculated suspensions.

CHAPTER I

1 INTRODUCTION

1.1 Background

The primary purpose of wastewater treatment is to remove the suspended and soluble organic constituents measured as chemical oxygen demand (COD) or biochemical oxygen demand (BOD). Biological treatment processes are used to degrade the organics in the wastewater before it is discharged (Murthy, 1998). Of the numerous methods available , the activated sludge system is one of the most popular and versatile and perhaps the most common biological process for wastewater treatment. According to Pavoni et al. (1972) this process inherently relies on two independent characteristics for the production of an acceptable effluent. The first is the assimilation of the suspended, colloidal and dissolved organic material by the active mass of microorganism to a final end product of carbon dioxide, water and inert material. The second phase, and ultimately the most significant, is the flocculation of the biomass and other suspended and colloidal material into units large and dense enough to settle out of solution so that a high-quality effluent can be obtained.

The substrate entering the activated sludge system can be differentiate into readily and slowly biodegradable substrates. According to Okutman et al. (2001) the slowly biodegradable portion constitutes the bulk and it is broken down to soluble, readily biodegradable compounds by hydrolysis. Okutman et al. (2001) expressed that the rate of hydrolysis is much slower than that of the utilization of the soluble substrate it generates. Thus, it is the hydrolysis rate that determines the overall rate of particulate organics degradation. According to Tchobanoglous et

al. (2003) the overall process in complete-mix activated-sludge (CMAS) requires hydraulic retention times (HRTs) between 3 and 6 hours.

Great effort has been made to understand the role of biopolymers and bioflocculation in sludge thickening, settling and dewatering, e.g., Novak and Haugan (1981), Urbain et al. (1993), Chao and Keinath (1979) , Goodwin and Forster (1985), Murthy (1998), Liao et al. (2001). But few effort has been made in understanding the kinetics of biopolymers production and bioflocculation. Indeed, the actual theories incorporated into the IWA/IAWQ consensus models indicate that the removal of particulate organics from the liquid in the activated sludge process is a two-step process, namely, rapid enmeshment of particles and hydrolysis followed by oxidation. La Motta et al. (2003, 2004) expressed that, in any case, this removal process should be described at least by a three-step process, namely, flocculation, hydrolysis and finally oxidation.

La Motta et al. (2003, 2004) demonstrated that flocculation plays an important role in the removal of particulate COD; once the oxidation of readily biodegradable soluble organic matter has been completed the microorganisms excrete polymers for trapping the particulate compounds, resulting in the formation of flocs that are heavy enough to be removed by sedimentation. They found that bioflocculation can be accurately describe by a first-order relationship, and that the time required for the bioflocculation of particulate organic matter is just a fraction of the time required for the bioflocculation-hydrolysis-oxidation of this material. La Motta et al. (2003, 2004) reported important removals of COD in a continuous-flow suspended growth reactor with hydraulic retention times (HRT) less than one hour. La Motta et al. (2003, 2004) proposed a three-step treatment process, namely, oxidation of readily biodegradable

soluble substrate- bioflocculation of particulate organic matter – settling of the flocculate suspension. However, the interactions between the bioflocculation and the consumption of readily biodegradable soluble substrates is not well understood. Moreover, the factors affecting the kinetics of readily biodegradable substrate, as a process independently of hydrolysis, need to be identified. La Motta el at. (2003, 2004) proposed a first-order model for the simulation of the dissolved COD consumption, but they did not compared against other order models, neither a different So/Xo ratios. Chudoba *et al*. (1992) showed that the initial substrate-total biomass (So/Xo) ratio plays an important role in batch cultivations, and the biokinetic parameters evaluated at high and low So/Xo ratios can differ significantly

1.2 Scope and Objectives

The main goal of this investigation is to study the kinetics of readily biodegradable soluble substrate, and the interactions between the bioflocculation process and such kinetics. The general objective of this thesis is to study the kinetics of dissolved COD removal, and specific objectives include:

- Determine the order of the reaction, and the kinetic coefficients for the consumption of DCOD.

- Evaluate the effect of the initial soluble substrate to biomass ratio in the kinetics of readily biodegradable soluble substrates.

- Compare the consumption of dissolved substrate using two different bacterial suspensions: flocculated and dispersed cells suspensions.

- Evaluate the effect of internal diffusion in the bacterial flocs on the removal of readily biodegradable soluble substrates

1.3 Thesis Organization

This document is organized into five chapters:

Chapter 1 presents the background and introduces the topic, discusses the problem, the dissertation scope and objectives, and the organization of the document.

Chapter 2 presents the literature review. Kinetics relationship, different rate expression for the utilization of soluble substrates, and other the topics related to the thesis are presented in this Chapter.

Chapter 3 presents the materials and methods use in the development of this research.

Chapter 4 presents the results obtained with the different evaluations carried out in this research. This Chapter also presents the analysis and discussion of such results

Chapter 5 states the conclusions and recommendations reached with the development of this thesis.

CHAPTER II

2. LITERATURE REVIEW

Traditionally, three main objectives have been recognized in the biological treatment of domestic wastewater. The first one is to transform dissolved and particulate biodegradable constituents into acceptable end products. The second one is to capture and incorporate suspended and nonsettleable colloidal solids into a biological floc or biofilm. And the last one is to transform or remove nutrients, such a nitrogen and phosphorus. is to remove or reduce the concentration of organic and inorganic compounds. These objectives may vary depending on the treatment required according to the final destination of the treated wastewater. For example, in the case of land application of wastewater, the main objective is to remove phosphorus and nitrogen, which are nutrients capable of stimulating the growth of aquatic plants.

In biological treatment the active role is played by microorganisms, which are responsible for the removal of particulate and dissolved carbonaceous BOD and the stabilization of organic matter found in wastewater. According to Tchobanoglous *et al.* (2003) microorganisms to oxidize (i.e., convert) the dissolved and particulate carbonaceous organic matter into simple end products and additional biomass. This process is represented by the following equation for the aerobic biological oxidation of organic matter:

$$v_1\left(\text{organic material}\right)+v_2O_2+v_3NH_3+v_4PO_4^{-3}\xrightarrow{microorganis}v_5\left(new\ cells\right)+v_6CO_2+v_7H_2O \qquad \ldots\ldots\ldots\ldots\ldots\ldots 2.1$$

where:

v_1 = the stoichiometric coefficient

O_2 = oxygen

NH_3 = ammonia

PO_4^{3-} = phosphate

According to La Motta et al. (2003, 2004), in addition to the microbial oxidation of organic matter there are other important physical processes mediated by bacterial action that participate in the removal of organic matter present in domestic wastewater. La Motta et al. (2003, 2004) demonstrated that when sewage contains a large proportion of particulate organics, flocculation plays an important role in the removal of particulate COD. Once the oxidation of readily biodegradable soluble organic matter has been completed the microorganisms excrete polymers for trapping the particulate compounds, resulting in the formation of flocs that are heavy enough to be removed by sedimentation; this process is known as a bioflocculation. Obviously, the time required for the bioflocculation of particulate organic matter is just a fraction of the time required for the bioflocculation-hydrolysis-oxidation of this material. La Motta et al. (2003, 2004) reported important removals of COD in a continuous-flow suspended growth reactor with hydraulic retention times (HRT) less than one hour, while similar removals in complete-mix activated-sludge (CMAS) plants are achieved with HRTs between 3 and 6 hours (Tchobanoglous et al. 2003).

There are two primary categories of biological processes used for wastewater treatment suspended growth and attached growth (or biofilm) processes. In suspended growth processes, the microorganisms responsible for organic matter decomposition are maintained in suspension within the liquid by appropriate mixing methods. Most of the wastewater treatment plants are operated with suspended growth processes with a positive dissolved oxygen concentration, but can turn anaerobic (no oxygen present) when the organic concentration is high.

For municipal wastewater the most common suspended growth process used is the activated-sludge process. According to Tchobanoglous *et al.* (2003), the activated sludge process was developed by Clark and Gage around 1913 at the Lawrence Experiment Station in Massachusetts, and by Ardern and Lockett in 1914 at the Manchester Sewage Work in Manchester, England. Ardern and Lockett retained biomass in the system by use of a batch aerated reactor and a fill and draw system; they found this suspension to be responsible for the improvement of the wastewater quality. In the activated sludge process the mixture of wastewater and sludge is agitated and aerated speeding the breakdown of the organic matter present in raw sewage. The sludge is separated from the wastewater and disposed of or returned to the system.

On the other hand, in the attached growth process, the microorganisms and bacteria treating the wastes are attached to some inert media (e.g., rock, gravel, sand, peat, designed ceramic or plastic and other synthetic materials) and use the dissolved organic material that diffuses into the film that develops on the media. Attached growth units act as secondary treatment devices in all cases. Raw wastewater must be treated first to remove the larger solids

and floating debris, because these solids can plug the filter. Examples of wastewater treatments that utilize attached growth processes include those that hold the media fixed in place, allowing the wastewater to flow over the bed such, as trickling filters, or those where the media is in motion relative to the wastewater, such as rotating biological disks. This method of wastewater treatment can be used for BOD removal, nitrification and denitrification.

This literature review will focus on suspended growth reactors and the associated processes of oxidation of dissolved organic matter, and bioflocculation and hydrolysis of particulate organic matter.

2.1 Kinetic relationships

Studies of kinetics of aerobic biological treatment yield the rate at which microorganism degrade a specific waste, and therefore provide the basic information required for sizing biological aerobic reactors. This study can be conveniently performed in a laboratory-scale batch reactor (See Figure 2.1). Figure 2.1 shows an example of four units operating in parallel, each with a capacity of 2 liters. Wastewater containing a seed of microorganisms (it can be biological sludge taken from an operating activated sludge plant or from settled sewage) is introduced into the reactors, and at the same time, compressed air is blown into the system. The biological is kept in a state of complete mixing due to the agitation provided by the air blown into the system.

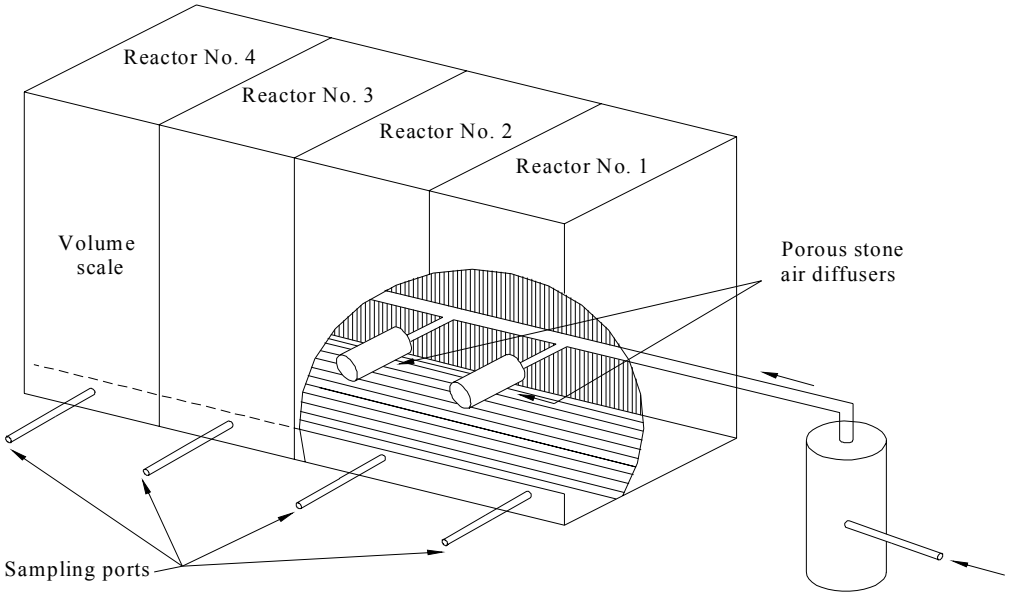

Figure 2.1 Batch Reactor

The substrate concentration S of the wastewater (measured as soluble BOD or COD, TOD, TOC….) is determined at selected time intervals by withdrawing samples for analysis. The mass of accumulated biological sludge is also determined at these same times intervals by measuring the concentration of volatile suspended solids. Typical curves showing the soluble substrate concentration S and variation of the amount of MLVSS with time are presented in Figure 2.2.

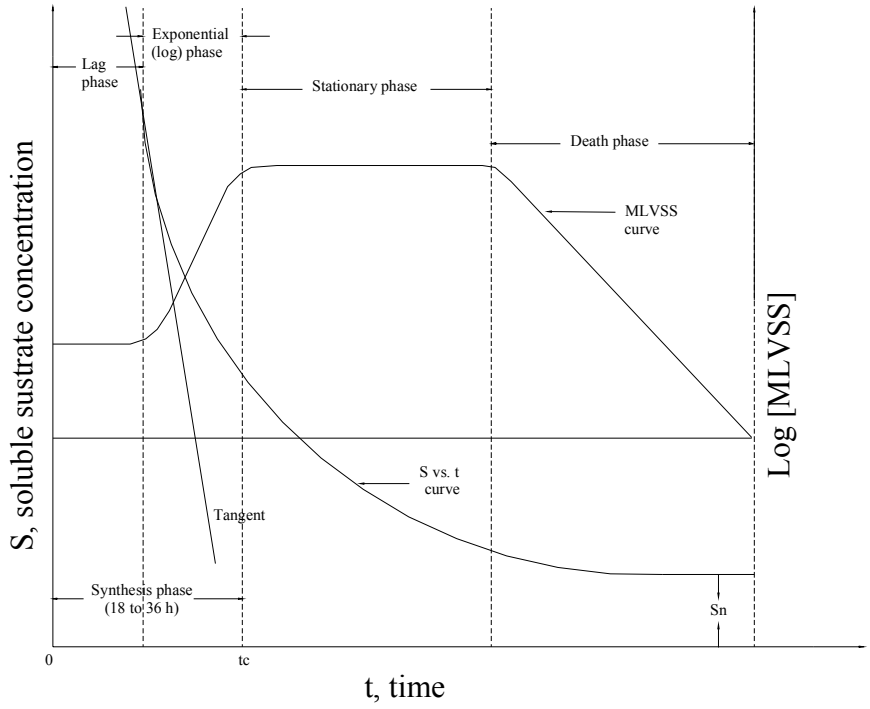

Figure 2.2 Typical soluble substrate concentration and MLVSS curves for a batch reactor (After Ramalho, 1983)

The soluble substrate concentration (S) of the wastewater, decreases with the time as the organic matter is oxidized. A plateau is eventually reached corresponding to the amount of nonbiodegradable matter (Sn). If BOD is used to measure substrate concentration, $Sn = 0$ since at infinite time all biodegradable substrate has been oxidized. On the other hand, if, for example, COD is utilized, it is possible to have $Sn > 0$, corresponding to substrate that is not biologically degradable but is chemically oxidized by $K_2Cr_2O_7$ (Ramalho, 1983)

The concentration of MLVSS increases (from time 0 to time t_C, at which most of the dissolved substrate concentration has been depleted. This growth corresponds to the synthesis of new microorganism cells, indicated in Fig. 2.2 as "synthesis phase". After time, there is not

enough food left to sustain microorganism growth. At this time, microorganisms start consuming their "fellow microorganisms" as food. As this "cannibalistic feast" proceeds, the concentration of MLVSS drops when the rate of destruction of microorganism cells exceeds that of synthesis of new cells. This corresponds to the " endogenous respiration phase". The maximum on the MLVSS curve corresponds to time t_C.

There are two fundamental differences between the operation of a batch reactor and a completely mixed reactor with cell recycle:

- Contrary to what happens in the batch reactor, BOD of the wastewater in the continuous reactor operating at steady-state conditions remains constant (Se). This corresponds generally to a low substrate concentration since the biological reactor is usually designed for removing most of the influent BOD.

- The concentration of MLVSS in the continuous reactor operating under steady-state conditions is kept constant ($X_{v,a}$) at a selected value. Maintenance of this constant X_v is obtained by providing the calculated amount of concentrated return sludge.

2.1.1 Rate of utilization of soluble substrates.

Perhaps the principal concern in wastewater treatment is the removal of substrate. The goal in biological wastewater treatment is, in most cases, to deplete the electron donor (i.e.,

organic compounds in aerobic oxidation). Lawrence and McCarty (1970) related the rate of substrate utilization to the concentration of microorganism in the reactor and to the concentration of the remaining substrate. They proposed an equation mathematically analogous to the formula that was proposed by Michaelis-Menten to describe enzyme kinetics and to the classical Monod equation describing the effect of substrate concentration on the growth rate coefficient (See Equation 2.2).

$$r_{su} = -\frac{kXS}{K_s + \mathrm{S}} \qquad (2.2)$$

The Monod's model differs from the "classical" growth models in the way that it introduces the concept of a growth-controlling ('limiting") substrate. The terms "nutrient limitation" and "nutrient limited growth" have been used to describe to different growth phenomena. First, they are used to indicate that a certain amount of biomass can be produced for a particular amount of substrate. (Liebig's law). Second, these terms are also used to indicate that the microbial growth rate (m) is dictate by the (low) actual concentration of a particular substrate, as described by Monod's model. Monod's model relates the growth rate to the concentration of a single growth-controlling substrate via two parameters, the maximum specific growth rate (mmax), and the substrate affinitive constant (*Ks*). (Kovaroca-Kovar, et al. 1998). This model which is a mixed order model is perhaps the most wide used model for describing the rate of substrate utilization:

$$\mu = \mu \max \frac{S}{Ks + S} \qquad (2.3)$$

where:

μ = specific growth rate, time^{-1}

μ max = maximum specific growth rate, time^{-1}

S = growth-limiting substrate concentration in solution, mass/unit volume (g/m^3)

K_s = half-velocity constant, substrate concentration at one half the maximum growth rate, mass/unit volume (g/m^3)

In a batch reactor the rate of growth can be defined:

$$r_g = \mu X \qquad (2.4)$$

where

r_g = rate of bacterial growth, mass/unit volume * time

X = concentration of microorganism, mass/unit volume

Combining Equations 2.3 and 2.4, the resulting expression for the rate growth is:

$$r_{su} = -\frac{\mu \text{ max} XS}{K_s + S} \qquad (2.5)$$

The following relationship has been developed between the rate of substrate utilization and the rate of growth (Tchobanoglous et al. 2003):

$$r_g = -Yr_{su} \qquad (2.5)$$

where

r_g = rate of bacterial growth, mass/unit volume * time

Y = maximum yield coefficient, mg/mg (defined as the ratio of the mass of cells formed to the mass of substrate consumed, measured during any finite period of logarithmic growth)

r_{su}= substrate utilization rate, mass/unit volume * time

If the value of r_{su} from Eq. 2.5 is substituted in Eq. 2.6, the rate substrate utilization can be defined as follows:

$$r_{su} = -\frac{\mu \text{maxX}S}{\text{Y}(K_s + \text{S})} \tag{2.7}$$

In Eq. 2.7 the term $\frac{\mu \text{m}}{\text{Y}}$ is often replace by the term k, defined as the maximum substrate utilization rate per unit mass of organisms (g/g * d)

$$k = \frac{\mu \text{m}}{\text{Y}} \tag{2.8}$$

If the term k is substituted for the term μm /Y in Eq 2.7, the resulting expression is similar to the Equation 2.2 proposed by Lawrence and McCarty (1970):

$$r_{su} = -\frac{k\text{X}S}{K_s + \text{S}} \tag{2.2}$$

Defining q as the specific substrate removal rate we obtain the following expression

$$q = \frac{r_{su}}{X} = -\frac{kS}{Ks + S} \tag{2.9}$$

To express the growth dynamics of a population that is limited solely by the concentration of a single substrate, other kinetic expressions have been proposed. Some models can be derived from non-Monod models if such models saturate at high values of *S* and are roughly linear when *S* is close to zero. (Simkins et al. 1984).

The actual growth rate lies was the first kinetic principle proposed for microbial growth by its systematic deviations of μ at low substrate concentrations, where the actual growth rate lies above the prediction, and at high substrate concentrations, where μ_{max} is approached too slowly,

were a matter of much debate. According to Penfold and Norris, (1912), the relationship between μ and s is best described by a "saturation" type of curve, that at high substrate concentrations the organisms should grow at a maximum rate (μ_{max}) independent of the substrate concentration. However, the development of structured (mechanistic) models for quantifying microbial growth kinetics is still limited because the mechanism of cell growth is very complex and is not yet completely understood. Therefore, most of the proposed growth models are unstructured and empirical. (Kovaroca-Kovar, et al. 1998).

Caperon, (1968); Droop, (1968) assume that the growth of the cells is relate to intracellular nutrient concentration, this formulation is commonly called cell quota model. (Davidson, et al. 1999).

Zero order and first order name's are derived from the chemical kinetics they resemble. Models "Monod with growth" and "Monod" , no growth refer to the manner these models where obtain and could be called Michaellis-Menten kinetics. Logistic and logarithmic models bear the names associated with the kinetics of growth that occurs as the substrate disappears. (Simkins, et al. 1984)

According with Simkins, et al. (1984) when the initial cell density is much greater than the number of new organisms which could be produced from the substrate present at time zero, ie., $Xo \rangle\rangle So$, the growth of the population during the course of an experiment becomes insignificant on a proportional basis, and the term ($So = Xo - S$) can be approximated as simply Xo, as is done for the zero order, Monod, no growth, and First order models. When the initial substrate concentration is much greater than the half saturation constant ($So \rangle\rangle Xo$) , most of

the substrate will disappear while the uptake systems of the cells are saturated. In zero order and logarithmic models this simplification is reflected. Alternatively, in the first order and logistic models the substrate may initially be present at much less than saturating levels ($So \langle\langle K_s$), in which case the uptake rate per cell becomes a linear function of substrate concentration.

According to Simkins, et al. (1984) these are some different kind of models :

Zero order

Differential form	$-\mathrm{d}S/\mathrm{d}t = k_1$	(2.10)
Integral form	$S - So = k_1 t$	(2.11)
Derived parameter	$k_1 = \mu \max Xo$	(2.12)
Necessary condition	$Xo \rangle\rangle So$ and $So \rangle\rangle K_s$	

Monod, no growth

Differential form	$-\mathrm{d}S/\mathrm{d}t = k_1 S/(K_s + S)$	(2.13)
Integral form	$K_s \ln(S/So) + S - So = k_1 t$	(2.14)
Derived parameter	$k_1 = \mu \max Xo$	(2.15)
Necessary condition	$Xo \rangle\rangle So$	

First order

Differential form	$-\mathrm{d}S/\mathrm{d}t = k_3 S$	(2.16)
Integral form	$S = So \exp = (-k_3 t)$	(2.17)
Derived parameter	$k_3 = \mu \max Xo/K_s$	(2.18)

Necessary condition $Xo \rangle\rangle So$ and $So \langle\langle K_s$ (2.19)

Logistic

Differential form $-\mathrm{d}S/\mathrm{d}t = k_4 S(So + Xo - S)$ (2.20)

Integral form $S = \dfrac{So + Xo}{1 + (Xo/So)\exp[k_4(So + Xo)t]}$ (2.21)

Derived parameter $k_4 = \mu\max/K_s$ (2.22)

Necessary condition $So \langle\langle K_s$

Monod with growth

Differential form $-\mathrm{d}S/\mathrm{d}t = [\mu\max S(So + Xo - S)]/(K_s + S)$ (2.23)

Integral form $Ks\ln(S/So) = (So + Xo + K_s)\ln(X/Xo) - (So + Xo)\mu\max t$ (2.24)

Derived parameter None

Necessary condition None

Logarithmic

Differential form $-\mathrm{d}S/\mathrm{d}t = \mu\max(So + Xo - S)$ (2.25)

Integral form $S = So + Xo[1 - \exp(\mu\max t)]$ (2.26)

Derived parameter None

Necessary condition $So \rangle\rangle K_s$

First order with non-biodegradable fraction

This equation was proposed by La Motta (2003,2004) and is similar to the equation described by McKinney and Eckenfelder (1970). According to La Motta (2003,2004) this equation fit better at low soluble substrate than the Monod equation, and is used to compare and to contrast the particle removal rate to.

$$r_{oxidation} = k_D(S_D + a_D)X \tag{2.27}$$

The first order model ($r_{su} = -kXS$) is satisfactory for describing substrate utilization rates when the biological treatment processes will be operated at relatively low substrate concentrations. (Tchobanoglous et al. 2003).

$$r_{su} = \frac{ds}{dt} \tag{2.28}$$

$$r_{su} = -kXS \tag{2.29}$$

2.1.2 Rate of soluble substrate production from biodegradable particulate organic matter.

According to Tchobanoglous et al. (2003) in municipal wastewater treatment only about 20 to 50 percent of the degradable organic material enters as soluble compounds. La Motta et al. (2003, 2004) reported that in the case of the Jefferson Parish in Louisiana, only 20% of the total

COD (TCOD) is truly dissolved organic material. Bacteria cannot consume the particulate substrates directly and employ extra cellular enzymes to hydrolyze the particulate organics to soluble substrates. Grady et al. (1999) presented a rate expression for particulate substrate conversion is as follows:

$$r_{sc,P} = -\frac{k_P(P/X)X}{(K_X + P/X)} \tag{2.30}$$

where:

$r_{sc,P}$ = rate of change of particulate substrate concentration due to conversion to soluble substrate, g/m^3 * d

k_P = maximum specific particulate conversion rate, g P / g X * d

X = biomass concentration, g/m^3

K_X = half-velocity degradation coefficient, g/g

The particulate degradation concentration is expressed relative to the biomass concentration, because the particulate hydrolysis is related to the relative contact area between the nonsoluble organic material and the biomass. Other associated hydrolysis substrate removal rates are presented in a later section.

2.1.3 Rate of biomass growth with soluble substrates.

The biomass growth is proportional to the substrate utilization rate by the synthesis yield coefficient, and the biomass decay is proportional to the biomass present. Thus, the following relationship between the rate of growth and the rate of substrate utilization might be applicable in both batch and continuous culture systems: (Tchobanoglous et al., 2003)

$$r_g = -Yr_{su} - k_d X \tag{2.31}$$

$$r_g = Y\frac{kXS}{K_s + S} - K_d X \tag{2.32}$$

where:

r_g = net biomass production rate, g VSS/m^3*d

Y = synthesis yield coefficient, g VSS/g bsCOD

k_d = endogenous decay coefficient, g VSS/g VSS*d

r_{su} = rate of substrate concentration change due to utilization, g/m^3*d

k = maximum specific substrate utilization rate, g substrate/g microorganisms * d

X = biomass (microorganism) concentration, g/m^3

S = growth-limiting substrate concentration in solution, g/m^3

K_s = half-velocity constant, substrate concentration at one half the maximum specific substrate utilization rate, g/m^3

If both sides of Eq. 2.8 are divide by the biomass concentration X, the specific growth rate is defined as follows:

$$\mu = \frac{r_g}{X} = Y\frac{kS}{Ks + S} - k_d \qquad (2.33)$$

where

μ = specific biomass growth rate, g VSS/g VSS*d

The specific growth rate corresponds to the change in biomass per day relative to the amount of biomass present, and it is a function of the substrate concentration and the endogenous decay coefficient. The endogenous decay coefficient accounts for the loss in cell mass due to oxidation of internal storage products for energy for cell maintenance, cell death, and predation by organism higher in the food chain. (Tchobanoglous et al., 2003)

The kinetic coefficients $k, K_s, Y,$ and K_d used in the previous equations to predict the rate of substrate utilization and biomass growth vary as a function of the wastewater source, microbial population, and temperature. Kinetic coefficient values are determined from bench-scale testing or full-scale plant test results. Typical kinetic coefficient values are reported in Table 2.1 for the aerobic oxidation of BOD in domestic wastewater. Tchobanoglous et al. (2003).

Table 2.1 Typical kinetic coefficients for the activated-sludge process (after Tchobanoglous, 2003)

Coefficient	Unit	Value Range	Typical
k	g bsCOD/g VSS*d	2-10.	5
Ks	mg/L BOD	25-100	60
	mg//LbsCOD	10-60.	40
Y	mg VSS/mg BOD	.4-0.8	0.6
	mg VSS/mg BOD	0.3-0.6	0.4
kd	g VSS/g VSS*d	0.06-0.15	0.1

Values reported are for 20 º C

2.2. Soluble Microbial Process

A study made by Barker et al. (1998) reveled important characteristic in the soluble microbial products that must be noted in this investigation. Soluble Microbial Products (SMP) have been found to comprise the majority of soluble organic material in the effluent from biological treatment processes. SMP exhibit several characteristics, such as toxicity and metal chelating properties, which affect the performance of the treatment system, and their presence has also been shown to adversely affect the kinetic activity and the flocculating and settling properties of sludge.

The term Soluble Microbial Product (SMP) has been adopted to defined the pool of organic compounds that are released into solution from substrate metabolism (usually with biomass growth) and biomass decay. The existence of residual microbial products produced by microbial cultures involved in wastewater treatment was demonstrated as early as 1961

(Graffney and Heukelekian, 1961). Since then many researchers have shown that the majority of the soluble organic matter in effluents from biological treatment processes is actually SMP. Traditionally, models of wastewater treatment system have been based on the Monod model which predicts that the effluent concentration of the rate limiting substrate should be independent of the influent substrate concentration. According to Barker and Stuckey (1999) this does not agree with observed measurements and the incorporation of SMP formation paved the way for more accurate modeling of wastewater treatment. The importance of SMP in all types of wastewater treatment is now fairly well accepted, but difficulties still occur in trying to measure SMP and draw conclusions when they are present in effluents from plants treating highly complex feeds. Hence, much of the work regarding SMP has been done on pure cultures and defined feeds. (Barker et al., 1998)

Boero el at. (1991) state that SMP result "from the intermediates or end products of substrate degradation and endogenous cell decomposition", whereas Noguera et al. (1994) define SMP "as the pool of organic compounds that result from substrate metabolism (usually with biomass growth) and biomass decay during the complete mineralisation of simple substrates" . Hence, they conclude that "for anaerobic systems intermediate compounds, such as volatile fatty acids, should be excluded from the definition of SMP because they are not of microbial origin". (Barker et al., 1998)

Chudoba (1985) classified the organic compounds produced by activated sludge micro-organisms into three categories:

1.- Compounds excreted by micro-organisms due to their interaction with the environment.

2.- Compound produced as a result of substrate metabolism and bacterial growth.

3.- Compounds released during the dissolution or destruction of cells (lysis) and degradation of micro-organisms.

According to Barker et al. (1998) microbiologists classify microbial product formation into three categories: growth-synonymous,; growth-associated, and growth-independent. Soluble Microbial Products fall into two different categories based on the bacterial phase from which they were derived:

1.- Utilization associated products (UAP). SMP that are associated with substrate metabolism and biomass growth and are produced at a rate proportional to the rate of substrate utilization.

2.- Biomass associated Products (BAP). SMP that are associated with biomass decay and are produced at a rate proportional to the concentration of biomass.

The most definitive list on the origin of SMP is provided by Kuo (1993). This author cites the following factors as causes of SMP production:

- Concentration equilibrium: organisms excrete soluble organic materials to establish a concentration equilibrium across the cell membrane.
- Starvation: bacteria excrete organic materials during starvation because they must obtain energy for maintenance by endogenous respiration or metabolism of cellular components when the substrate is essentially absent.

- Presence of energy source: the presence of an increased concentration of exogenous energy source can stimulate the excretion of SMP
- Substrate-accelerated death: sudden addition of a carbon and energy source to bacteria starved for carbon and energy may accelerate the death of some bacteria. SMP may be produced as a result of this process.
- Availability of required nutrient: if essential nutrients are present in very low concentrations, SMP may be produced to scavenge the required nutrient.
- Relieving environmental stress: SMP are produced in response to environmental stress, such as extreme temperature changes and osmotic shocks. The author also speculates that SMP are produced in response to toxic substances.
- Normal bacterial growth and metabolism: SMP, such as exocellular enzymes, are no only produced during stressed conditions but also during normal growth and metabolism.

In this context are presented some characteristics of Soluble Microbial Product of particular interest, such as molecular weight (MW) distribution, biodegradability and toxicity. MW distributions of organics in samples can be determined by either serial or parallel processing of samples through an array of pressurized stirred cells containing ultrafiltration membranes. The next characteristic, biodegradability of SMP, shows that over 90% of the residual COD measured in batch or continuous flow treatability studies is subject to biological degradation. Finally, toxicity in SMP may actually be created in the biological treatment process itself. In others words, SMP may actually be more toxic than the original organic compounds present in the wastewater. (Barker et al., 1998).

In addition to contributing to the BOD and COD of the effluent, SMP can have further implications on process performance, although the effect of high concentrations of these products is not yet fully known. The effects of various parameters, physical, chemical and biological influence the production of SMP. All the investigations regarding the effects of process parameters tend to point to the fact that a considerable decrease in the residual COD can be achieved through optimization of the biological treatment process itself, rather than by increasing the size of the treatment plant. This must be the preferable investment and higher operation and maintenance costs without necessarily lowering the effluent concentration.

Whether the substrate affects the quantity and type of SMP produced is closely linked to whether bacterial type affects the quantity of SMP. That effect depending on the bacterial species (and the dilution rate). The concentration of SMP ranged from 4 to 9 % of the initial substrate concentration (Lao, 1988).

Soluble Microbial Products are produced at a rate proportional to the concentration of the biomass due to the release of organic material from cell lysis. Hence, an accumulation of biomass in the system leads to an increased amount of SMP and this is why an increase in effluent COD is observed at high sludge ages (Hao and Lao, 1988). Also, the amount of this material increased with decreasing process temperature (Barker and Sutckey, 1999).

Pribyl et al. (1997) demonstrated that a continuous flow system with a completely mixed aeration tank produced consistently higher concentrations of SMP than a sequencing batch

reactor (SBR). However, Artan and Orhon (1989) modeling the effect of reactor hydraulics found that there was no practical difference between the performances of completely mixed and plug flow activated sludge system, because they produced similar amounts of microbial products under similar operating conditions.

2.3. Hydrolysis

A major fraction of organic material in municipal wastewater is in the particulate form and has to be hydrolyzed before it can be taken up and be degraded by bacteria (Levine et al.,1985). According to Morgenroth *et al.* (2002) hydrolysis refers to the breakdown of organic substrate into smaller products that can subsequently be taken up and degraded by bacteria. Two types of hydrolysis can be differentiated: a) Hydrolysis of primary substrate where organic substrate present in the original wastewater is broken down; b) Hydrolysis of secondary substrate that refers to the break-down of substrate that has been produced by the bacteria (e.g. hydrolysis of internal storage products, of substances released by the bacteria during normal metabolism, or of particles produced during decay of bacteria)

Henze (2000) expressed that particle size and particle composition determine rate and mechanism of hydrolysis and degradation in a wastewater treatment system. Strictly speaking, the definition of slowly biodegradable organic matter (Xs) as used in the activated sludge models is only indirectly related to particle size. Morgenroth et al. (2002) concluded that in mathematical models, the process of hydrolysis must be adequately described to be able to predict spatial and

temporal availability of organic substrate for nutrient removal process (denitrification and biological phosphorus removal).

According to Okutman *et al*. (2001) there is a general consensus on the necessity to differentiate between readily and slowly biodegradable substrates, in domestic sewage. The slowly biodegradable portion constitutes the bulk and it is broken down to soluble, readily biodegradable compounds by hydrolysis. The products of this mechanism can be used for the biosynthesis of heterotrophic biomass. The hydrolysis process involves regulation of extracellular enzyme synthesis in the cells. It takes place by enzymes secreted by the cells before the substrate can be taken up by the microorganism and being metabolized.

The rate of hydrolysis is much slower than that of the utilization of the soluble substrate it generates. It is commonly described by means of a surface limited-type of a reaction kinetics (Henze el al. 1987).

$$\frac{\mathrm{d}X_S}{\mathrm{d}t} = -k_h \frac{X_S / X_H}{K_X + X_S / X_H} X_H \qquad (2.37)$$

Only a limited amount of experimental work has been carried out on the quantitative evaluation of the hydrolysis rate coefficients (Henze & Mladenovski 1991). Concerning the hydrolysis of particulate organics Eliosov et al. (1994) suggested the following equation for the kinetics of particulate organics degradation to describe the rate of active biomass growth on particulate substrate:

$$\frac{1}{X_a}\frac{dXa}{dt} = Y\frac{K_m X_s / X_v}{K_{f_s} + X_s / X_v} \qquad (2.38)$$

where

X_a = active biomass concentration, mg VSS1^{-1}

X_s = stored substrate concentration, mg 1^{-1}

X_v = MLVSS concentration coefficient, mg 1^{-1}

K_m = maximum specific growth rate, day $^{-1}$

K_{fs} = half saturation coefficient

Y = yield coefficient

Equation 2.38 was later modified by Dold et al. (1980) by using the kinetics of surface limiting reactions:

$$\frac{1}{X_a}\frac{dX_a}{dt} = Y\frac{K_m X_s / X^n{}_a}{K_{fs} + X_s /{}^n{}_a} \qquad (2.39)$$

where:

n = coefficient, depending on cells volume to surface ratio

Equation 2.39, with the assumption that n = 1, was used by Henze et al. (1987). The yield coefficient in this equation is the ratio of the rate of biomass growth to the rate of stored substrate degradation. Since the stored substrate is the same as the degradable particulate organics and assuming that n = 1, Equation 2.39 can be rewritten as follows:

$$\frac{dX_{pd}}{dt} = \frac{K_m X_{pd}}{K_{fs} + X_{pd} / X_a} \qquad (2.40)$$

where:

X_{pd} = concentration of biodegradable particulate organics, mgVSS1^{-1}

Some investigators (e.g. Goronszy and Eckenfelder, 1991; Henze and Mladenovki, 1991) used the first order kinetics with respect to biodegradable particulate substrate utilization:

$$\frac{dX_{pd}}{dt} = -K'_p X_{pd} \qquad (2.41)$$

where:

K'_p = "first order" hydrolysis rate coefficient, day^{-1}

2.4. Role of Bioflocculation on the Removal of Particulate Organic Matter.

As mentioned before the total chemical oxygen demand (TCOD) can be defined as the sum of particulate COD (PCOD) and soluble COD present (DCOD). As presented by La Motta *et al*. (2003, 2004) the PCOD is made up of organic suspended solids and organic colloids present in the wastewater, and the dissolved COD is defined as the COD remaining after sweep flocculation of the sample with zinc hydroxide. The actual theories incorporated into the IWA/IAWQ consensus models indicate that the removal of particulate organics from the liquid in the activated sludge process is a two-step process, namely, rapid enmeshment of particles and hydrolysis followed by oxidation. However, observations made at the University of New Orleans experimental station indicate that flocculation of particulate organics and their subsequent separation by settling plays an important role in the removal of PCOD from wastewater (La Motta, *et al*., 2003, 2004)

Bioflocculation is the ability of microorganisms to self-associate in a suspended growth environment. Under normal operating conditions activated sludge flocculates naturally. This process occurs as a result of extracellular polymeric substance (EPS) secreted by microorganisms present in the mixed liquor (Das et al., 1993). The effect of EPS on bioflocculation has been largely studied and considerable efforts have been made to understand their role in biosolids-liquid separations in the activated sludge and solids contact processes (Liao et al., 2001). However, the precise role of EPS is not well understood, and contradictory studies have been presented in this matter. Indeed, while Chao and Keinath (1979) and Urbain et al. (1993) claimed that the settling properties of the sludge are enhanced when the EPS content in the sludge increases, Goodwin and Forster (1985) showed an opposite effect.

The most common way of describing the floc formation through polymer effects is polymer bridging (Busch and Stumm, 1968; Parker et al., 1970;; Urbain et al., 1993). Hogg (1999) described flocculation by means of polymer bridging as a dynamic process involving polymer adsorption, particle-to-particle collisions leading to floc formation and growth, and floc degradation in the presence of mechanical agitation.

2.4.1 Flocculation Models

Parker et al. (1970, 1971) proposed a rate expression describing the overall kinetic of flocculation in turbulent mixing. Their model expressed the net flocculation in a turbulent

environment as the balance of the opposing processes of aggregation and floc breakup. This model is presented next.

$$\frac{dn}{dt} = K_B \cdot X \cdot G^m - K_A \cdot X \cdot n \cdot G \qquad (2.42)$$

where X is the MLSS concentration (g/L), G the root-mean-square velocity gradient (s^{-1}), K_A a floc aggregation coefficient (L/g), K_B a floc breakup rate coefficient (number. S^{m-1}/g), *m* the floc breakup rate exponent (dimensionless), and n is the primary particle number concentration (particles /L).

Other researchers have supported the development presented by Parker and his co-workers, e.g. Wahlberg et al. (1994), Manrique (2000) and La Motta et al. (2003). Wahlberg et al. (1994) presented an integrated form of Equation 2.42 for the calculation of flocculation in a batch flocculator:

$$n_t = \frac{K_B \cdot G}{K_A} + \left(n_o - \frac{K_B \cdot G}{K_A} \right) \cdot e^{-K_A \cdot X \cdot G \cdot t} \qquad (2.43)$$

where n_o is the initial concentration of primary particles (numbers/L) and n_t is the primary particle number concentration in the reactor at time t. Wahlberg et al. (1994) tested Equation 2.6.2 with activated sludge samples obtained at different 21 full-scale facilities. Their study presents that maximum removal of suspended solids by flocculation was achieved within 10 minutes under batch conditions in most cases. They expressed that a similar performance

improvement could be obtained in the field using a completely-mixed flocculation zone with a residence time of at least 20 minutes

La Motta et al. (2003, 2004) used equations similar to Equation 2.42 and 2.43 to evaluate the removal of SS and Particulate COD (PCOD) in continuous flow and batch flocculators. For a batch reactor, operated a constant G, they presented:

$$C = a + (C_O - a) \cdot e^{-k \cdot t \cdot X} \tag{2.44}$$

where C (mg/L) is the concentration of unflocculated particles remaining in the supernatant at reaction time t (min) after 30 minutes settling, a is the residual concentration of particles (mg/L), k is the reaction rate coefficient, C_O is the initial concentration of influent particles (mg/L), and X is the MLSS concentration (mg/L).

For a continuous flow mixed reactor (CFSTR), operated at constant G, La Motta et al. (2003, 2004) presented the following relationship:

$$C = \frac{C_i \cdot (1+\alpha) + a \cdot k \cdot \bar{t} \cdot X}{(1+\alpha) + k \cdot \bar{t} \cdot X} \tag{2.45}$$

here α is the recycle ratio (recycle flow rate/plant flow rate) and C_i is the concentration of unflocculated particles concentration in the influent to the CFSTR.

$$C_i = \frac{C_o + \alpha \cdot C_R}{1 + \alpha} \tag{2.46}$$

where C_R is the concentration of particles of the recycle sludge after 30 minutes of sedimentation. According to La Motta et al. (2003) C_i should be measured by mixing the influent to the aeration chamber and the recycle sludge in proportion to Q and αQ, respectively, and by measuring the suspended solids concentration of the supernatant of the mixture after 30 minutes of settling.

In a pilot plant study using a CFSTR, La Motta *et al*. (2003, 2004) found that significant removal of suspended solids could be achieved at low detention times. They found that less than 30 mg/L could be obtained with hydraulic retention times (HRT) as short as 10 minutes, and 88% removal could be achieved during 30 minutes of flocculation.

CHAPTER III

3. EXPERIMENTAL PHASE

Batch reactors have been widely used to determine the biodegradation kinetics of specific organic compounds by activated sludge (Ellis and Eliosov, 2004). Chudoba *et al*. (1992) showed that the initial substrate-total biomass (So/Xo) ratio plays an important role in batch cultivations, and the biokinetic parameters evaluated at high and low So/Xo ratios can differ significantly. According to Grady *et al.* (1992) batch kinetics tests can be classified as intrinsic (high So/Xo ratio) and extant (low So/Xo ratio). In this research several batch tests experiment were performed at different soluble-substrate-biomass ratios in order to evaluate its impact on the kinetics and the order of the reaction. Similarly the consumption of dissolved substrate was compared using two different bacterial suspensions, the first one under normal conditions, and the second one with a deflocculating agent added in order to evaluate the effect of internal diffusion in the bacterial flocs on the removal of readily biodegradable soluble substrates.

In this research biomass from a complete mixed activated sludge (CMAS) system was tested in sequencing batch reactors (SBR). The CMAS system used is an experimental pilot plant located at the full scale Marrero Wastewater Treatment Plant (WWTP). The batch reactors were installed at the environmental laboratory located at the Research and Technology Park of the University of New Orleans, New Orleans, LA. The next section presents a description of the experimental pilot plant, the lab experiment and

techniques used in this research, a description of the SBRs, and the series of bench-scale experiment performed in order to reach the proposed objectives.

3.1 Pilot Plant Description

As mentioned before, the pilot plant is located at the Marrero WWTP, Marrero, Louisiana, which is a 34,000-m^3/d (9 MGD) trickling filter/solids contact (TF/SC) process that treats mainly domestic sewage. The pilot plant was built independently by other researchers in the team to simulate the TF/SC process existing at the Marrero wastewater treatment plant.

The activated sludge pilot plant contains the following components: a rotating screen, a trickling filter, an inlet mechanism, an aeration tank, and finally, a secondary clarifier. The unit was designed for a flow rate of 7.5 m^3/d (2000 gal/d) and a hydraulic retention time (HRT) in the solids contact chamber (SCC) that can vary between 15 and 120 minutes. Figure 4.1 shows a sketch of the units. Also Pictures 1 and 2 in Appendix A show an overview of the pilot plant.

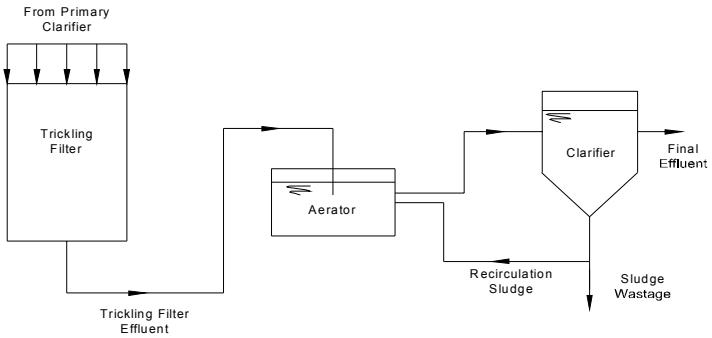

Figure 3.1 Pilot plant diagram

It is important to notice that during the development of the experiments in this research, the pilot plant was operated bypassing the trickling filter, i.e, the wastewater was fed directly from the rotating screen to the aeration tank. During the data gathering-process the HRT in the aeration tank varied between 30 and 50 minutes, and the sludge retention (SRT) time varied between 0.71 and 1.34.

3.2 Laboratory Experiments and Techniques

Four parameters were measured: total and dissolved COD (TCOD and DCOD), and total and volatile suspended solids (TSS and VSS). The next sections provide a short description of the lab procedures.

3.2.1 Total and Dissolved Chemical Oxygen Demand

The chemical oxygen demand (COD) is used as a measure of the oxygen equivalent of the organic matter content of a sample. The COD test was selected to estimate the concentration of organic matter in the liquid, because it is much faster than BOD test.

Retana (1999) developed a correlation between BOD and COD using a similar wastewater to the one used in this research. He collected samples at the three different Jefferson Parish West Bank WWTPs namely Marrero, Harvey, and Bridge City. This correlation is:

$$BOD_{(mg/l)} = 0.56\ COD_{(mg/l)} - 2.77 \qquad (3.1)$$

The COD values are, in general, higher than BODs because more compounds can be chemically oxidized that can be biochemical oxidized. To perform the COD tests was used Method 5220C of the Standard Methods (APHA, 1998).

To perform the dissolved COD samples were flocculated chemically by adding, to a 100 ml wastewater sample, 1 ml of zinc sulfate solution, and sodium hydroxide solution until it reaches a pH higher than 10.5. The new solution (wastewater sample, Zn and NaOH) were mixing vigorously with a magnetic stirrer of 20 mm long for approximately one to two minutes. After few minutes, the sample was allowed to settle; very carefully 25 ml were taken of the supernatant with a pipette and then passed through a Hach No. 30 glass qualitative filter paper with a pore size of 0.45 μm using vacuum filtration. The dissolved COD of the sample was defined to be the COD of the supernatant filtrate. APHA (1995) section 5220B.

3.2.2 Total and Volatile Suspended Solids

The TSS test is used to measure and quantify the amount of solids suspended in a specific sample. Method 2540 D of Standard Methods (APHA, 1998) was used to perform the Total Suspended Solid (TSS) tests. After filtration, the solids remaining in the 0.45-μm pore size filter paper were dried at 103^{o} C $\pm$ 1^{o} C. Volatile suspended solids (VSS) were ignited at 550^{o} C as indicated in Method 2540 E of the Standard Methods *(APHA, 1998)*. The solids remaining represent the fixed fraction, and the volatile fraction

of the sample is represented by weight lost.

3.3 Description of the Batch Reactors

The batch reactors are 11.45 x 11.45 cm (4.5 x 4.5 in) square beakers with a total capacity of 2000 ml. Five square Phipps & Bird's B-KER jars with a sampling port located at 10 cm settling-distance level were used in the experiments. This beaker jars are molded of strong, durable 0.635 cm (¼ inch) thick acrylic; their square shape provides thorough mixing action, and the valve-controlled port allows the gentle withdrawal of the sample without the need of raising the paddles of the jar mixer. A six-paddle Phipps & Bird jar stirrer was used to provide mixing in the reactors. This lab instrument is equipped with six stainless steel rectangular flat-blades of 2.54 x 7.62 cm (1.0 x 3.0 in) that rotate in a horizontal plane about the centerline of their length. These paddles are spaced six inches apart and are adjustable to a maximum depth of nine inches. The bottoms of the paddles, during the tests, were placed approximately 5 cm (1.97 in) above the bottom of the jars. The tester uses a reliable electronic motor control system that offers regulated variable speeds of all paddles simultaneously, from 1 - 300 rpm, with the exact speed clearly displayed on a digital readout. The speed used for these experiments was 37 rpm.

One of the five reactors operated without oxygen as a control unit. The initial conditions of each analysis were measured in this reactor. Oxygen was distributed to the other four reactors through a 3.2 mm (1/8 in) diameter plastic clear air line tubing using air diffusers. The 3.81 cm (1.5 in) long (with adapter) and 1.524 cm (0.6 in) diameter porous diffusers were placed at the bottom of each reactor to maintain complete-mixed conditions and an adequate oxygen level in the reactor to ensure aerobic conditions.

During the development of the experiment the dissolved oxygen (DO) was measured using a fisher scientific accumet ab40 oxygen meter. The pipes and the diffuser were arranged so that similar DO level were measured in the four reactors; typical DO measured values ranged between 2.5 and 6 mg/L. Figure 4.2 shows and overview of the batch reactors and the air supply system.

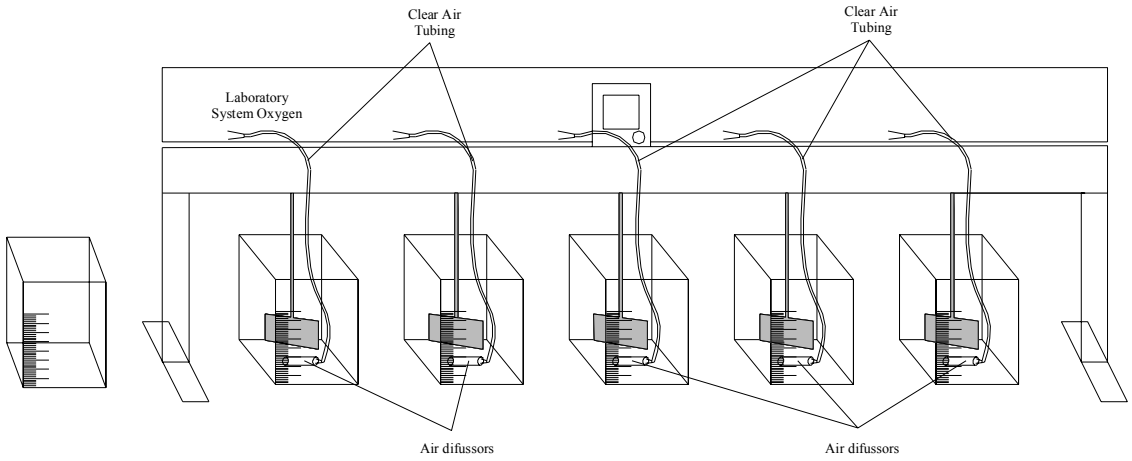

Figure 3.2. Batch Reactors

Activated sludge samples were collected from the SCC at the Marrero pilot plant, and transported to the environmental lab in less than 40 minutes. Once at the lab these samples were analyzed for TSS and VSS. To run the batch experiments the activated sludge samples from Marrero (ASSM) samples were diluted with an artificial wastewater. The artificial wastewater was prepared by mixing dechlorinated water with a known concentration of a soluble substrate. Sodium acetate and methanol were used as two different source of carbon to simulate the dissolved substrate on the wastewater. The dechlorinated water was prepared by filtering tap water through a sand filter and an activated carbon filter. About 5 liters of working solution were

prepared for each experiment (about 1000 ml were poured in each batch reactor) by mixing different amount of artificial wastewater and ASSM according the desired concentration in the working solution, and the respective concentrations in the artificial wastewater and ASSM. The total COD and the TSS of each sample were measure before each analysis, the COD values of the artificial wastewater were between 250 and 450, and the TSS values of the ASSM varied between 3500 and 6500 mg/L. The initial TCOD and DCOD in the working solution varied between 200 and 600 mg/L, and the initial TSS varied between 300 and 3000 mg/L. The four reactors were mixed mechanically with a velocity gradient (G) of about 40.

3.4 Steps used in the batch experiments.

The following steps were used in each batch experiment. The artificial wastewater was prepared with dechlorinated tap water, and sodium acetate or methanol. Then, the total and dissolved COD of the solution were measured. These CODs should be the same but in the sodium acetate case it was variable. This mean that not all the concentration of sodium acetate was dissolved, it had colloidal particles too. For this reason the dissolved substrate solution was prepared with dechlorinated tap water and methanol.

1. The values of TSS and VSS in the ASSM were measured.
2. With the known values of TCOD of the artificial water and TSS of the sludge, the volume of sludge to get a desired TSS or DCOD concentration in the batch reactor was calculated using the following relationships:

$$V_{(ASSM)} = \frac{TSS(total) x V(total)}{TSS_{(ASSM)}} \qquad (3.2)$$

$$V_{(methanol)} = \frac{DCOD_{(total)} x V_{(total)}}{DCOD_{(methanol)}} \quad (3.3)$$

where:

V= Volume

TSS = Total suspended solids

DCOD = Dissolved chemical oxygen demand

This relationship shows the amount of sludge and the artificial wastewater that should be used to prepare the working solution.

3. 1000 ml of the working solution were gently poured into each beaker. The stirrer was placed at a velocity of 37 rpm.
4. The first beaker, which was not placed under the stirrer, was used to determine the initial conditions of the working solution (TCOD, DCOD, TSS and VSS). TSS and VSS were measured immediately after the mixture was prepared. Total and Dissolved COD were measured after 30 minutes of settling. This reactor is referred as time-zero.
5. The other four reactors were operated at different reactant time in the presence of oxygen. Each reactor had a different time, typically 5, 10, 15 and 20 minutes respectively; however, in some experiment the time was extended. Once the time was expired, the reactor was removed from the stirrer, and TSS and VSS samples were collected. Total and Dissolved COD samples were collected after 30 minutes of settling.

6. DO levels and pH were measured in a regular basis during the development of the experiments. It was not necessary to control the pH because the variations in the ASSM and in the working solution were in an acceptable range, i.e., 6.25 to 7.8.

The same procedure was used when the deflocculating agent was added, the only difference was that before placing the working solution in the biker, the solution was stirred with the deflocculant for 5 minutes.

3.4.1 Removal of Dissolved Substrate using Sequencing Batch Reactors at Different So/Xo ratios.

The main objective of this experiment is to identify and understand the dissolved substrate removal kinetics in suspended growth reactors. The sludge samples used for this experiment were collected from the aeration basin of the pilot plant. The sludge sample was mixed with the substrate used (sodium acetate or methanol as was the case) and poured very carefully into batch reactors. The operational conditions of the batch reactors are detailed in Table 3.1

Table 3.1 Operational Conditions of the batch reactors.

Parameters	Batch Reactor No. 1	Batch Reactor No. 2	Batch Reactor No. 3	Batch Reactor No. 4
Reaction Time (min)	5 5 5 5 5	10 10 10 20 10	15 15 20 30 30	20 30 40 60 60
TSS sludge (mg/L)	300 - 500 800 2000 - 3500 6000	300 - 500 800 2000 - 3500 6000	300 - 500 800 2000 - 3500 6000	300 - 500 800 2000 - 3500 6000
TCOD sustrate (mg/L)	250 - 400	250 - 400	250 - 400	250 - 400
G (s -1)	37 - 40	37 - 40	37 - 40	37 - 40

The working solution (sludge + substrate) was mixed in the Phipps & Bird Jar Tester, using a velocity gradient (G) between 37 and 40. The reactor at time 0 was not mixed neither oxygen was applied. In this reactor was measured the initial condition of the working solution. The other four reactors were placed in the stirrer with the working solution, and oxygen was supplied. TSS and Total and Dissolved COD were measured according to the procedure previously described. TSS was measured to evaluate the biomass growth, and the TCOD and DCOD were measured to determine the consumption of the oxygen dissolved at different intervals of time, and therefore to evaluate the effect of HRT on the removal of readily soluble substrates. As indicated in Table 3.1 the experiments were performed with different soluble-substrate-biomass ratios in order to evaluate its impact on the kinetics and the order of the reaction.

3.4.2 Chemical De- Flocculation Test

This experiment was designed to evaluate the effect of internal diffusion and bacterial flocs in the removal of readily soluble substrates. The sludge used for this experiment was collected from the aeration tank of the activated sludge pilot plant. The working solution was prepared based on the TSS concentration of the ASSM, and TCOD concentration of the artificial wastewater. Sodium acetate and methanol were used as carbon source. A cation exchange resin (CER) was used a deflocculating agent. According to Frolund et al. (1996) the resin removes divalent cations from the sludge matrix, and produces the destruction of the floc structure. The CER selected was a DOWEX 50 x 8, 20 - 50 mesh in the sodium form manufactured by J.T. Baker Chemical Co. The CER was added according to the dosification proposed by Frolund et al. (1996). This mixture was agitated with a magnetic stirrer at velocity of 50 rpm for five minutes. After agitation, the mixture with the CER was poured into the 2 L reactors and placed in the jar tester, at 35-40 rpm with oxygen provide by the laboratory system. Samples of approximately 100 mL were taken at 5, 10, 15, 20 minutes for dissolved COD analysis.

CHAPTER IV

4. ANALYSIS AND DISCUSSION OF RESULTS

4.1 Evaluation of substrate removal kinetic for flocculant suspension at different So/Xo ratios

Lawrence and McCarty (1970) related the rate of substrate utilization to the concentration of microorganism in the reactor and to the concentration of the remaining substrate. They proposed an equation mathematically analogous to the Michaelis_Menten equation for enzyme kinetics and to the classical Monod equation describing the effect of substrate concentration on the growth rate coefficient. This Equation was presented in Chapter 2, and is recapitulated below:

$$r_{su} = -\frac{kXS}{K_s + \mathrm{S}} \qquad (2.2)$$

This model which is a mixed order model is perhaps the most wide used model for describing the rate of substrate utilization. Such model includes two kinetic coefficients, i.e, "k" (maximum specific substrate utilization rate) and "K_s"(half-velocity constant). In this section the model proposed by Equation 2.2 is applied to three different data sets with different initial substrate to biomass ratios that are called low, medium and high So/Xo ratios with values for the initial ratios equal to 0.08, 0.28 and 0.96 respectively (this is an arbitrarily classification).

This experiment was designed to test the variation of the kinetic constants and the order of the reaction with different So/Xo ratios. Pitter and Chudoba (1990), Chudoba *et al*. (1992), and Grady et al. (1996) expressed that the outcome of the batch experiment may be influenced by the initial substrate (S_o) to biomass (X_o) ratio. According to Chudoba *et al*. (1992) kinetic parameters evaluated at high and low So/Xo ratios might differ significantly.

Table 4.1 presents the data gathered the 12 of July, 2004. For the batch test, a flocculent suspension-biomass sample was collected from contact chamber of the pilot plant at Marrero, and brought to the environmental lab within 40 minutes. This sample presented a VSS value of 6013 mg VSS/L, and it was diluted using a solution prepared with a dissolved substrate and dechlorinated tap water. The mixture of sludge and substrate had an initial concentration of 1250 mg VSS/L and the COD initial value (time 0) was 344 DCOD mg/L yielding an initial So/Xo relationship equal to 0.28. The substrate used was methanol with a COD value of 500 DCOD mg/L. The Jar Tester was used to analyze the variations of the volatile suspended solids (X) and the DCOD (Se). Five batch reactors where placed with approximately 1L of the mixture and oxygen provided by the lab system; the mixture was agitated at 37 rpm. DO and pH were monitored during the experiment, the pH varied in an acceptable 7.2 to 7.6 range, and the DO was controlled at 4.0 mg/L on each reactor. Approximately 200 ml samples were taken at different time intervals, and the TSS and dissolved COD were measured for each sample. This information is summarized in Table 4.1.

Table 4.1. Batch Experiment, So/Xo= 0.28 (July 12, 2004)

Unit	Time (min)	So (DCOD, mg/L)	Se (DCOD, mg/L)	X (mg VSS/L)	q (min^{-1})
1	0	344	344	1250.00	
2	5	344	123	1320.00	0.033484848
3	10	344	40	1337.00	0.012415856
4	15	344	20	1325.00	0.003018868
5	30	344	10	1280.00	0.000520833

Table 4.1 present the values of the specific substrate removal rate (q) which is calculated using the next relationship:

$$q = \frac{r_{su}}{X} \approx \frac{S_0 - S_e}{X\ (\Delta t)} \qquad (4.1)$$

where X is the biomass concentration as mgVSS/L, and *t* is the reaction-aerated time in the batch reactor. Figure 4.1 presents the removal of the dissolved substrate and the evolution of the biomass with respect to the reaction time; a 97% dissolved substrate removal was reached in the experiment after 30 minutes of reaction time and 30 minutes of settling. Slightly growth in the biomass can be observed in Figure 4.1.

Figure 4.2 shows the relationship between Se and the specific substrate removal rate (q). This curve apparently follows a first-order reaction with respect to q; the evaluation of the first-order kinetics is presented in a later section.

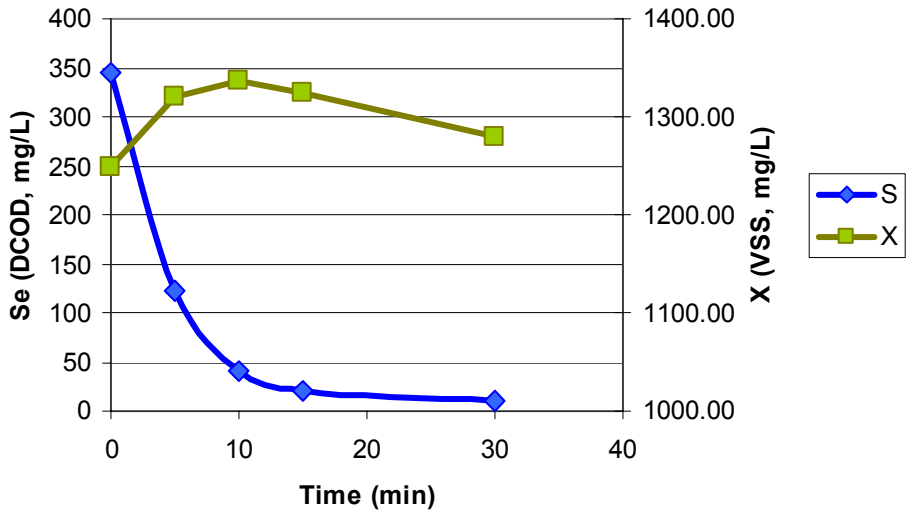

Figure 4.1 Dissolved substrate removal and biomass evolution (July 12, 2004)

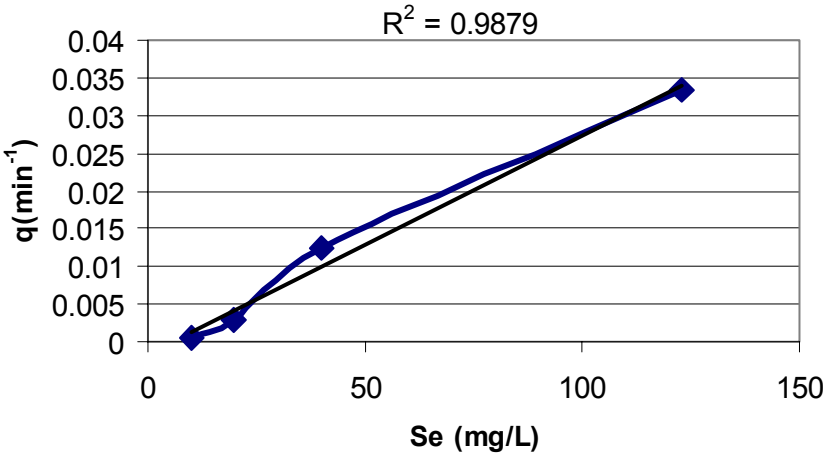

Figure 4.2 Se vs. the specific substrate removal rate (July 12, 2004)

In Figure 4.2, a very good correlation is shown between a straight line and the points, with a coefficient of determination, R^2 of 0.99. The rate of the reaction for the specific substrate removal rate can be expressed as:

$$q = \frac{r_{su}}{X} = KS_e + a \qquad (4.2)$$

where *K* and a are first order constants.

The order of the reaction for the rate of utilization of soluble substrates was compared for a zero-order reaction (see Figure 4.3a), for a first-order reaction with respect to r_{su} (see Figure 4.3b), for a second-order reaction (see Figure 4.3c), and for the mixed-saturation type reaction described by Equation 2.2 (see Figure 4.4). Appendix B provides a description of the theoretical basement for Figure 4.4.

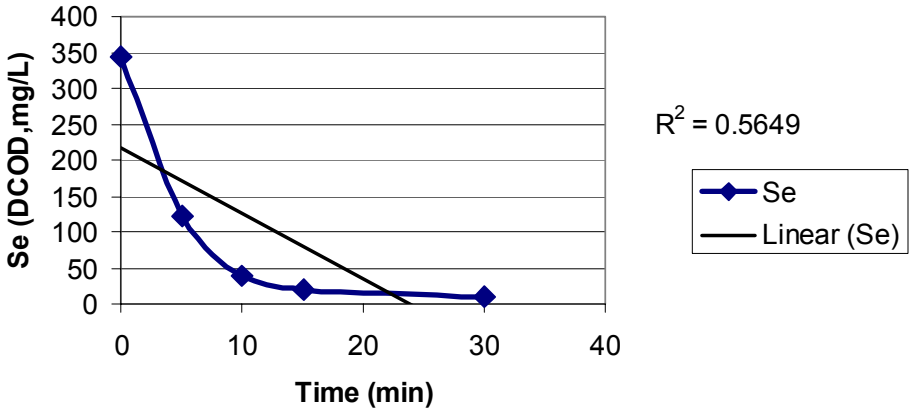

Figure 4.3a Se vs Time, So/Xo= 0.28 (July 12, 2004)

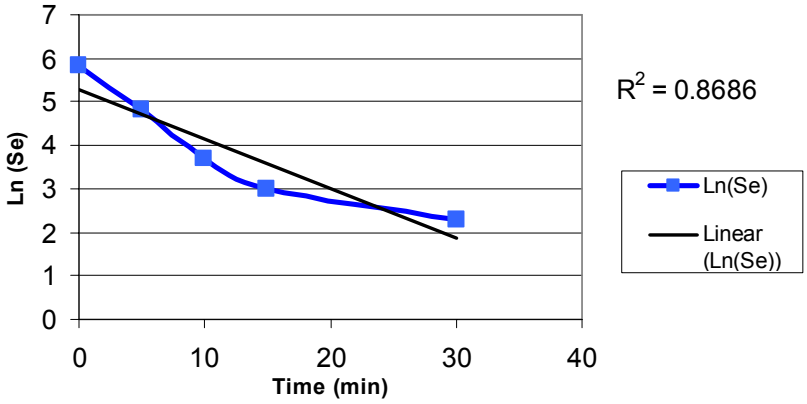

Figure 4.3b Ln(Se) vs Time, So/Xo= 0.28 (July 12, 2004)

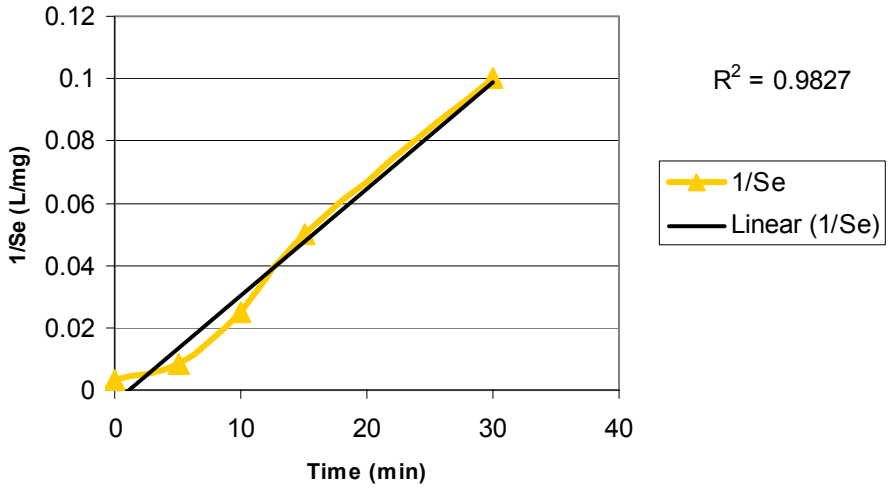

Figure 4.3c 1/Se vs Time, So/Xo= 0.28 (July 12, 2004)

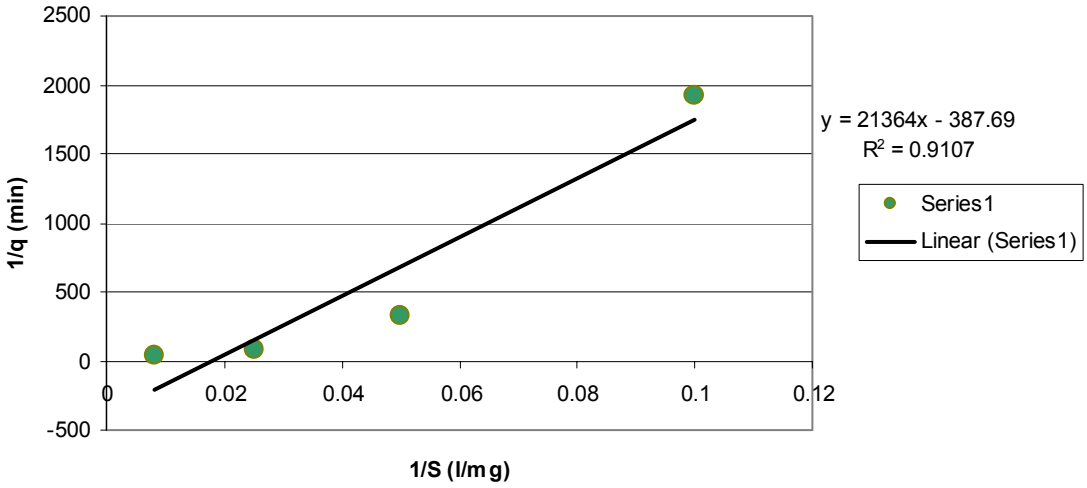

Figure 4.4 1/q vs. 1/Se

From the evaluation of Figures 4.2, 4.3 and 4.4 it can be concluded that for the data set presented in Table 4.1 the order of the reaction for the rate of utilization of soluble substrates is better described for a first order reaction as the one presented in Equation 4.2.

The values of the kinetic constant "k" (maximum specific substrate utilization rate) and "K_s" (half-velocity constant) can not be determined from Figure 4.4, because the intercept with the y-axis gave a negative value (see Appendix B). The only conclusion that can be determined from Figure 4.4 regarding the kinetic coefficients is that the reaction proceeded with a very high value for the maximum specific substrate utilization rate.

The values of "Y" (synthesis yield coefficient) and "k_d" (endogenous decay coefficient) are determined plotting the specific growth rate (μ) versus the specific substrate utilization rate (q) according to the procedure presented in Appendix B. Figure 4.5 shows the relationship between the two rates. The value of μ is approximate with the following equation:

$$\mu = \frac{r_g}{X} \approx \frac{X_0 - Xt}{\overline{X}(t)} \qquad (4.3)$$

where Xt is the biomass concentration as mg VSS/L at time t, $\overline{X}$ is the average biomass, and t is the HRT in the batch reactor.

The value of synthesis yield coefficient (Y) correspond to the value of the slope in Figure 4.5, i.e, Y = 0.289; and the value of the endogenous decay coefficient (k_d) corresponds to the negative intercept with the ordinate, i.e., k_d = - 0.0021 min^{-1}. It is important to notice that the values of the decay coefficient using this procedure are extremely sensitive to the variability of the points, and therefore it is difficult to obtain a reliable value, in fact a negative value of k_d is meaningless.

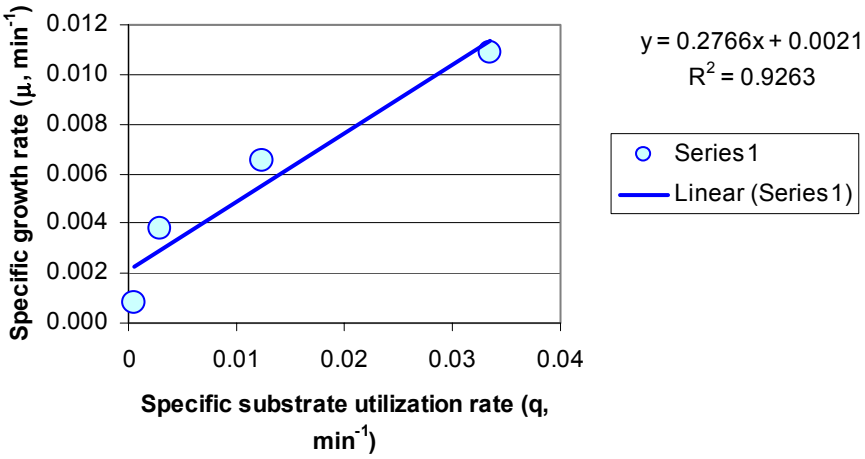

Figure 4.5 Plot of specific growth rate with specific substrate utilization rate

Table 4.2 presents the data gathered on July 15, 2004. Similar to the previous sample the working solution was prepared with fresh sludge collected at the Marrero pilot plant and artificial wastewater prepared with methanol as a single carbon source. The mixture of sludge and substrate had an initial concentration of 3100 mg VSS/L and the COD initial value (time 0) was 250 DCOD mg/L yielding an low initial So/Xo relationship equal to 0.08. Batch experiments were conducted according to the procedure presented in Chapter 3.

Table 4.2. Batch Experiment, So/Xo= 0.08 (July 15, 2004)

Unit	Time (min)	So (DCOD, mg/L)	Se (DCOD, mg/L)	X (mg VSS/L)	q (min^{-1})
1	0	250	250	3100.00	
2	2.5	250	113	3125.00	0.017536
3	10	250	40	3125.00	0.003114667
4	15	250	20	3100.00	0.001290323
5	30	250	1	2900.00	0.000436782

Figure 4.6 shows the removal of the dissolved substrate and the evolution of the biomass with respect to the residence time for the data presented in Table 4.2. In this cases the dissolved substrate was completely depleted after 30 minutes of residence time giving a 100% removal efficiency. The biomass growth was negligible in the reactor, and started decreasing in concentration after 20 minutes.

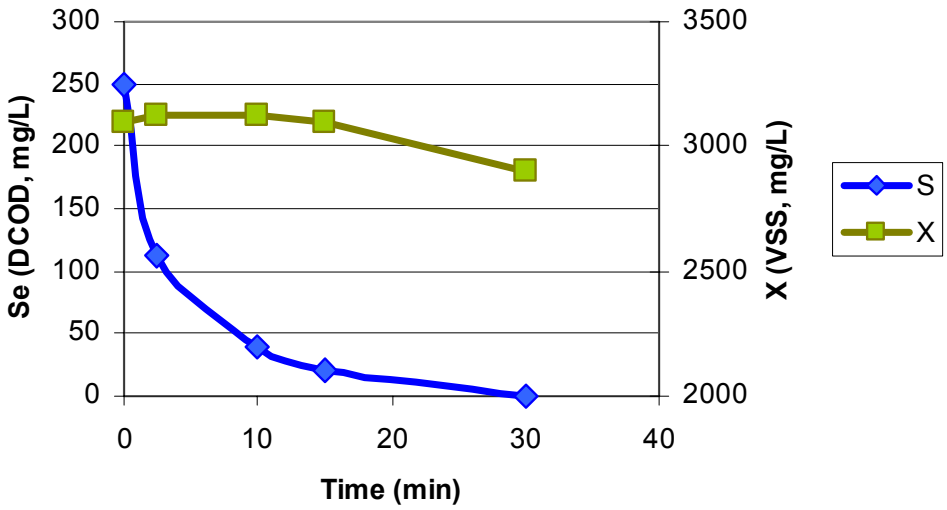

Figure 4.6 Dissolved substrate removal and biomass evolution, So/Xo = 0.08 (July 15, 2004)

Figure 4.7 shows the relationship between Se and the specific substrate removal rate (q) for the data presented in Table 4.2. Like in the previous case, the data follow a first order reaction with respect to q. A good correlation is shown between a straight line and the points, with a coefficient of determination, R^2 of 0.96. The rate of the reaction for the specific substrate removal rate can be represented with a first-order relationship as the one presented in Equation 4.3.

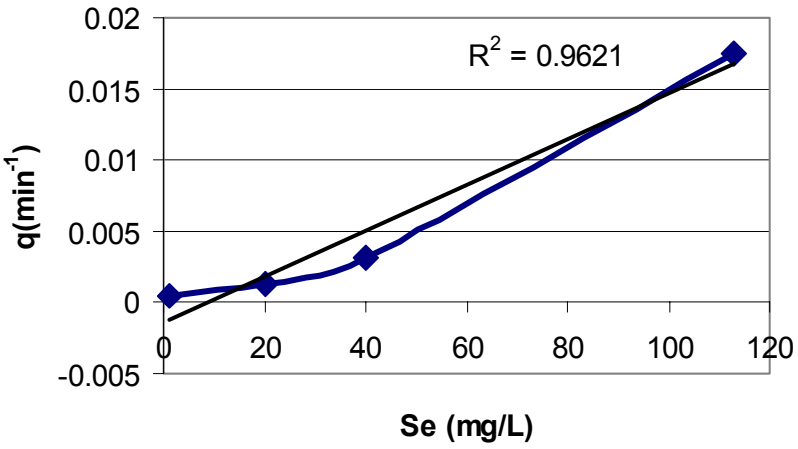

Figure 4.7 Se vs. the specific substrate removal rate, So/Xo= 0.08 (July 12, 2004)

Similarly to the previous data set, the order of the reaction for the rate of utilization of soluble substrates was compared for a zero-order reaction (see Figure 4.8a), for a first-order reaction with respect to r_{su} (see Figure 4.8b), for a second-order reaction (see Figure 4.8c).

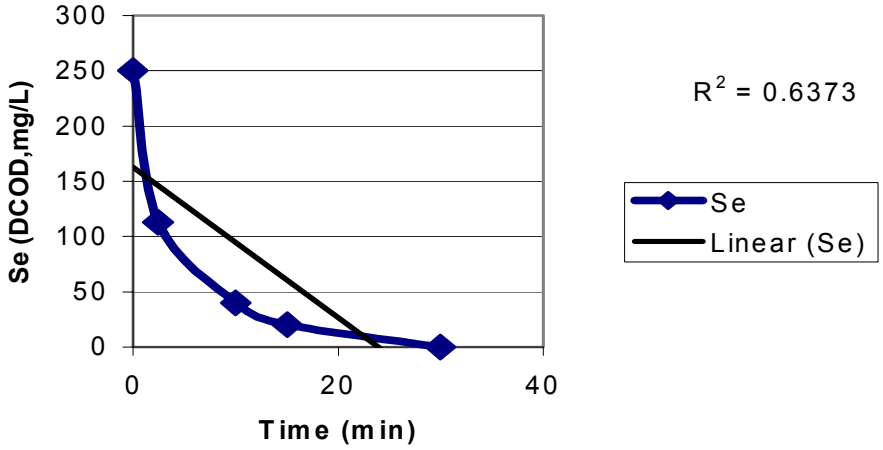

Figure 4.8a Se vs Time, So/Xo= 0.08 (July 15, 2004)

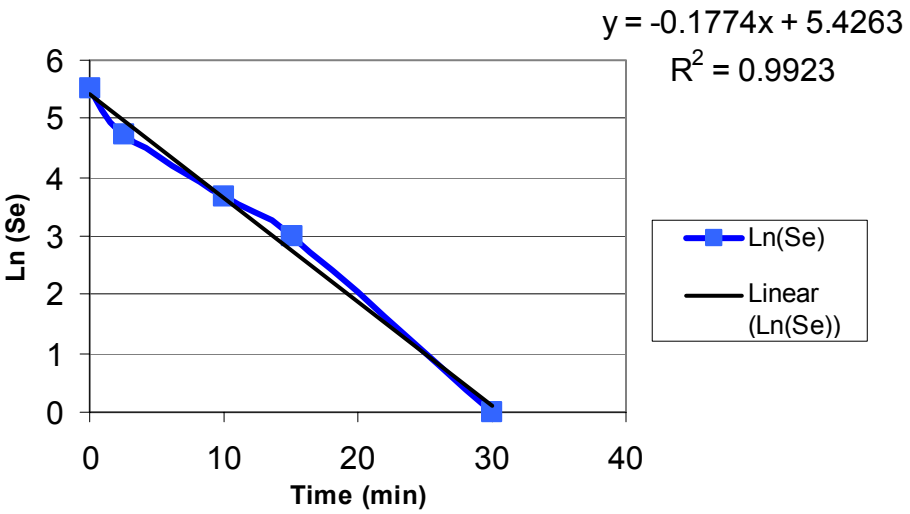

Figure 4.8b Ln(Se) vs Time, So/Xo= 0.08 (July 15, 2004)

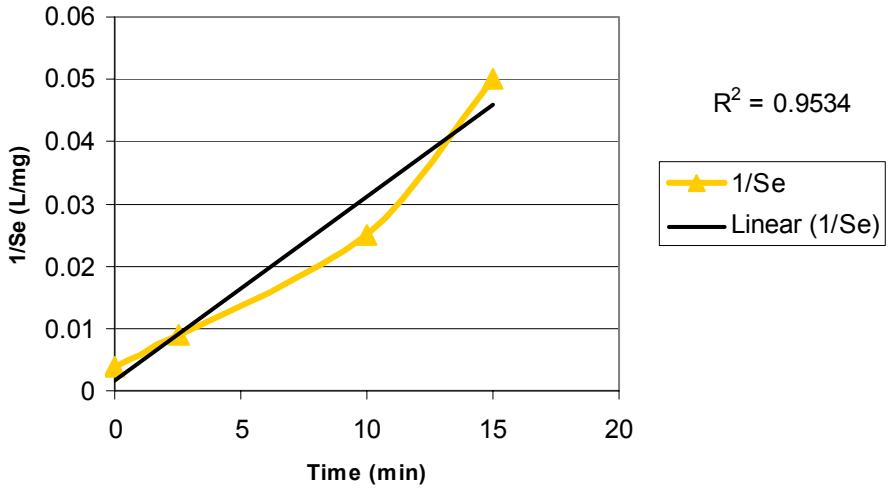

Figure 4.8c 1/Se vs Time, So/Xo= 0.08 (July 15, 2004)

It can be concluded from Figure 4.8 that the rate of utilization of soluble substrates very close follows a first-order kinetic for the case of a low So/Xo relationship. This result was expected according to the results obtained in Figure 4.7 with the specific substrate removal rate since the biomass growth was negligible during the experiment. A very good correlation is

observed in Figure 4.8b with a coefficient of determination, R^2 of 0.99. A more detailed study on the determination of first-order kinetic coefficients for the data set presented in Table 4.1 and 4.2 is presented in the next section.

Even though it was observed that the data gathered at low So/Xo ratio can be accurately described for a first order relationship, the data presented in Table 4.2 was analyzed and compared with the mixed-saturation type reaction described by Equation 2.2 according to the procedure describe in Appendix B. Figure 4.9 shows the relationship between the inverse values of the dissolved substrates and the specific substrate removal rate.

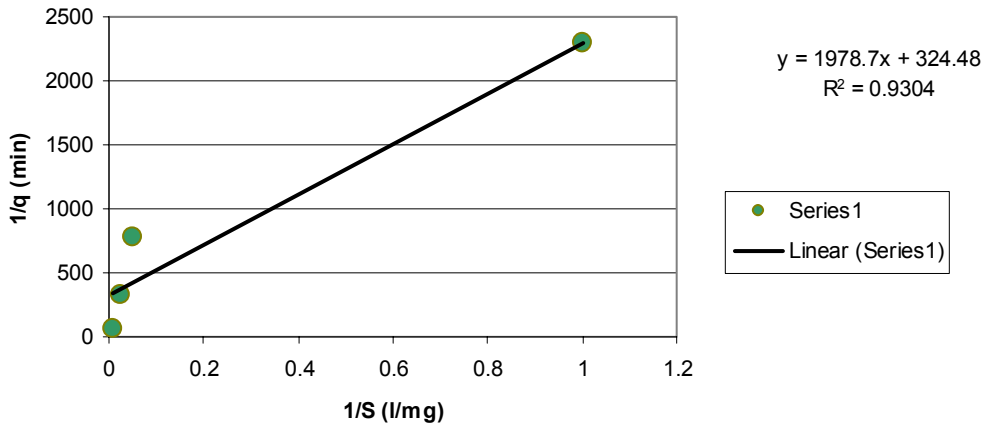

Figure 4.9 1/q vs. 1/Se for So/Xo= 0.08, (July 15, 2004)

The values of the kinetic constant "k" (maximum specific substrate utilization rate) and "K_s" (half-velocity constant) for the low So/Xo ratio are determined from Figure 4.9.

$1/k = 324.48$ min$= 0.2253$ d$\rightarrow$ $k = 4.45$ d^{-1}

$K_s/k = 1978.7$ min mg/L $\rightarrow$ $K_s = 6.1$ mg/L

As previously shown in Figure 4.5 the values of "Y" (synthesis yield coefficient) and "k_d" (endogenous decay coefficient) are determined plotting the specific growth rate (μ) versus the specific substrate utilization rate (q). Figure 4.10 shows the relationship between the two rates for the Low So/Xo ratio.

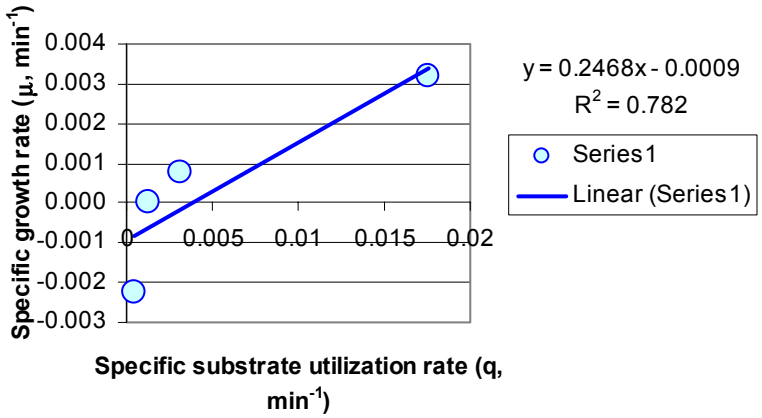

Figure 4.10 Plot of specific growth rate with specific substrate utilization rate, So/Xo= 0.08

Compared with the values obtained for the medium So/Xo ratio, i.e, So/Xo = 0.28, the value f the synthesis yield coefficient (Y) for the case of the low So/Xo ratio is lower. In this case a value of Y = 0.25 is obtained from Figure 4.10. The value of the endogenous decay coefficient (k_d) corresponds to the negative intercept with the ordinate, i.e., k_d = - 0.001 min^{-1}.

So far the kinetic removal of dissolved substrate have been evaluated for a medium and a low So/Xo ratio, i.e., 0.28 and 0.08 respectively. Now the same procedure presented for those ratios is going to be presented for a relatively high So/Xo. Table 4.3 presents the information for a So/Xo ratio equal to 0.96, in this cases the mixture of sludge and substrate had initial concentrations of 260 mg VSS/L and 250 mg DCOD/L. Like in the previous cases the artificial

wastewater was prepared with methanol as carbon source. Table 4.3 shows the information of the supernantat DCOD (Se) and the biomass concentration (X) after 5, 10, 30 and 60 minutes of reaction time in the aerated-batch reactor. The values of the specific substrate utilization rate are also presented in this table.

Table 4.3. Batch Experiment, So/Xo= 0.96 (June 30, 2004)

Unit	Time (min)	So (DCOD, mg/L)	Se (DCOD, mg/L)	X (mg VSS/L)	q (min^{-1})
1	0	250	250	260.00	
2	5	250	185	300.00	0.043333333
3	10	250	125	340.00	0.035294118
4	30	250	40	400.00	0.010625
5	60	250	15	380.00	0.002192982

Figure 4.11shows the removal of the dissolved substrate and the evolution of the biomass with respect to the residence time for the data presented in Table 4.3. In this cases the remaining dissolved substrate was still present in the solution even after 60 minutes of HRT. At 30 minutes the removal efficiency of the systems was only 84% compared to the 97% and 100% obtained in the previous cases. A considerably increase in the biomass is observed in Figure 4.11, after 30 minutes the biomass has increased a 54% which is very large compared with the increase in biomass obtained for the case with the medium and the low So/Xo ratios that were 7% and 1% respectively.

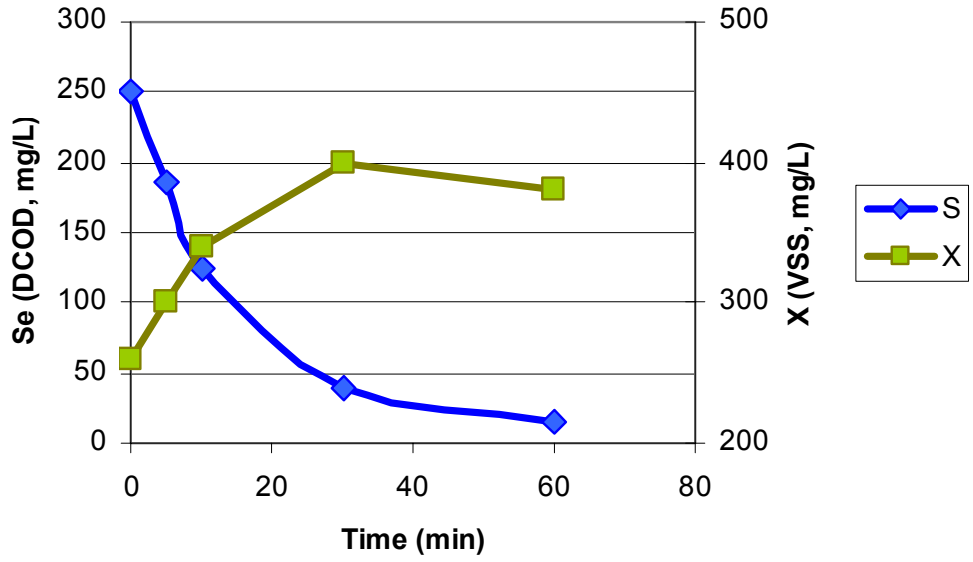

Figure 4.11 Dissolved substrate removal and biomass evolution, So/Xo = 0.96 (June 30, 2004)

Figure 4.12 shows the relationship between Se and the specific substrate removal rate (q). Like in the previous cases, the Figure shows a good correlation between a straight line and the points. However, the tendency of the curve seems to approach a saturation or mixed-order relationship. The curve seems to approach a zero-order kinetics a high values of S, and a firs-order kinetics a low values. T

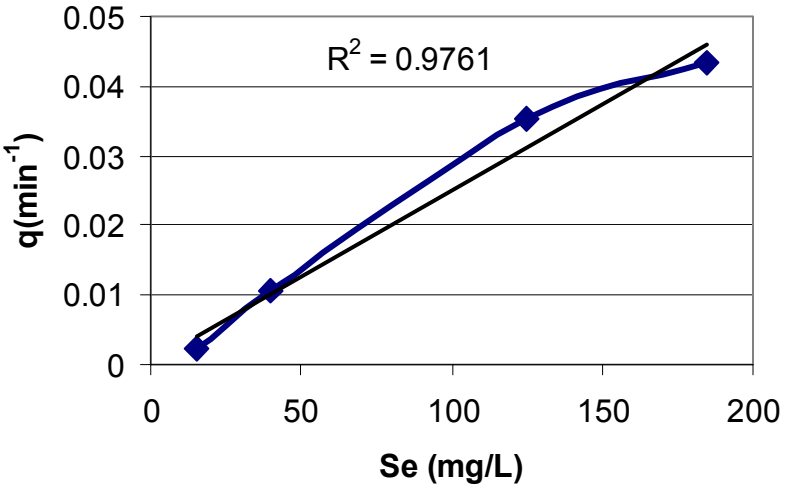

Figure 4.12 Se vs. the specific substrate removal rate (June 30, 2004)

As previously done the order of the reaction for the rate of utilization of soluble substrates was compared for a zero-order reaction (see Figure 4.13a), for a first-order reaction with respect to r_{su} (see Figure 4.13b), for a second-order reaction (see Figure 4.13c), and for the mixed-saturation type reaction described by Equation 2.2 (see Figure 4.14).

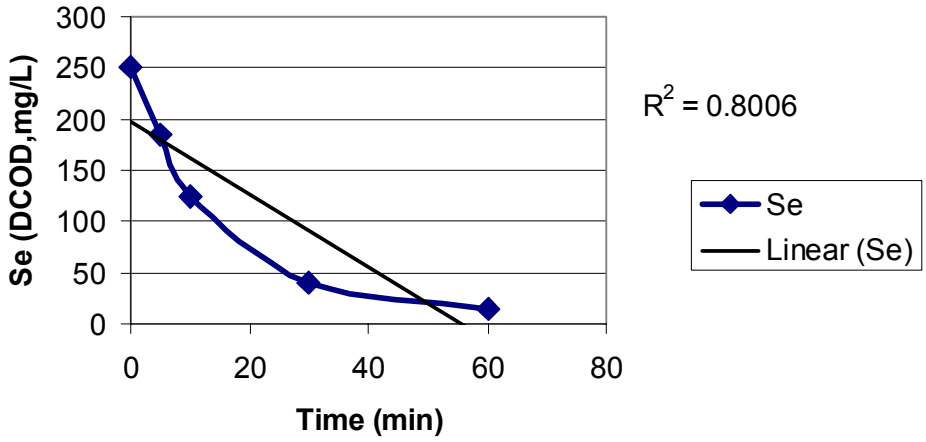

Figure 4.13a Se vs Time, So/Xo= 0.96 (June 30, 2004)

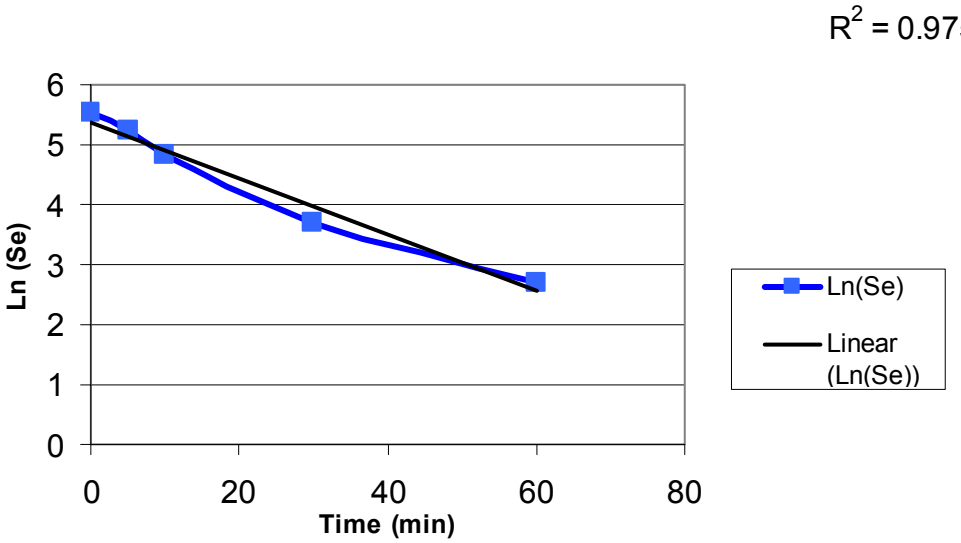

Figure 4.13b Ln(Se) vs Time, So/Xo= 0.96 (June 30, 2004)

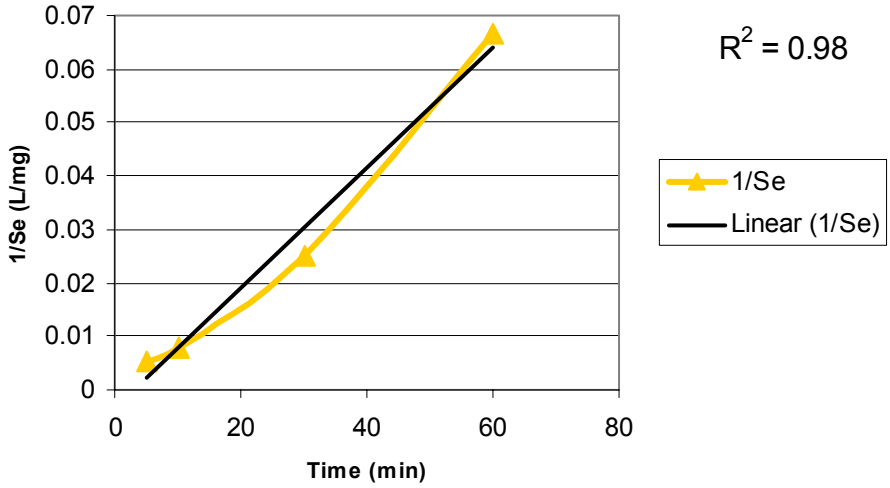

Figure 4.13c 1/Se vs Time, So/Xo= 0.96 (June 30, 2004)

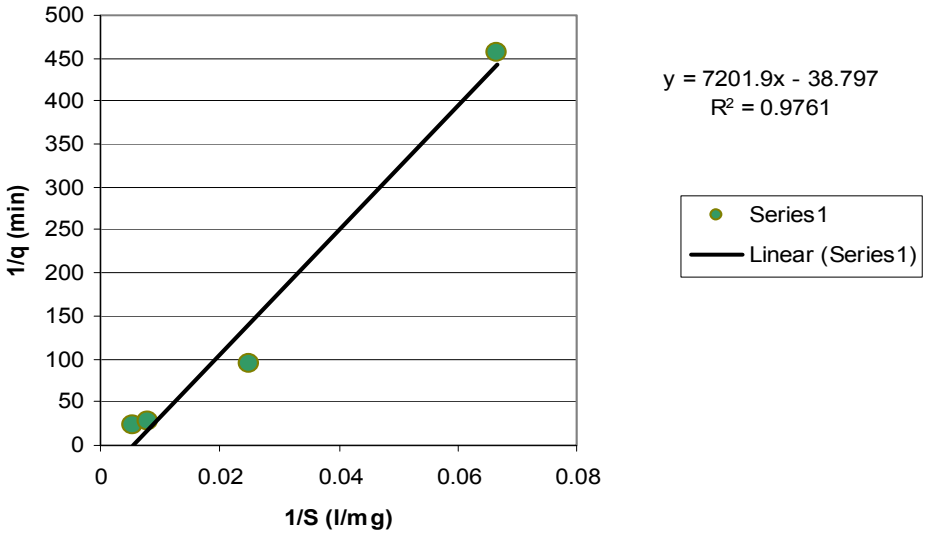

Figure 4.14 1/q vs. 1/Se, So/Xo= 0.96 (June 30, 2004)

Based on the R^2 obtained with Figures 4.13 and 4.14 it can be concluded that the order of the reaction for the rate of utilization of soluble substrates can be accurately described for a mixed-order relationship as the one proposed by Equation 2.2. or by a second-order reaction.

Even though the data seems to follow a mixed-order kinetics, the values of the kinetic constant "k" (maximum specific substrate utilization rate) and "K_s" (half-velocity constant) can not be determined from Figure 4.14, because the intercept with the y-axis gave a negative value (see Appendix B).

The values of "Y" (synthesis yield coefficient) and "k_d" (endogenous decay coefficient) are determined plotting the specific growth rate (μ) versus the specific substrate utilization rate (q). Figure 4.15 shows the relationship between the two rates.

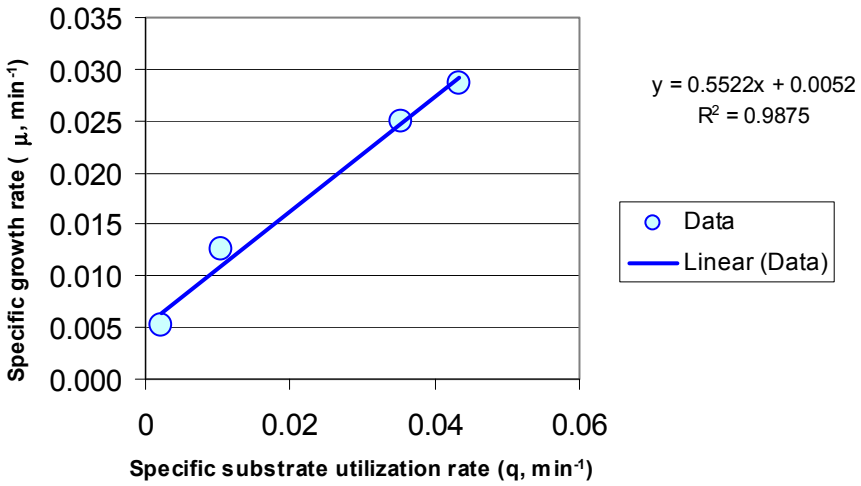

Figure 4.15 Plot of specific growth rate with specific substrate utilization rate, So/Xo= 0.96 (June 30, 2004)

According to the slope of the fitted straight line in Figure 4.15 the value of synthesis yield coefficient (Y) for the high So/Xo ratio is equal to 0.55; and the value of the endogenous decay coefficient (k_d) corresponds to the negative intercept with the ordinate, i.e., k_d = - 0.005 min^{-1}, which is meaningless. The value of the yield coefficient is higher than the values obtained in the previous two cases. It means that in the case of the high initial substrate to biomass ratio more biomass is produced per mass of substrate utilized and the process is more efficient in converting substrate to biomass.

4.1.1 Kinetic Constant Variability at Different So/Xo ratios.

Lawrence and McCarty (1970) related the rate of substrate utilization to the concentration of microorganism in the reactor and to the concentration of the remaining substrate. They proposed an equation mathematically analogous to the Michaelis_Menten equation for enzyme kinetics and to the classical Monod equation describing the effect of substrate concentration on the growth rate coefficient (See Equation 2.2). This model which is a mixed order model is perhaps the most wide used model for describing the rate of substrate utilization. Such model includes two kinetic coefficients, i.e, "k" (maximum specific substrate utilization rate) and "K_s"(half-velocity constant). In the previous section the mixed-order model proposed by Lawrence and McCarty (1970) was applied to three different data sets with different initial substrate to biomass ratios that were called low, medium and high So/Xo ratios with values for the initial ratios equal to 0.08, 0.28 and 0.96 respectively. The value of synthesis yield coefficient (Y) and the value of the endogenous decay coefficient (k_d) were also found for three different data sets with different initial substrate to biomass ratios. The determination of the kinetic coefficients for the mixed-order model was unsuccessful, and the values of the maximum specific substrate utilization rate and the half-velocity constant only could be found in the case of the low Xo/So ratio. The fact that these kinetic constant could not be found is an indication that the data sets are not well described for mixed-order kinetics as the one proposed by Lawrence and McCarty.

As indicated before, the values of synthesis yield coefficient (Y) and the value of the endogenous decay coefficient (k_d) were also found for three different data sets with different initial substrate to biomass ratios. The values obtained for the decay coefficient are meaningless

since they are extremely sensitive to the variability of the points, and therefore it was difficult to obtain a reliable value. Table 4.4 summarizes the values of the yield coefficient found for the three rations, Figure 4.16 shows the relationship between Y and So/Xo.

Table 4.4 Kinetic Coefficients Y and k_d at different So/Xo ratios

	So/Xo		
	0.96	0.28	0.08
Y	0.57	0.39	0.32

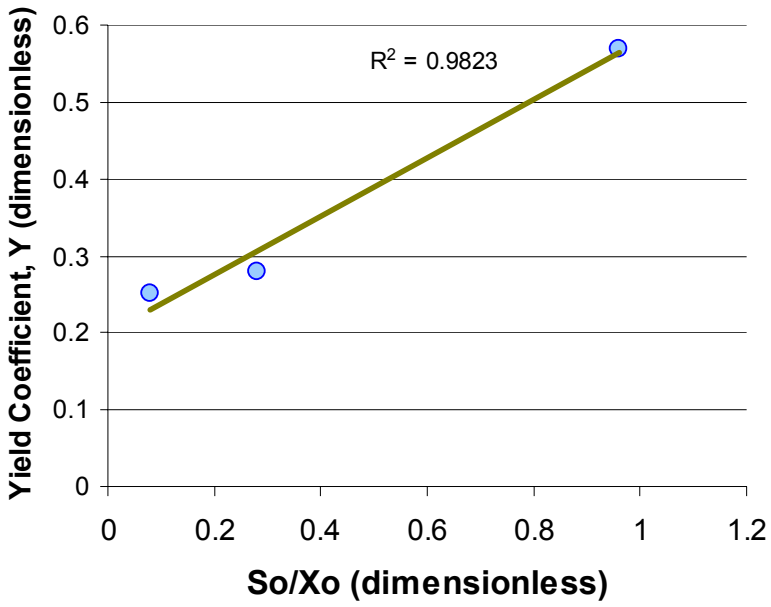

Figure 4.16 synthesis yield coefficient (Y) vs. So/Xo

Figure 4.16 shows an excellent correlation between the yield coefficient and the initial biomass to substrate ratio for the case of the methanol as single carbon source. This Figure presents a directly proportional relationship between Y and So/Xo, Y increases as So/Xo increases.

According to Benefield and Randall (1980) from the total substrate utilized for microoganism there is a fraction of the substrate which is channeled into the synthesis function, i.e, provides the building blocks for cell growth, and there is a fraction of the substrate which is channeled into the energy function. Furthermore, the fraction utilized for energy can be subdivide into energy utilized for synthesis and energy utilized in maintenance. The relationship between metabolism and substrate removed can be expressed mathematically as

$$\Delta S_{removed} = \Delta S_{\substack{synthesis \\ and\ energy \\ for\ synthesis}} + \Delta S_{\substack{energy\ of \\ ma\,\mathrm{int}\,enance}} \qquad (4.4)$$

Since the Yield coefficients represent the efficiency of conversion of a substrate to either products or biomass, the low Y is an indication that more substrate is used in energy for maintenance that in cellular synthesis. Figure 4.16 shows that when the substrate surrounding the biomass is low, the microorganism used the few substrate available mostly for the primary function of maintenance; as more substrate becomes available the microorganisms use this availability for promoting cell growth.

4.1.2 Dissolved Substrate Removal as a First Order Kinetic.

La Motta et al. (2003, 2004) proposed an first-order kinetic equation to express the concentration of dissolved COD remaining in the supernatant of the mixed liquor after time *t* in a batch reactor:

$$S_D = a_D + (S_{DO} - a_D)e^{-K_D tX} \qquad (4.5)$$

where S_D is the DCOD remaining in solution after time *t,* mg/L; a_D the non-biodegradable COD fraction which cannot be biodegraded by bacteria during the process, mg/L; K_D is first-order oxidation constant, L/ mg VSS x min; and *X* is the biomass concentration in mg VSS/L. In terms of the rate of substrate utilization r_{su} Equation 4.5 can be expressed as:

$$r_{su} = K_D(S_D - a_D)X \qquad (4.6)$$

Equation 4.6 is similar to Equation 4.3 proposed for the rate of the reaction for the specific substrate removal rate.

Equation 4.5 was fit to the experimental data presented in Tables 4.1, 4.2 and 4.3 using the Software "Data Fit™". A very good correlation was obtained for the cases of medium and low So/Xo ratios, i.e., Tables 4.1 and 4.2. The analysis did not work for the case with the high So/Xo ratio (Table 4.3) . In this case the program gave a floating point error; apparently due to the variability of the biomass concentration (*X*). Figure 4.17 shows the values of Se versus time for the data presented in Table 4.1, and the curve fitted with the software.

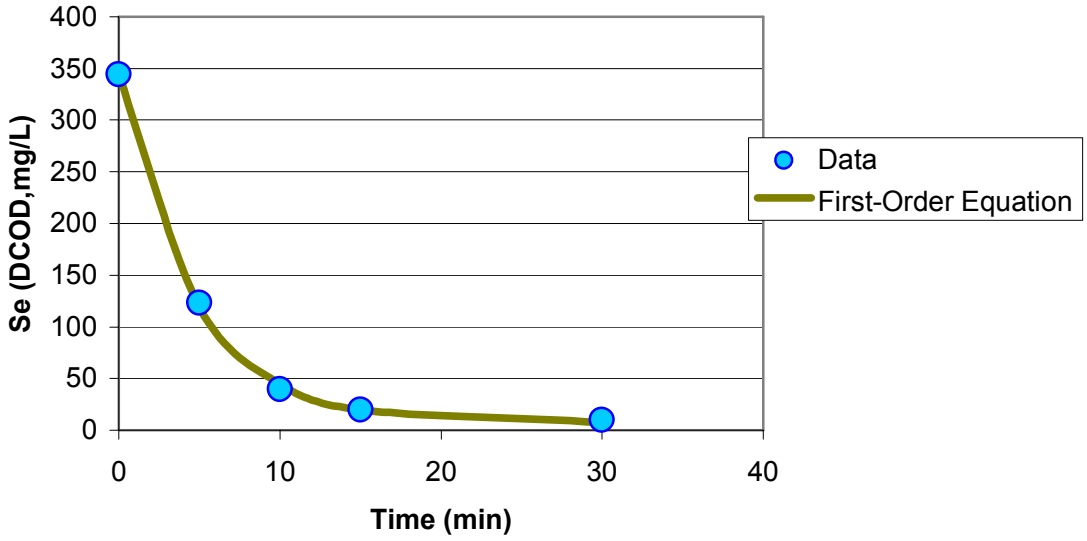

Figure 4.17 DCOD vs Time, medium So/Xo

The values obtained with the curve-fitting analysis are:

$R^2 = 0.9995$

$a_D = 7.375$ mg/L

$K_D = 1.7 \times 10^{-4}$ L/ mgVSS min

Since the biomass concentration was kept relatively constant during the batch experiment, Equation 4.5 can be reduced to Equation 4.7, which can be expressed as:

$$S_D = a_D + (S_{DO} - a_D)e^{-K_{Dx}t} \qquad (4.7)$$

where $K_{Dx} = K_D . X$, and has unit of min^{-1}. Assuming an average biomass concentration equal to 1300 mg/L, we obtain:

$$K_{Dx} = 0.22 \text{ min}^{-1}.$$

Figure 4.18 shows the values of Se versus time for the data presented in Table 4.2, and the curve fitted with the Data Fit™.

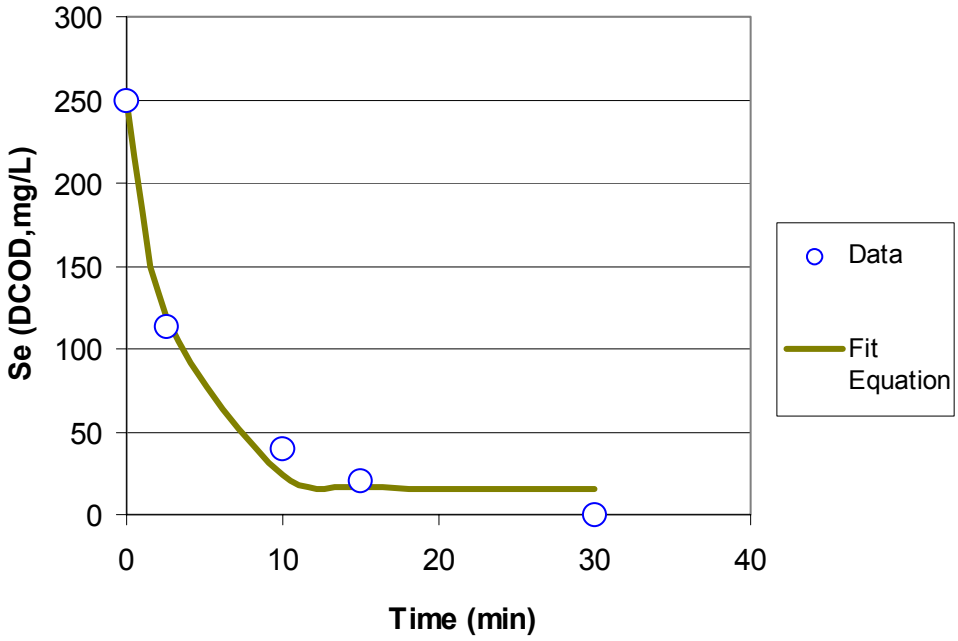

Figure 4.18 DCOD vs Time, Low So/Xo

The values obtained with the curve-fitting analysis are:

$R^2 = 0.9873$

$a_D = 15.17$ mg/L

$K_D = 1.1 \times 10^{-4}$ L/ mgVSS min

In this case the biomass growth in negligible and Equation 4.5 can be reduced to Equation 4.7. Using an average biomass concentration equal to 3000 mgVSS/L, we obtain:

$K_{Dx} = 0.33$ min^{-1}.

The values of the first-order kinetic coefficient, K_D, shows little variation between the experiment with low and medium So/Xo, and also show a good agreement with the values

presented by La Motta et al. (2003,2004) using methanol as a dissolved substrate. The value of the first order kinetic constant K_{Dx} agree with an earlier study presented by Balmat (1957) who evaluated the removal of soluble BOD who find first order coefficients between 0.17 and 0.39 min^{-1}. Moreover, the results obtained herein support the utilization of a first-order kinetic model for medium and low values of So/Xo.

4.2 Effect of Diffusion and Biological Oxidation inside the floc matrix in the Removal of Dissolved COD.

This experiment was designed to evaluate the effect of biological flocculation and internal diffusion in bacterial flocs in the kinetics of dissolved substrate consumption. Similar batch experiment were run in parallel using two different bacterial suspensions, the first one under normal conditions, and the second one with a deflocculating agent. A DOWEX 50 x 8, 20 - 50 mesh cation exchange resin (CER) was used as a deflocculating agent; this CER removes divalent cations from the sludge matrix destroying the biochemical bridges used by the EPS to flocculate the suspended and colloidal particles which became free in the suspension (Frolund et al. 1996).

In a similar study to the one proposed herein La Motta et al. (2003) found that flocculation itself does not have an important effect in the removal of DCOD, they found no difference between a flocculated and a deflocculated reactor when evaluating the kinetic of a dissolved substrate. On the other hand, Logan and Hunt (1988) argued that since bioflocculation is a microbial characteristic, there should be some advantage of growth within an aggregate; they

said that for a culture of microorganism to bioflocculate when substrate is nearly depleted implies that the cell associations may confer some advantage over freely dispersed cells relative to increasing substrate uptake by aggregated cells. Based on a theoretical analysis Logan and Hunt found that bioflocculation increases the rate of substrate transport to cells in permeable flocs compared to dispersed cells. However, they found that the permeability of the floc may define the direction of this relationship between flocculated and dispersed cells. Steiner et al. (1976) expressed that activated sludge flocs remove both colloidal matter and soluble BOD by adsorption. La Motta *et al*. (2003, 2004) clearly demonstrated the role of bioflocculation in the removal of particulate COD, and the polymers bridging theory supports the idea that bioflocculation is a mechanism for trapping organic particulates previously to hydrolysis. As expressed by Okutman et al. (2001) the rate of hydrolysis is much slower than that of the utilization of soluble readily biodegradable substrate. They results presented in the previous Section indicate that dissolved substrate is still present in the solution after 20 and 30 minutes of residence time when relatively high initial substrate concentration was used; moreover, La Motta *et al*. (2003, 2004) found remaining DCOD after 20 and 30 minutes of HRT in batch and continuous flow reactors when evaluating artificial and municipal wastewater, but at those times they found a good removal of particulate COD by bioflocculation. They point that the author is trying to answer is why the biomass produced polymers and promoted the aggregation by bioflocculation when there was still soluble COD in the suspension? As expressed by Logan and Hunt (1988): Is there any advantage of growth within an aggregate regarding the consumption of readily biodegradable substrate? The experiments presented herein were designed with the goal of providing an answer to these questions.

Table 4.5 presents the data gathered for the suspension with and without CER. For this experiment flocculated sludge was collected at the aerobic contact chamber and brought to the environmental lab within 40 minutes. Once at the laboratory the samples were analyzed following the procedures describe in Chapter 3. The working solution was prepared with the fresh sludge and artificial wastewater prepared with methanol as a single carbon source.

The mixture of sludge and substrate had an initial concentration of 1250 mg VSS/L and the COD initial value (time 0) was 344 mg DCOD /L yielding an initial So/Xo relationship equal to 0.28. The mixture with the CER, which was added according to the dosification proposed by Frolund et al. (1996), was agitated with a magnetic stirrer at velocity of 50 rpm for five minutes. After agitation the DCOD was again measured. Interestingly, the result for the initial DCOD for the deflocculated sample was 20 mg/L higher after the agitation process; this value is reported in Table 4.5 as So for the deflocculated sample. The increase in the DCOD may be due to the release of EPS and soluble microbial products (SMP) into the suspension after flock break up.

Table 4.5 DCOD at different HRT for flocculated and deflocculated samples for a Medium So/Xo ratio

Unit	Time (min)	So Flocculated (DCOD, mg/L)	Se Flocculated (DCOD, mg/L)	So Deflocculated (DCOD, mg/L)	Se Deflocculated (DCOD, mg/L)
1	0	344	344	364	364
2	5	344	123	364	104
3	10	344	40	364	38
4	15	344	20	364	15
5	30	344	10	364	14

Figure 4.19 shows the effect of the residence time in the supernatant DCOD for both the flocculated and the deflocculated sample. The statistical software Data Fit^{TM} was used to fit the first-order model with an asymptotic non-biodegradable COD fraction, in both cases excellent R^2, higher that 0.99, were obtained. This first-order model represented by Equations 4.6 and 4.7 was selected because it accurately predicted the data for the cases with medium and high So/Xo as the one presented in Table 4.5, and also for its simplicity. Equation 4.7 is used instead of Equation 4.5 because, as shown in Section 4.1 the biomass growth is negligible under medium and high So/Xo ratios.

For the flocculated sample the values obtained with the curve-fitting analysis are:

$R^2 = 0.999$

$a_D = 7.4$ mg/L

$K_{Dx} = 0.22$ min^{-1}.

For the deflocculated sample the values obtained with the curve-fitting analysis are:

$R^2 = 0.995$

$a_D = 14.1$ mg/L

$K_{Dx} = 0.28$ min^{-1}.

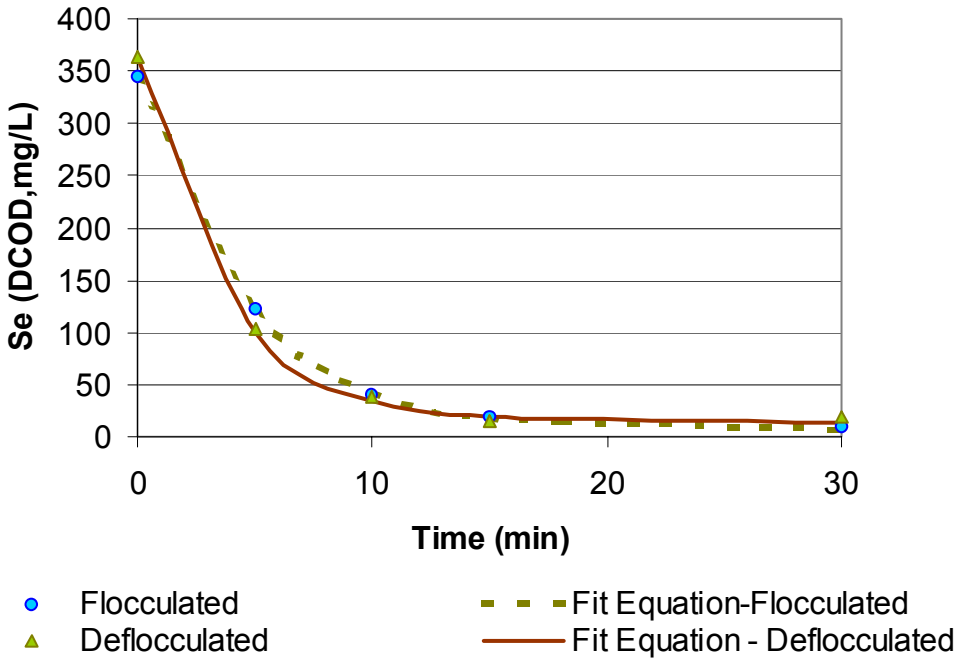

Figure 4.19 Effect of Bioflocculation on the Kinetics of DCOD Consumption for a Medium So/Xo ratio.

Both the first-order kinetic constant, K_{Dx}, and the asymptotic non-biodegradable COD coefficient, a_D, were higher for the deflocculated sample. The fact that the kinetic constant has a higher value is an indication that the dissolved COD consumption in the deflocculated sample, where there are freely dispersed cells, proceeds at a faster rate; this is and indication that bioflocculation results in a slight limitation in the amount of substrate that is supplied to the bacteria inside the flocs, which reduces the biodegradation rate. On the other hand, the fact that the asymptotic non-biodegradable COD coefficient is also higher for the deflocculated sample looks like a contradiction to the previous statement. However, if EPS were release as soluble substance after the CER was added, the high value of a_D would be an indication of low biodegradability for this type of substance. This result would agree with a study presented by

Pavoni et al. (1973) on the biodegradability of exocellular polymer substance, where they found a very low level of biodegradability for EPS. The high value of a_D may also be due to the release of SMP during the floc break up during the agitation of the sample with the CER. According to Gaudy and Blachly (1985) over 90% of the residual COD measured in batch reactor is subject to biological degradation; however, this residual COD experimented lower conversion rates than the original substrate used in its generation.

The experiment presented in the previous paragraphs was repeated using a lower So/Xo ratio. The new experiment was carried out following a similar procedure to the one described in the previous paragraphs, and in Chapter 3. The mixture of sludge and substrate had an initial concentration of 2300 mg VSS/L and the COD initial value (time 0) was 220 mg DCOD/L giving a So/Xo value equal to 0.10. After the agitation with CER the deflocculated sample presented a raise in the DCOD; triplicate values indicated that the DCOD was equal to 267 mg/L, 40 mg/L more than the value measured previous to agitation. The increase in the DCOD may be attribute to the release of EPS and SMP products into the suspension after flock break up. Table 4.6 presents the data gathered for the suspension with and without the cation exchange resin.

Table 4.6 DCOD at different HRT for flocculated and deflocculated sample for a Low So/Xo ratio

Unit	Time (min)	So Flocculated (DCOD, mg/L)	Se Flocculated (DCOD, mg/L)	So Deflocculated (DCOD, mg/L)	Se Deflocculated (DCOD, mg/L)
1	0	220	220	267	267
2	5	220	113	267	90
3	10	220	60	267	62
4	15	220	42	267	35
5	30	220	0	267	22

Figure 4.20 presents the kinetics of the DCOD for both cases, i.e, the flocculated and the deflocculated samples for the low So/Xo ratio.

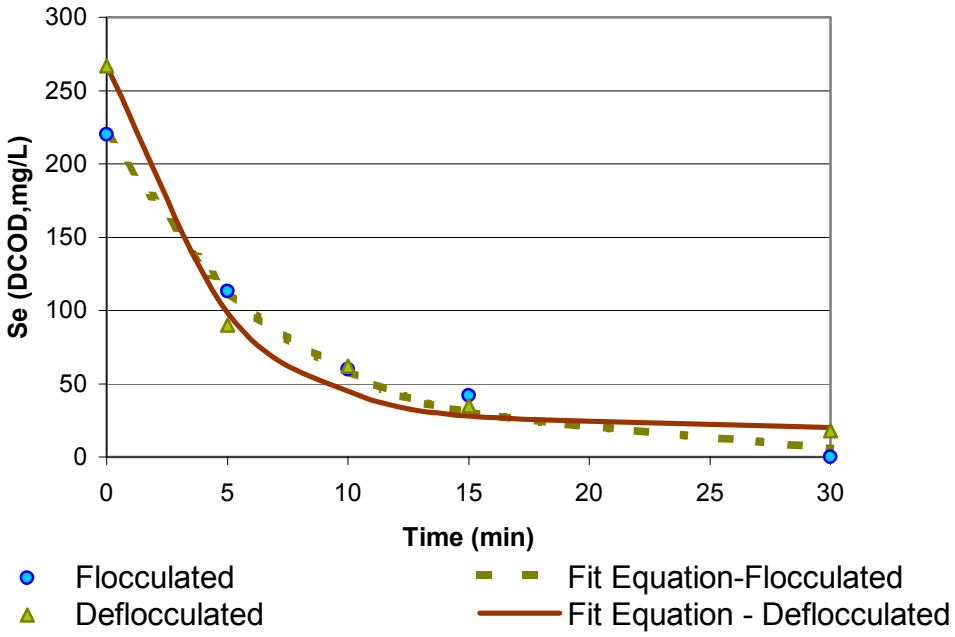

Figure 4.20 Effect of Bioflocculation on the Kinetics of DCOD Consumption for a Low So/Xo ratio.

Equation 4.7 was fitted to the data using the software Data Fit™, with very good correlation in both cases. The results of the fitting analysis are presented in Table 4.7

Table 4.7 First-order Kinetic Constant for Flocculated and Deflocculated Samples for a low So/Xo ratio.

	Flocculated	Deflocculated
R^2	0.987	0.989
a_D (mg/L)	2	20
K_{Dx}, min^{-1}	0.14	0.23

Like in the case for the medium So/Xo ratio, both the first-order kinetic constant, K_{Dx}, and the asymptotic non-biodegradable COD coefficient, a_D, were higher for the deflocculated sample, supporting the discussion presented previously.

Table 4.8 presents a summary of the first-order kinetic constants obtained with the flocculated and deflocculated samples.

Table 4.8 First-order Kinetic Constant.

	Flocculated			Deflocculated	
So/Xo	0.28	0.08	0.1	0.28	0.1
$K_{Dx} = K_{Dx} . X$ (min^{-1})	0.22	0.33	0.135	0.28	0.23
X (mg/L)	1300	3000	2292	1300	2292
K_D (L/mgVSS min)	1.7E-04	1.1E-04	5.9E-05	2.2E-04	1.0E-04

Figure 4.21 shows the relationship between the first-order kinetic constant K_D and the initial soluble substrate to biomass ratio. Even though the relationship is not completely well defined it can be said that the value of the kinetic constant tends to increase as So/Xo increases.

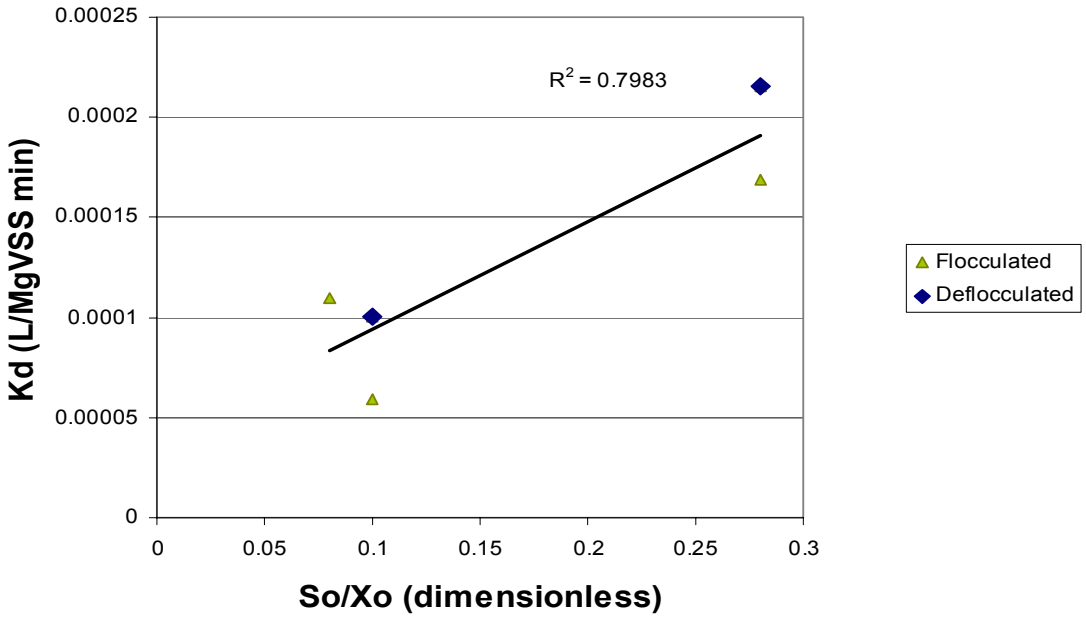

Figure 4.21 First-Order Kinetic Constant K_D versus So/Xo

Figure 4.22 shows the values obtain for $K_{Dx} = K_D . X$ for different So/Xo ratios. It can be concluded from this Figure that the value of K_{Dx} is independent from the initial soluble substrate to biomass ratio.

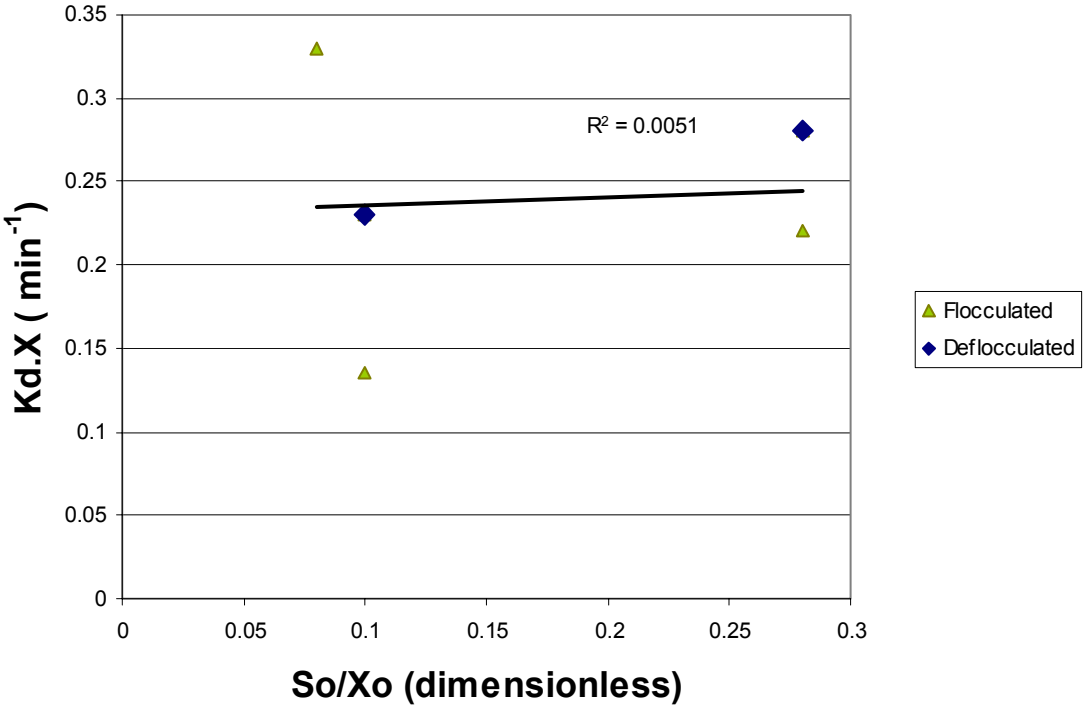

Figure 4.22 First-Order Kinetic Constant K_{Dx} versus So/Xo

CHAPTER V

5.1 CONCLUSIONS

The main purpose of this investigation was to study the kinetics of readily biodegradable soluble substrate simulated with methanol as a single carbon source and measured as dissolved chemical oxygen demand. The kinetics of soluble substrates was evaluated with different models and at different So/Xo ratios, and also using flocculated and dispersed cells suspensions. The specific conclusions that can be drawn from this research are:

- The removal of readily biodegradable soluble substrate at medium and low So/Xo rations (values less than 0.3) can be well described by a first-order kinetics with an asymptotic non-biodegradable portion as the one presented next:

$$r_{su} = -K_D(S_D - a_D)X$$

  Values of the product $K_D X$ varied between 0.14 and 0.33 min^{-1}. Most of the municipal wastewater presents a ratio So/Xo less than 0.3, and therefore the first-order rate expression presented herein for the removal of readily biodegradable soluble substrate can be used in conjunction with bioflocculation kinetics or hydrolysis kinetics for the simulation of complex dissolved-particulate substrates.

- For values of So/Xo less than 0.3 the growth of biomass is negligible, and thus can be neglected in the determination of the kinetics of soluble substrates.

- A directly proportional relationship was found between the yield coefficient, Y, and the So/Xo ratio. This is an indication that at low So/Xo ratio the cells are basically using the substrate for maintenance and not for growth. This is a contradiction of the classical Monod Equation which does not consider the fact that microorganism may need substrate even when they do not grow. As the So/Xo ratio increases the biomass uses more substrate for the synthesis of new cellular material.

- The value of the first-order kinetic constant K_D tends to increase as the value of the initial soluble substrate to biomass increases. However, the value of the product of K_D times the biomass concentration is independent of the So/Xo ratio.

- First-order kinetics can describe very well the consumption of readily biodegradable soluble substrate for both freely dispersed cells, and flocculated suspensions.

- The dissolved COD consumption for freely dispersed cells proceeds at a faster rate than for flocculated suspensions. This is an indication that the diffusion in the flocs results in a limitation in the amount of substrate that is supply to the bacterias that are inside the floc.

- The value of the first-order kinetic constant K_D tends to increase as the value of the initial soluble substrate to biomass increases. However, the value of the product of K_D times the biomass concentration is independent of the So/Xo ratio.

5.2 RECOMMENDATIONS

This study provides a better understanding of the kinetics of readily biodegradable soluble substrates and the effect of bioflocculation on such kinetics. However, in order to better understand such kinetics and its interaction with other kinetic process in the wastewater treatment, the following studies and ideas are recommended:

- The experiment presented in this study should be repeated using more complex dissolved substrates. If possible non-artificial soluble substrate should be used.

- Particulate and dissolved substrates should be combined in different proportions in order to evaluate the effects that such interaction have in the kinetics of the dissolved substrate.

- The deflocculated sample presented a higher kinetic constant, but also a higher value for the "non-biodegradable coefficient". This phenomenon is not well understood and should be further study.

- Experiment presented herein, and by other researchers, e.g., La Motta (2003, 2004) indicate that at reaction times of 20 to 30 minutes there is still soluble substrate available, but the biomass has already produced polymers for promoting bioflocculation. Studies should be conducted starting with dispersed cells and soluble substrate to determine what triggers polymer production in suspended growth reactors. Gradually particulate substrate can be included in the wastewater in order to evaluate the bacterial response in terms of polymers production.

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APPENDIX A

Picture A1. TF/SC Pilot Plant Overview

Picture A2. Solid Contact Process

APPENDIX B

To determine the coefficients *Y, k, Ks*, and *kd*, which must be available to use biological kinetic models, bench-scale reactor or pilot-system are used.

In determine these parameters, the usual procedure is to operate the units over a range of effluent substrate concentrations; therefore, several different θ_c (at least five) should be selected for operation ranging from 1 to 10 days. Using the data collected at steady-state conditions, mean values should be determined for *Q, So, S, X*, and r_{su} .

Equating the value of r_{su} given by Eq. 8-8 to the value of r_{su} given by Eq 8-41 results in the following expression:

$$rsu = -\frac{kXS}{K_s = S} = -\frac{S_o - S}{\theta} \qquad \text{(B-1)}$$

Dividing by *X* yields

$$\frac{kS}{K_s + S} = \frac{So - S}{\theta} \qquad \text{(B-2)}$$

The linearized form of eq. B-2, obtained by taking its inverse, is

$$\frac{X\theta}{So - S} = \frac{K_s}{k}\frac{1}{S} + \frac{1}{k} \qquad \text{(B-3)}$$

The values of K_s and k can be determined by ploting the term [$X\theta$/(*S*o-*S*)] versus (1/*S)*. The values of *Y* and k_d may be determined using the following equation, by plotting *(1/HRT*) versus (-r_{su}/*X*).

$$\frac{1}{HRT} = -Y\frac{r_{su}}{X} - k_d \qquad \text{(B-4)}$$

The slope of the straight line passing through the plotted experimental data points is equal to Y, and the intercept is equal to k_d.

APPENDIX C

Y and k_d Determination by Batch Test

A conventional method that calls for operating at least four bench-scale, continuous-flow, biological reactors at different sludge ages is difficult and time consuming to obtain Y and k_d. These parameters mainly affect activated sludge production and have relatively little effect on predicted effluent quality, therefore, Y and k_d are important for BPR design.

It is easy to determine Y and k_d by running a batch test, which procedure is similar to the used for T_bOD determination. Therefore, from the same batch test, T_bOD, Y, and k_d can be determined simultaneously. Since there is little difference in Y and k_d values (VSS basis) for conventional treatment plants (McClintock et al. 1992).

Data Analysis:

Some experimental runs may suffer from variability in VSS analyses used to measure biomass growth. The variability in the VSS measurements, if the samples are not carefully taken at each time may be even greater than the net growth of microorganisms, making the kinetic study inaccurate. Hence. before taking samples the reactor contents must be mixed vigorously to disperse the mixture uniformly. Should be analyzed Triplicate VSS and duplicate COD samples. It may be desirable to increase the F/M above typical values. In this way, a more noticeable biomass growth may be attained. Idealized cell growth and substrate removal curves are shown in Figure C.1. In experimental runs with municipal wastewater, the net growth of microorganisms begins to decrease after several hours and becomes negative after the substrate is consumed. The experimental data are plotted and a smooth "best fit" curve is drawn through the points to average out some of the variability in the test data.

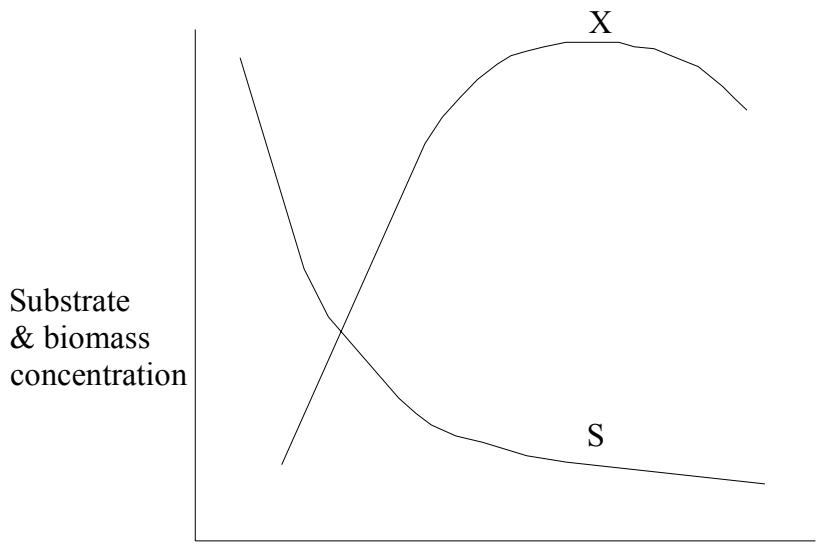

Figure C.1 . Generalized substrate consumption and biomass growth with time.

From the initial portion of the curve where the biomass is in the logarithmic growth phase are chosen values of S and X. These data are transformed into estimates of U, the substrate utilization rate, and μ, the specific growth rate, for each time period (Δt from *i* - 1 to *i*) using the following equations:

$$U\ i = \frac{(S_{i-1} - S_i) / \Delta t_i}{(X_{i-1} + X_i) / 2} \qquad \text{(C.1)}$$

$$\mu\ i = \frac{(X_i - X_{i-1}) / \Delta t_i}{(X_i + X_{i-1}) / 2} \qquad \text{(C.2)}$$

Based on Equation B.5, μ and U can be plotted and a regression line can be drawn as shown in Figure B.2. The endogenous decay rate, k_d, is the Y-intercept. Since k_d is extremely sensitive to the variability of the data points, it may be difficult to determine a reasonable value for k_d using this method. Forcing a regression line to fit through the independently determined k_d makes the resulting slope a more reliable estimate of Y.

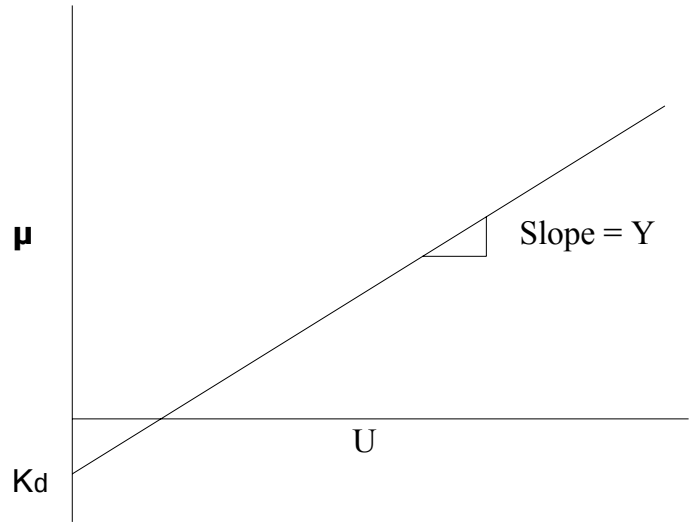

Figure C2 . Plot of specific growth rate (u) with specific substrate utilization rate (U)

VITA

Shirley Poliszuk Quintero was born in Maracaibo, Venezuela, on July 3rd , 1974. In May 1998, she graduated from University Rafael Urdaneta at Maracaibo, Venezuela, with a Bachelor of Science in Civil Engineering. In October 1998, she started working as a design engineer at "Consuvial" a consulting engineering firm located at Maracaibo where she worked for about three years. In Spring 2003, she started at University of New Orleans, pursuing a Masters of Science in Environmental Engineering, achieving her academic goal on December 2004.

During her Master studies, she was a graduate research assistant for one and a half years at the Department of Civil and Environmental Engineering at University of New Orleans working in a wide variety of activities related to wastewater processes. Her academic emphasis has been in the areas of water and wastewater processes of the environmental engineering field.