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Fecal coliform decay and regrowth kinetics in an anaerobic dairy wastewater environment

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FECAL COLIFORM DECAY AND REGROWTH KINETICS IN AN ANAEROBIC DAIRY WASTEWATER ENVIRONMENT

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The kinetics of fecal coliforms (FC) decay and regrowth were analyzed under laboratory conditions using filtered dairy wastewater under anaerobic conditions.

The mean specific growth rates during the regrowth phase, μ_r , in the batch study were 1.79, 1.46, and 1.27d^{-1} for initial organic carbon concentrations of 478, 235 and 127 mg/L COD, respectively. The substrate concentrations had a significant impact on the FC regrowth. A maximum specific growth rate (μ_{max}) of 1.92 d^{-1}, and half-saturated coefficient (k_s) of 60.92mg/L were determined from these data.

The mean dark FC decay rate coefficients, k_d , at 35^oC in the batch study were 2.19, 2.52 and 3.29 d^{-1} for organic carbon concentrations of 478, 235 and 127 mg/L COD, respectively. The effect of substrate concentrations on the FC dark decay rate coefficient was significant (P-value=0.0004). A simple linear regression equation of k_d= 3.460-0.00497 *S was obtained for the batch study.

The decay rate coefficients of FC, determined from non-steady state data, for hydraulic retention times of 1.7, 3.5, and 6.9days at 25^oC were 1.34, 1.57, and1.38 d^{-1}, respectively.

The mean μ_r values in the CSTR at 35^oC were 0.83, 2.85, 2.68, 2.29, 2.11 d^{-1} for the hydraulic retention times of 1.7, 3.5 (Trial 1), 3.5 (Trial 2), 3.5 (Trial 3) and 6.9days, respectively. μ_{max} of 4.00d^{-1}, and k_s of 275.12mg/L were obtained for the CSTR studies. μ_{max} of 3.03 d^{-1}, and k_s of 169.01mg/L was obtained for the combined data from batch and CSTR studies.

The mean k_d-μ_d values determined from the non-steady state data for hydraulic retention times of 1.7, 3.5 (Trial 1), 3.5(Trial 2), 3.5 (Trial 3), and 6.9days at 35^oC were 4.67, 1.72, 0.72, 1.63, and 5.87d^{-1}, respectively. These results indicate that the 3.5days hydraulic retention time reactors were near steady state conditions.

CHAPTER 1

INTRODUCTION

By definition, fecal coliforms (FC) are a group of bacteria that inhabit the intestinal tract of warm blooded animals, are non-sporiform, gram negative, rod-shaped and ferment lactose.

FC are of great concern to the public due to indicators for disease transmission. FC are commonly used by public health officials to reflect the potential presence of pathogenic microorganisms. The standard established for primary contact recreation is 200 fecal coliform/100ml as a geometric mean of five samples taken over 30-day period, with a maximum of 400 fecal coliform/100ml (USEPA, 1976). This standard is frequently exceeded in surface waters that receive runoff from agricultural land and from non-agricultural forested land (Drapcho and Beatty, 1995).

The goal of many investigations of the decay of fecal bacteria is to relate some easily measured environmental parameters with survival so that a general predictive model can be developed (Manicini, 1978, Sarikaya and Saatci, 1987, Auer and Niehaust, 1993). The results of many studies suggest that the decay phenomenon of FC is probably due to complex interactions among a number of factors (Scarpino, 1962, Manicini, 1978). Factors that influence the decay of FC are temperature, sunlight, pH, competitive organisms, available nutrients, and organic compounds. Models have been developed to mathematically describe the relationship between FC decay rate and the decay factors.

Lagoon wastewater systems have long been used as a wastewater treatment method for the destruction of organic compounds. The reduction of FC bacteria numbers in dairy waste lagoons is not well documented. Scott (2000) investigated the dark FC decay rate in dairy wastewater as function of temperature in batch reactors. FC regrowth was observed at the initial

phase of inoculation in Scott's study. The value of the dark FC decay rate coefficient in Scott's study, k_d (20^oC) of $0.133d^{-1}$ was lower than values reported in the literature for diluted wastewater mixtures. Scott concluded that substrate concentration might affect the net decay rate coefficient of FC in batch study. Therefore, the first objective of this study is to find the relationship between substrate concentration and FC regrowth and decay rate coefficient in batch study. The FC decay rate in Scott's study may represent combined effect of FC regrowth and true decay. In order to develop a rigorous FC decay model, the regrowth coefficient and the true decay rate coefficient should be separated. Therefore, a second objective of this study is to use continuous stirred tank reactor (CSTR) to obtain the regrowth coefficient and the true decay rate coefficient of FC. This can be accomplished by solving mass balance equations for simple CSTR at steady state.

The sum of reactions occurring in CSTR which involve biomass growth and decay was represented by Grady et al. (1999),

$$\sum r_{XB} = \mu X_B - k_d X_B \tag{1.1.1}$$

Where $\sum r_{XB}$ =sum of reactions affecting heterotrtrophic biomass, mg/L.d;

μ=specific grow rate coefficient, d^{-1};

k_d=decay rate coefficient, d^{-1};

X_B =heterotrophic biomass, mg/L.

Performing a mass balance with respect to biomass for a simple CSTR, a governing equation can be obtained:

$$\frac{dX_B}{dt} = \frac{X_{B\,in}}{\tau} + \mu X_B - k_d X_B - \frac{X_B}{\tau} \tag{1.1.2}$$

Where τ=hydraulic retention time (HRT), d.

When CSTR is at steady state with no biomass recycle and no biomass in the influent flow, Equation (1.1.2) can be reduced to:

$$\mu = \frac{1}{\tau} + k_d \tag{1.1.3}$$

This equation shows that at steady state, specific growth rate will increase with decreasing hydraulic retention time.

Performing a mass balance with respect to soluble organic substrate for a simple CSTR, a governing equation can be obtained:

$$\frac{dS_S}{dt} = \frac{S_{S\,in}}{\tau} - \frac{X_B}{Y\tau} - \frac{S_S}{\tau} \tag{1.1.4}$$

Where Y=biomass yield, mg/mg;

S_{Si} = influent soluble COD, mg/L;

S_S =effluent soluble COD, mg/L.

When CSTR is at steady state with no biomass recycle and no biomass in the influent flow, Equation (1.1.4) can be reduced to:

$$X_B = \frac{Y(S_{Si} - S_S)}{1 + k_d\tau} \tag{1.1.5}$$

Reverting and rearranging terms in the equation yields:

$$\frac{(S_{Si} - S_S)}{X_B} = \frac{1}{Y} + \frac{k_d\tau}{Y} \tag{1.1.6}$$

This equation is in the form of a linear equation with slope k_d/Y and intercept 1/Y. If CSTRs are operated at several hydraulic retention times and S_{Si}, S_S, and X_B at steady state are measured, the kinetic parameters k_d and Y can be determined. Solving Equation (1.1.3), regrowth rate coefficient (μ) can also be obtained.

Lineweaver-Burk Linearization method (Equation 1.1.7) of the Monod equation can be used to determine the kinetic parameters of k_s and μ_{max}, from pairs of Ss and μ data. The slope and intercept of linear regression of $1/\mu$ versus 1/Ss are k_s/μ_{max} and $1/\mu_{max}$.

$$\frac{1}{\mu} = \frac{k_s}{\mu_{\max}} \times \frac{1}{Ss} + \frac{1}{\mu_{\max}} \tag{1.1.7}$$

Where k_s= half-saturated coefficient, mg/L;

μ_{max}=maximum specific growth rate, d^{-1}.

In summary, the objectives of this study are to determine the decay and regrowth kinetics in an anaerobic dairy wastewater environment by:

(1) Using batch reactor to study the relationship between substrate concentration and net decay rate coefficient of FC.

(2) Using CSTR to separate the true decay rate coefficient and regrowth coefficient.

CHAPTER 2

REVIEW OF LITERATURE

2.1 FC

The coliform bacteria group consists of several genera of bacteria belonging to the family *enterobacteriaceae*. These mostly harmless bacteria live in soil, water, and the digestive system of warm-blooded animals. Fecal coliform bacteria, which belong to this group, are present in large numbers in the feces and intestinal tracts of humans and other warm-blooded animals, and can enter water bodies from human and animal waste. Except for pathogen strains of Escherichia coli (E.coli) (i.e.O157: H7 EHEC, which causes internal bleeding), FC generally do not pose a danger to people or animals but they indicate the presence of other disease-causing bacteria, such as those that cause typhoid, dysentery, hepatitis A, and cholera.

E.coli is the principal component of the fecal coliforms group. Scott (2000) found that 100% of FC in dairy wastewater was E.coli, suggesting that FC net decay rates determined in dairy wastewater may represent E.coli net decay rates.

2.2 Factors Affecting FC Decay

On expulsion from the host to receiving water, FC is in an alien environment, where FC may regrow, but the proliferation is only temporary and FC decay very soon. The survival of FC in an aquatic environment depends upon their ability to tolerate a set of alien biological, physical and chemical conditions. The most important factors considered to be controlling the rate of decay are temperature, solar intensity, and pH (Mayo, 1989, Auer and Niehaust, 1993, Howell et al., 1996).

2.2.1 Temperature Factor

Many studies have found that the decay rate coefficient of FC was significantly correlated with temperature. Increase in temperature was shown to lower the survival rate or increase the decay rate of FC (Flint, 1987, Howell et al., 1996). Scott (2000) studied the effect of four different temperatures on the dark decay rate coefficients of FC in coarse-filtered dairy wastewater. Scott reported that the decay rate coefficient increased significantly with increase in temperature from 18 to 32^{o}C and a temperature correction factor (θ) of 1.149 was obtained. Graham and Sieburth (1973) also found that increasing the incubation temperature from 15 to 25°C without added nutrients led to the decline of E.coli in artificial seawater. The studies of Gordon (1972) on the survival of fecal indicator bacteria in ice-covered rivers suggested that the maximum survival under natural conditions occurred in water at 0°C under ice cover. However, Auer and Niehaust (1993) reported no consistent relationship was observed between the dark decay rate coefficients of FC and temperatures of 10-35°C.

The effect of temperature on the decay of FC can be explained by the hypothesis of metabolism causing FC decay. Lessard and Sieburth(1983) and Mezrioui et al.(1995) reported that low temperatures prolonged bacterial survival by reducing the metabolic activity of the bacterial cells if nutritional requirements are not continually replenished. Rates of biochemical reactions, and thus microbial growth rates, tend to increase as temperatures rise. High metabolic rates place added demands on cellular nutrient reserves, which may not be renewed in, dilute, natural systems, leading to an increase in the decay rate of FC (Auer and Niehaust, 1993).

2.2.2 Sunlight Factor

Many studies have found that solar radiation is the dominant influence on culturable densities of fecal indicator bacteria in open waters, with the inactivation (loss of cultuarability)

rate in sunlight being typically 2 or more orders of magnitude greater than that in the dark (Kapuscinski and Mitchell, 1983, Gameson , 1986, Chamberlin and Mitchell, 1978). Mayo (1989) pointed out that many of the factors affecting bacterial decay were directly or indirectly influenced by solar radiation. Gameson and Saxon (1967) concluded that the rate of decay at any time of the year was approximately proportional to the intensity of the short-wave radiation received by the sample. However, Lessard and Sieburth (1983) found that there was no significant difference in decay rate between light and dark diffusion chambers, nor were decay rates correlated with light intensity.

Solar radiation diminishes with pond depth. The decay rate coefficient varies significantly with pond depth from 0-0.2 meters, but varies little with pond depth more than 0.2 meters (Mayo, 1989) (Figure 2.2.1). Sunlight is attenuated through a water column at a rate dependent on the water clarity. Hence water clarity becomes an important factor influencing fecal indicator inactivation rates.

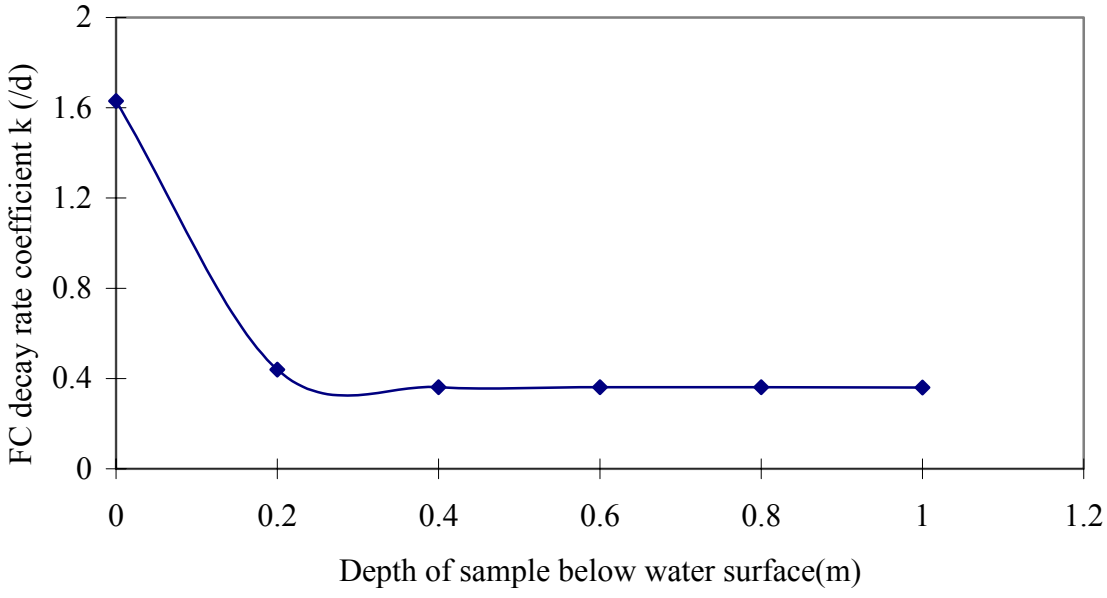


Figure 2.2.1 Relationship between FC decay coefficient and pond depth (Mayo, 1989)

Several mechanisms have been proposed to support the hypothesis of damage from sunlight causing FC decay. Chamberlin and Mitchell (1978) explained that solar radiation might only injure coliforms, making them more susceptible to the activities of microbial predators. Curtis et al. (1992) reported that the damage to bacterial cells caused by the wavelengths present in visible light mainly affects the cytoplasmic membrane whereas the damage caused by ultraviolet radiation affects the DNA. Curtis et al. indicated that damage to the membrane of an organism is ecologically important, since it makes the organism more sensitive to the effects of other factors such as high pH.

2.2.3 pH Factor

Extremes in pH are detrimental to organism survival. Mayo (1995) has showed that the fecal coliform decay rate coefficient increases with increasing pH (pH>7.0). However, the correlation was poor (R=0.27), suggesting that factors other than pH might play a major role in FC reduction. Parhad and Rao (1974) observed that E.coli counts declined rapidly at pH above 9.3.The results of Parhad and Rao indicated that the increasing pH, whether it occurred as a result of algal growth or because of the addition of alkalis, was responsible for the gradual reduction and eventual elimination of E.coli in sterilized wastewater. Generally a neutral pH environment favors extended bacterial survival; and acid and alkaline conditions in water can greatly increase FC decay rates (Mefeters, 1972).

2.2.4 Regrowth Factor

Hendricks (1972) found that E.coli grew at a specific growth rate of $0.696 day^{-1}$ at 30°C in a chemostat with autoclaved river water taken 750 m below a sewage outfall, when lab-strain E.coli were inoculated. Savage and Hanes (1971) showed that below 10mg/L of initial BOD, no growth was observed for total and fecal coliform bacteria. Lessard and Sieburth (1983) observed

that E.coli growth roughly corresponded to increase in temperature, DOC and polysaccharides in salt marsh.

Howell et al. (1996) found no FC regrowth at 4^{o}C, but salient regrowth at 25 and 35^{o}C occurred shortly after deposition from day 0 to 3. Higher temperature increases fecal coliform decay, but it can also promote FC regrowth in aquatic environments (Doran and Linn, 1979).

2.2.5 DO Factor

Microbial decay rates in anaerobic environments have been reported to be lower than in aerobic environments (Grady et al., 1999). Research findings, such as that of Curtis et al. (1992), suggest that the ability of light to damage FC was highly sensitive to, and completely dependent on, oxygen. The rate of damage was proportional to oxygen concentration. However, Mayo (1995) reviewed that dissolved oxygen concentration did not play any role in the survival or decay of FC (correlation coefficient R=-0.06). Pearson et al. (1987) observed that there was no influence of DO even at 100% saturation on E.coli.

2.2.6 Organic Carbon Factor

McGrrew (1962) reported that glucose concentration of 4 mg/L as carbon provided sufficient maintenance energy and could even stimulate growth of E.coli. Mayo (1995) reviewed that the fecal coliform decay rate coefficient was not affected by the volumetric soluble BOD_5 loading rate. This finding was in agreement with work by Mills et al. (1992), and Skerry and Parker (1979) but differed from that of Canter et al. (1969) and Gran (1968), who suggested that low fecal bacterial competitiveness for nutrients might lead to their reduction in waste stabilization ponds. Gray (1975) also observed that E.coli showed remarkable survival skills under conditions in which all possible nutrients were removed from the suspending medium, an indication that starvation was probably unlikely to influence coliforms removal in ponds. Enteric

bacteria are assumed to exist in fresh water lakes and streams under starving conditions with growth being limited primarily by the nonavailability of a suitable carbon source (Hendricks, 1972). Scarpino (1962) suggested that cysteine and other amino acids act to increase survival of cells of E.coli in seawater by a chelation mechanism.

The effect of organic carbon on the decay of FC can also be explained by the metabolism of FC. Klock (1971) reported that resulting from the organism's normal dependence on host-prepared precursors or their own limited production of exoenzymes, FC could consume little or no organic compounds in the environment. Under these circumstances, it is hypothesized that FC reverts from an adsorptive metabolism and proliferation in the host to a near endogenous metabolism in wastewater relying on its contained food resources for survival. Over an extended period, exhaustion results with an accompanying population decay. This explanation indicates that the metabolism of FC restricts their food source (Klock, 1971).

2.2.7 Other Factors

Other factors, such as sedimentation, predators, algae, and salinity influence the decay rate coefficient of FC. Auer and Niehaust (1993) reported that sedimentation may be a major mechanism of FC removal from lake water. McCambridge and McMEEKIN (1981) reported that the decline in the numbers of E.coli cells in estuarine water samples was found to be significantly greater in the presence of both naturally occurring microbial predators and solar radiation than when each of these factors was acting independently. Howell et al. (1996) has found that saline can reduce cell death by osmotic shock. Davis (1994) has stated that the existence of a greater variety of algal species indicates a more complex environment, which in some cases is correlated with increased coliform reduction rates. Little influence upon decay of enteric bacteria is exerted by a single algal species. Rice et al. (1991) reported that significantly

higher coliforms growth responses were associated with waters that had been exposed to ozonation, which may suggest competition from other microbes plays a large role in FC growth survival in natural waters.

2.3 FC Decay Models

FC decay models reported in the literature may be useful for the first prediction of decay rate coefficient in different kinds of aquatic environments if similar conditions can be found. Since many factors in waste stabilization ponds and other natural aquatic environments affect the FC decay rate coefficient, a comprehensive model should consider the effects of different factors.

2.3.1 First-order Kinetic Model

In this model, FC decay is immediate upon release into environment. This phenomenon will occur where the environment is totally unsuitable for FC and decay rate coefficient is constant with time (Crane and Moore, 1985). The rate of FC decay is

$$r = -kN \qquad (2.3.1)$$

Where r= the decay rate of FC, CFU/100mL.d;

N = FC concentration at time t, CFU/100mL;

k=first-order rate coefficient for decay of FC, d^{-1}.

The first-order model appears to accurately describe the decay under most of the conditions; however, the decay rate is a highly variable parameter spanning several orders of magnitude (Table 2.3.1). This variability is due to the interaction of environmental factors on bacterial decay rate and different FC measure methods.

Table 2.3.1 Microbial decay rate coefficient in the literature

Water Source	Season or T (^oC)	pH	k, (day^{-1})	θ	Reference
Fresh water	20	-	0.80	1.07	Mancini (1978)
Fresh water	10-12	7.5	0.29	-	McFeters et al. (1974)
Lake water	20	-	0.22	-	Bhagat et al. (1972)
Lake water	10-35 20	- -	0.61±0.11 0.73	1	Auer and Niehaust (1993)
River water	4 15 25 37	-	0.54 0.77 1.27 2.34	1.04	Flint (1987)
Clean river	18.3	7.5	0.18	-	Klock (1971)
Stream water	18.5	-	1.10	-	Dutka (1980)
Stream water	10	2.5 4.0 5.0 6.0 7.0 10.0 12.0	6.39 0.58 0.40 0.30 0.32 0.71 6.39	-	McFETERS and Stuart (1972)
	5 10 15 20 25	- - - - -	0.15 0.23 0.50 0.99 1.39	1.12	
	4-6	8.37 8.10	1.73 1.39	-	
Sterilized estuarine water	22	-	0.46	-	McCAMBRIDGE and McMEEKIN (1981)
Natural estuarine water	May July Nov	-	0.54 0.71 0.36	-	Faust (1975)
Sea water	20	-	1.40	1.07	Mancini (1978)
Sea water	8.9 10.7 13 14.5	- - - -	0.51 1.73 2.24 2.52	1.04	Vasconcelos and Swarts (1976)
Sea water	20	-	2.69		Savage and Hanes (1971)

Table 2.3.1 continued

Sea water Salinity 0.85% 2.5% 5.0%	-	-	 0.45 1.29 4.60	-	Carlucci and Pramer (1959)
Artificial sea water	28		6.91		Scarpino (1962)
Physiological saline water (8.5g NaCl /L water)	4 25 35	- - -	0.08 0.14 0.17	1.03	Howell (1996)
Marine water	0 8 16 20	- - - -	1.01 1.18 1.51 2.11	1.09	Lessard and Sieburth (1983)
	8 16 20	- - -	1.06 1.44 1.80	1.08	
Bay water	20 30	-	0.64 0.87	1.03	Canale et al. (1973)
Storm water	17.1-18.2	-	0.35	-	Dukta and Kwan (1980)
Storm Water runoff	10 20	5.0	0.25 1.45	1.19	Geldreich et al. (1968)
Storm Water runoff	10 20	- -	0.23 1.35	1.19	Geldreich (1969)
BOD dilution water	20	6.8 7.0 7.2 7.6	0.22 0.43 0.54 1.10	-	Hanes and Fragala (1967)
	20	6.8 7.0 7.2 7.6	0.22 0.27 0.77 1.33	-	
Polluted river	19.4	7.5	0.29	-	Klock (1971)
Sewage effluent	18.0	6.8-7.6	0.43	-	Slanetz and Bartley (1965)
			0.36	-	
			0.71	-	
Sewage effluent	20		0.91		Davies- colley (1994)

Table 2.3.1 continued

Polishing Ponds	W Sp Su F	7.3	0.14 0.77 1.19 0.33	-	Toms et al. (1975)
	W Sp Su F		0.17 0.29 0.36 0.08	-	
Anaerobic lagoon sewage	25-27	-	1.7	-	Mara and Silva (1979)
Series lagoon-raw sewage		-	0.48	-	
Facultative lagoon-raw sewage		-	0.49	-	
Primary clarifier	12.7 7.9 17.9 14.4 25.2 25.5	7.7 8.0 7.7 8.2 7.4 8.4	0.31 0.20 0.37 0.20 0.70 0.38	-	Klock (1971)
Raw domestic wastewater treatment lagoon	12.7 7.9 17.9 14.4 25.2 25.5	7.67 8.03 7.65 8.16 7.36 8.40	0.31 0.20 0.37 0.20 0.70 0.38	-	
Anaerobic digestion	35	7.5	1.55	-	
Wastewater lagoon	18.3	7.5	0.38	-	
Facultative and maturation pond	20	-	2.60	1.19	Marais (1974)
Beef manure lagoons	7 25 25	- - -	0.56 0.39 0.83-1.76	1.02- 1.07	Coles (1973)
	21-33	-	1.35	-	
	21-33	-	0.38	-	
Swine lagoon effluent	23-28	7	0.28	-	Krieger (1976)
	23-28	7	0.42	-	
Stabilization Pond	20	8	-0.57	-	Malina and Yousef (1964)
	8		-0.63	-	

Table 2.3.1 continued

Stabilization pond	26-33	9.4 >9.6 7.5 to10.4	3.45 6.91 3.31	-	Parhad and Rao (1974)
Stabilization pond	20	-	0.71	1.17	Mills et al. (1992)
Stabilization ponds	8 15 23 30	- - - -	0.05 0.07 0.53 0.65	1.14	Mezriousi (1995)
	8 15 23 30	- - - -	0.08 0.21 0.31 0.42	1.08	
Stabilization ponds	20-33		0.28		Polprasert et al. (1981)
Stabilization ponds	26	9.5	0.44	-	Mayo (1995)
Stabilization ponds	26-31	-	1.16	-	Data form Polprasert et al. (1983) analyzed by Sarikaya and Saatci (1987)
Stabilization ponds	28.6-32.6	-	0.09	-	Sarikaya et al. (1983)
Stabilization ponds	26.2-34.3	-	0.11	-	Mayo (1989)
			0.32		
			0.74		
Sewage effluent	8-10 15-20	- -	0.48 0.67	1.04	Sinton et al. (1994)
Meat works effluent	8-10 15-20	- -	0.46 0.94	1.09	
Dairy wastewater in batch reactors	17.8 21.8 29.6 31.9		0.11 0.15 0.45 0.79	1.15	Scott (2000)

(Note: θ=temperature correction factor.)

Based on equation (2.3.1), Marias (1974) obtained the following model for a series of stabilization ponds. The FC concentration of the effluent from the nth pond in the series is:

$$N_n=N_0/ [(kR_1+1) (kR_2+1)\dots (kR_n+1)] \quad (2.3.2)$$

Where R=the retention time based on influent flow;

N_n = FC concentration of the nth pond, CFU/100mL;

N_0 = initial FC concentration, CFU/100mL.

Due to regrowth, predators, and other factors, Howell et al. (1996) reported that equation (2.3.1) couldn't accurately reflect FC decay. Howell et al. found that the following model was more appropriate when FC regrowth existed.

$$\ln (N+1) = \beta_0 + \beta_1 X^{\beta 2} + e \quad (2.3.3)$$

Where X = the number of days after FC deposition, d;

N= FC concentration, CFU/100mL;

β_0, β_1, and β_2 = model parameters to be estimated;

e=random error.

2.3.1.1 Temperature Effect

Functions based on the Arrhenius or Van's Hoff equations, simplified as shown in equation (2.3.4), are often utilized to illustrate the relationship between temperature and FC decay rate coefficients. Temperature-dependent models have been developed by Klock (1971), Marais(1974), Mancini and Ridgewood(1978), and Mills et al.(1992).

$$k = k_{20}\, \theta^{(T-20)} \quad (2.3.4)$$

Where k= FC decay rate coefficient at $T=T^oC$, d^{-1};

k_{20}= FC decay rate coefficient at 20^oC, d^{-1};

θ= temperature correction factor which describes the relationship between temperature and decay rate coefficient.

A temperature correction factor of 1.149 was reported by Scott (2000) for dark FC decay in dairy wastewater. θ values of 1.0-1.195 have been reported (Table 2.3.1).

2.3.1.2 Solar Intensity or Pond Depth Effect

Moeller and Calkins (1980) stated that up 10% of surface ultraviolet solar radiation may penetrate 15 meters in clear seawater, while in wastewater, only about 3-5% of surface ultraviolet solar radiation may penetrate below 20 cm. Scott (2000) found that 90% of solar radiation was absorbed in top 12.2 cm of anaerobic dairy lagoon. Mayo (1995) emphasized that the overall decay rate coefficient should consist of dark decay rate coefficient and light decay rate coefficient just as described in equation (2.3.5).

$$k=k_d+k_I \tag{2.3.5}$$

Where k_d =decay rate coefficient in dark, d^{-1};

k_I= decay rate coefficient due to light, d^{-1}.

Sarikaya and Saatci (1987) developed a model (2.3.6) describing light FC decay rate coefficient affected by solar intensity and waste stabilization pond depth.

$$k_I = \frac{k_s S_0}{k_e H}(1-e^{-k_e H}) \tag{2.3.6}$$

Where k_s =decay rate coefficient for light, cm^2/cal;

k_e =light attenuation coefficient ,m^{-1};

S_0=solar intensity received at pond surface, cal/ cm^2/d;

H=pond depth, m.

The light attenuation coefficient (k_e) in waste stabilization ponds is 7.8-16m^{-1} (Sarikay and Saatci ,1987). Scott (2000) obtained a value of 13.17 m^{-1} for the light attenuation coefficient k_e, which was measured *in situ* in an anaerobic dairy wastewater lagoon.

Table 2.3.2 Values of k_s in the literature

k_s(cm^2/cal)	Reference
3.6-6.77*10^{-4}	Mayo(1995)
0.00824	Auer and Niehaus(1993)
0.00524	Sarikaya and Saatci(1987)
0.00-0.011	Lantrip(1983)

Mayo (1995) expanded the equation (2.3.6) by adding the surface layer effect coefficient, which is shown in equation (2.3.7).

$$k_I = \frac{k_s S_0}{k_e H}(1-l_0)(1-e^{-k_e H}) \tag{2.3.7}$$

Where l_0 =surface layer effect coefficient, dimensionless.

The surface layer effect coefficient (l_0) generally varies from 0 to 0.03 (Qin et al.1991).

Manicini (1978) developed equation (2.3.8) to describe the effect of solar intensity on the FC decay rate coefficient in seawater, which utilized I_A to substitute $k_s S_0$ as used in equation (2.3.7).

$$k_I = \frac{I_A}{k_e H}(1-e^{-k_e H}) \tag{2.3.8}$$

Where I_A=Average daily surface solar radiation, Langleys/hr.

Auer and Niehaust (1993) developed another form of model to describe the effect of solar intensity, which is shown in equation (2.3.9).

$$k_I = \frac{I_{o,avg}\alpha}{Z_e \eta}[1-e^{(Z_e \eta)}] \tag{2.3.9}$$

Where $I_{o,avg}$ =average irradiance immediately below the water surface over the incubation period, cal $cm^{-2}d^{-1}$;

α= irradiance proportionality constant, cm^2cal^{-1};

Z_e =epilimnion depth, m;

η= light attenuation coefficient, m^{-1}.

2.3.1.3 Salinity Effect

Manicini (1978) developed a model to consider the effect of salinity on the FC decay rate coefficient in seawater, which was shown in equation (2.3.10).

$$k = [k_d + 0.006(\%seawater)] * \theta^{(T-20)} \quad (2.3.10)$$

2.3.1.4 pH Effect

Mayo (1995) proposed a model including the effect of pH,

$$k = k_d + k_I + k_{pH}\, pH \quad (2.3.11)$$

Where k_{pH}=FC decay rate constant for pH, d^{-1}.

Mayo obtained a value of -0.0063 for k_{pH}.

2.3.1.5 Sedimentation Effect

Auer and Niehaust (1993) included sedimentation into the FC decay model, as shown in equation (2.3.12).

$$k = k_d + k_I + \frac{\nu}{Z_e} \quad (2.3.12)$$

Where Z_e =epilimnion depth, m;

ν = sedimentation velocity (m/d).

Based on the field and laboratory research, the value of 1.38m/d for sedimentation velocity (ν) was obtained by Auer and Niehaust.

2.3.2 Non-first-order Kinetic Model

Polprasert et al. (1981) developed a multiple linear regression equation to relate the bacterial decay rate coefficient to other parameters in the waste stabilization ponds, such as temperature, algae concentration, and influent COD loading rate.

$$e^k = R\lambda w_1^T w_2^C w_3^{OL} \quad (2.3.13)$$

Where R, w_1, w_2, w_3= multiple regression coefficient;

λ=species constant for FC,dimensionless;

C=algal concentration, mg/L;

OL=influent COD loading rate, kg COD/ha•d.

Based on the field research and regression analysis, Polprasert et al. obtained the parameters as follow:

$$e^k = 1.1274(0.6435)(1.0281)^{20}(1.0016)^{200}(0.9994)^{200} \quad (2.3.14)$$

CHAPTER 3

MATERIALS AND METHODS

3.1 Research Systems

3.1.1 Dairy Wastewater Filtration

Dairy wastewater was collected from the flush water from the LSU AgCenter dairy feed barn in Baton Rouge, LA. The barn floor surface is flushed twice daily with well water. The fresh dairy wastewater was filtered through cotton polyfill after collection. Then, the wastewater was filtered through a 0.2μm ultrafiltration cartridge (A/G Technology Corporation). Permeate from the cartridge contained 0 –100 CFU /100mL of FC. Permeate was stored in a 4^{o}C refrigerator for further use. A diagram of the filtration procedure is shown in Figure 3.1.1.

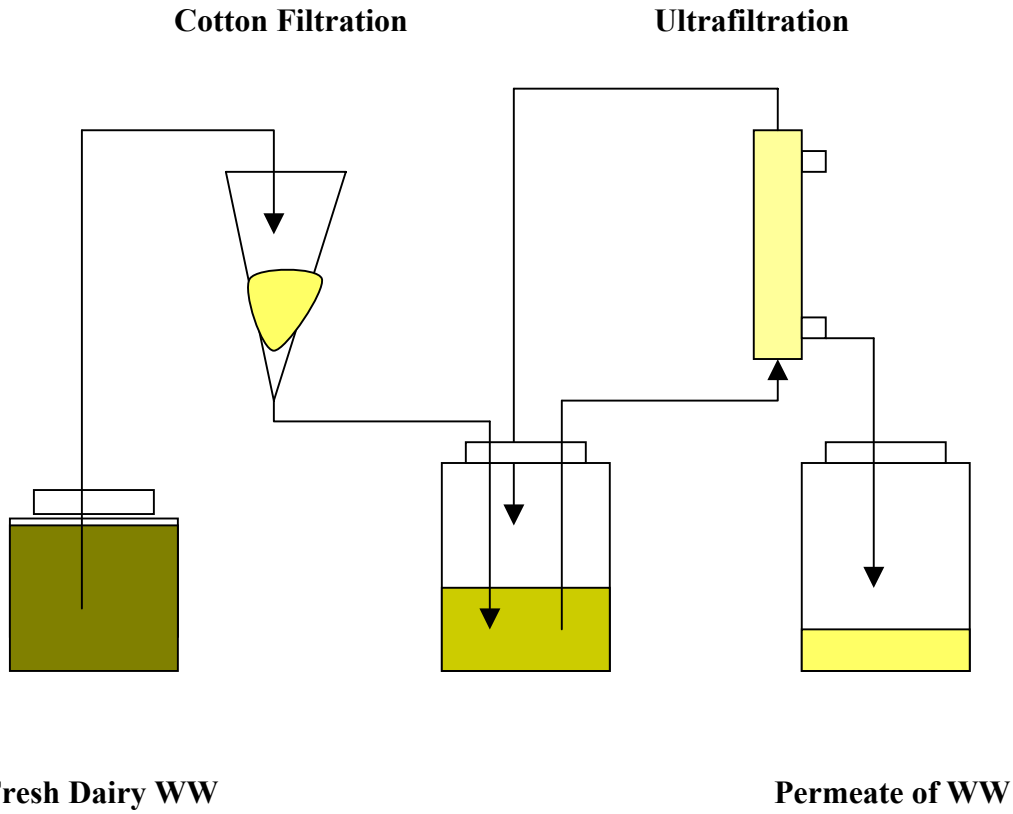


Figure 3.1.1 Diagram of dairy wastewater filtration

3.1.2 Batch Reactor

Batch reactors were operated under three different initial COD concentrations at 35^{o}C. Filtered dairy wastewater was diluted with water to achieve 1/1, 1/2, and 1/4 dilutions. Fresh dairy wastewater filtered through cotton was inoculated to each reactor. For each dilution, 2-L plexiglass reactors with 2 liters working volume were placed in the air bath shaker (New Brunswick Scientific, C24 incubator shaker). All batch reactor studies were conducted under anaerobic conditions provided by bubbling nitrogen gas through liquid.

3.1.3 Continuous Stirred Tank Reactor (CSTR)

CSTRs were operated under two different temperatures (25^{o}C and 35^{o}C) and three different hydraulic retention times (1.7days, 3.5days, and 6.9 days), for a total of six treatments. Fresh dairy wastewater filtered through cotton was inoculated to each reactor. For each treatment, 2-L plexiglass reactors with 2 liters working volume were also placed in an air bath shaker. The stir speed was 100rpm. CSTRs were operated under anaerobic conditions. Filtered dairy wastewater was used without dilution in these studies

3.2 Analytical Methods

3.2.1 Membrane Method for FC Test

3.2.1.1 Materials

Dehydrated M-FC medium was used (Fisher Scientific).

3.2.1.2 Theory

The FC concentration was analyzed by using the membrane filtration method according to the Standard Methods of Examination of Water and Wastewater (APHA, 1995).

The membrane filter (MF) technique is highly reproducible, can be used to test relatively large volumes of sample, and yields numerical results more rapidly than the multiple-tube procedure. The membrane filter technique is extremely useful in monitoring drinking water and a

variety of natural waters. However the MF technique has limitations, particularly when testing waters with high turbidity or noncoliform (background) bacteria.

The MF procedure uses an enriched lactose medium and incubation temperature of 44.5+/-0.2^{o}C for selectivity and gives 93% accuracy in differentiating between coliforms found in the feces of warm-blooded animals and those from other environmental sources (APHA, 1995). Because incubation temperature is critical, an incubator that will hold the 44.5^{o}C temperature within 0.2^{o}C, over a 24-h period is used. This elevated temperature is intended to heat shock non-fecal bacteria and suppresses their growth. As the fecal coliform colonies grow they produce acid through the fermentation of lactose, which reacts with the aniline dye in the agar thus giving the colonies their blue color.

3.2.1.3 Procedure

a. Culture dishes were prepared by mixing 3.71g M-FC media and 1.5 g agar into 100ml distilled water. The mixture was heated to 100 ^{0}C, and then cooled down to 50-60 ^{0}C. Approximate 5.5 ml culture was pipetted into each petri dish.

b. Sample volumes that would yield counts between 20 and 60 fecal coliform colonies per membrane were used. When the bacteria density of the sample was unknown, several dilutions were filtered to establish fecal coliform density. Estimated volume expected to yield a countable membrane was estimated, and two additional quantities representing one-tenth times this volume were selected, respectively.

c. 10 ml dilution was filtered through 0.45μm filter. The filter was removed by tweezers disinfected by fire to the petri dish with culture. The filter was pushed by using the tweezers to remove the bubbles.

d. All the prepared plates were placed in the incubator at 44.5 ^{o}C for 24 hours (Fisher Scientific Isotemp Standard Incubator 600 series).

e. Colonies produced by fecal coliform bacteria on M-FC medium were various shades of blue. Pale yellow colonies might be atypical E.coli or other microorganisms which are not counted. Nonfecal coliform colonies were gray to cream-colored.

3.2.1.4 Calculation of FC Concentration

The FC concentration was computed from the sample quantities that produced colony counts within the desired range of 20 to 60. The FC concentration with unit of CFU/100mL was obtained by dividing the count by the orders of magnitude of dilution.

3.2.2 COD Test

Samples were analyzed for chemical oxygen demand (COD) analysis (Standard Method 5220 D, APHA, 1995) using standard range micro COD vials (Bioscience, Inc.). Total COD and Soluble COD were tested in this study. Soluble COD was determined by filtering sample through 1μm glass fiber filter (Gelman) prior to analysis.

3.2.3 DO Test

Dissolved oxygen (DO) was tested by using a Fisher Scientific DO meter (OxyGuard).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Batch Reactor Studies

In the literature, most decay rate coefficients of FC were tested under batch conditions. Without regrowth of FC, the mass balance with respect to FC concentration in a batch reactor is

$$\frac{dN}{dt} = -kN \tag{4.1.1}$$

The decay rate coefficient is calculated as the slope of linear regression of ln (FC concentration) versus time.

Although some researchers (Auer and Niehaus, 1993, Howell et al., 1996) observed the regrowth phenomena of FC, they didn't take it into account. In this batch study, the regrowth rate of FC and relationship between decay rate coefficient and substrate concentration were studied. Three dilutions 1/1, 1/2, 1/4 of filtered dairy wastewater were used, resulting in mean total COD values of 478, 235, 127 mg/L, respectively. The incubation temperature was 35^{o}C.

4.1.1 Regrowth Rate Coefficient of FC in Batch Reactor

The results of the nine batch reactor experiments are shown in Figure 4.1.1-4.1.3. Mean DO values were 0.1mg/L. From the figures, it can be seen that FC concentration increased after inoculation for all three dilutions. FC concentration reached the maximum around 0.5day after inoculation, and declined drastically after that. Total COD and soluble COD declined with time (Figure 4.1.1-4.1.3). Figure 4.1.4 shows the comparison of the FC concentrations change with time for the three dilutions. Figure 4.1.5 shows the comparison of the ln(FC concentration) change with time for the three dilutions.

The initial FC concentration for the 1/2 dilution was more than double the initial concentration for undiluted (1/1) and 1/4 runs. This indicates the FC concentration in the raw wastewater used for the innoculum may have been highly variable. Despite the variation in initial FC concentration, the FC concentration increased initially then decreased for all dilutions.

The decay of FC for the regrowth period is assumed negligible. The mass balance with respect to FC concentration in the batch reactors during the regrowth period is

$$\frac{dN}{dt} = u_r N \tag{4.1.2}$$

Where μ_r=FC specific regrowth rate coefficient of the regrowth period, d^{-1}.

The value of μ_r can be determined by linear regression as the slope of ln (FC concentration) versus time during regrowth period. The results of this analysis are shown in Figure 4.1.7. The fitted equations are listed in Table 4.1.1.

Despite the limited data available, linear regression of ln (FC concentration) versus time was performed to determine FC regrowth rate coefficient (Table 4.1.1). PROC REG (SAS version 8.1) was performed to test the effect of initial FC on the regrowth rate coefficient. The obtained P-value of 0.754, which is much larger than α=0.05, indicates that initial FC had no effect on FC regrowth. PROC REG was also used to test the effect of the three COD concentrations on the FC regrowth rate. The obtained P-value of 0.02, which is smaller than α=0.05, indicates that the COD concentrations had a significant impact on the FC regrowth.

The Lineweaver-Burk Linearization method was used to determine the kinetic parameters of k_s and μ_{max} in the batch studies for the regrowth period. Linear regression of $1/\mu_r$ versus 1/S (Figure 4.1.7) yields

$$\frac{1}{\mu_r} = 31.73\frac{1}{S} + 0.52 \tag{4.1.3}$$

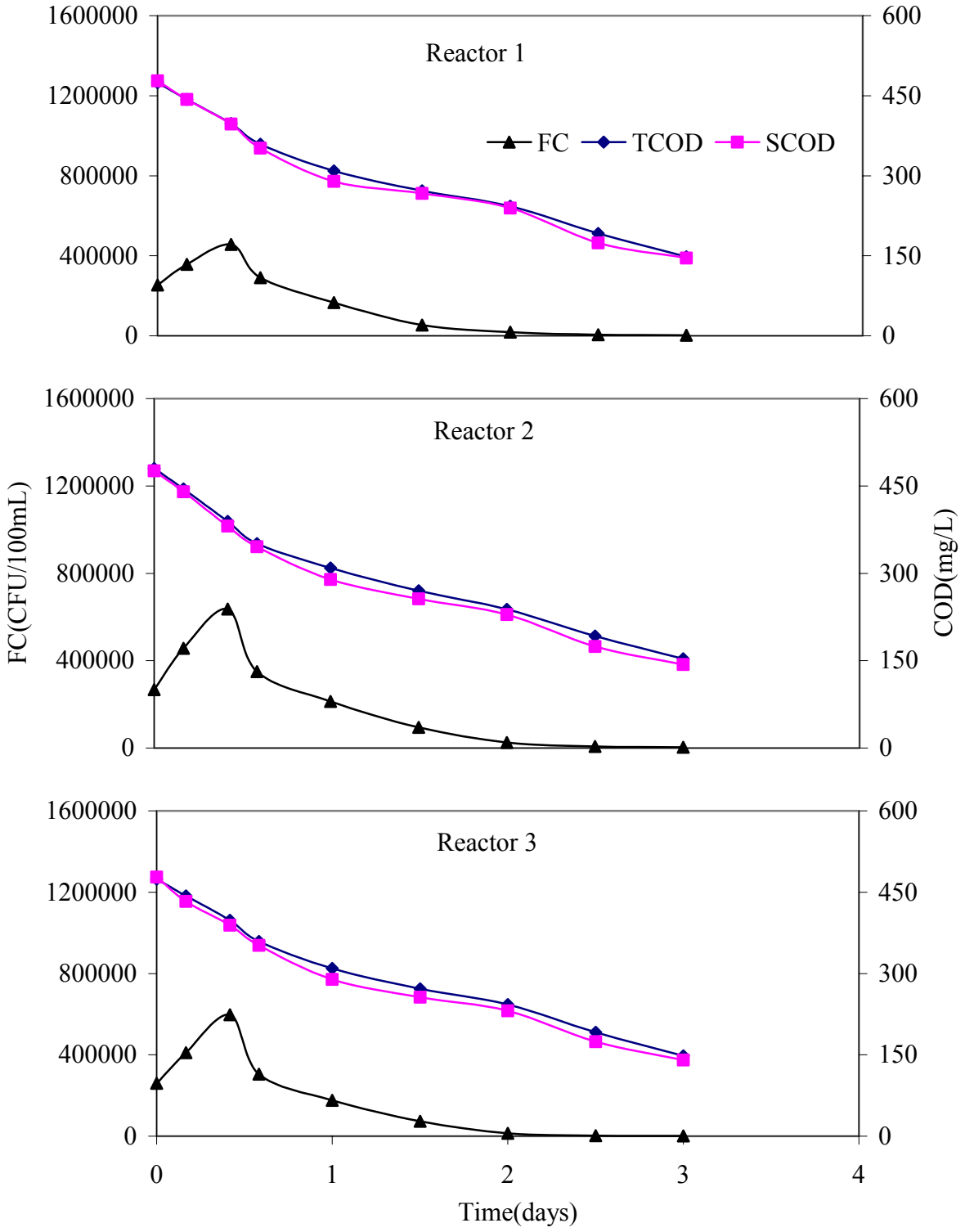


Figure 4.1.1 Batch reactors (Dilution 1/1)

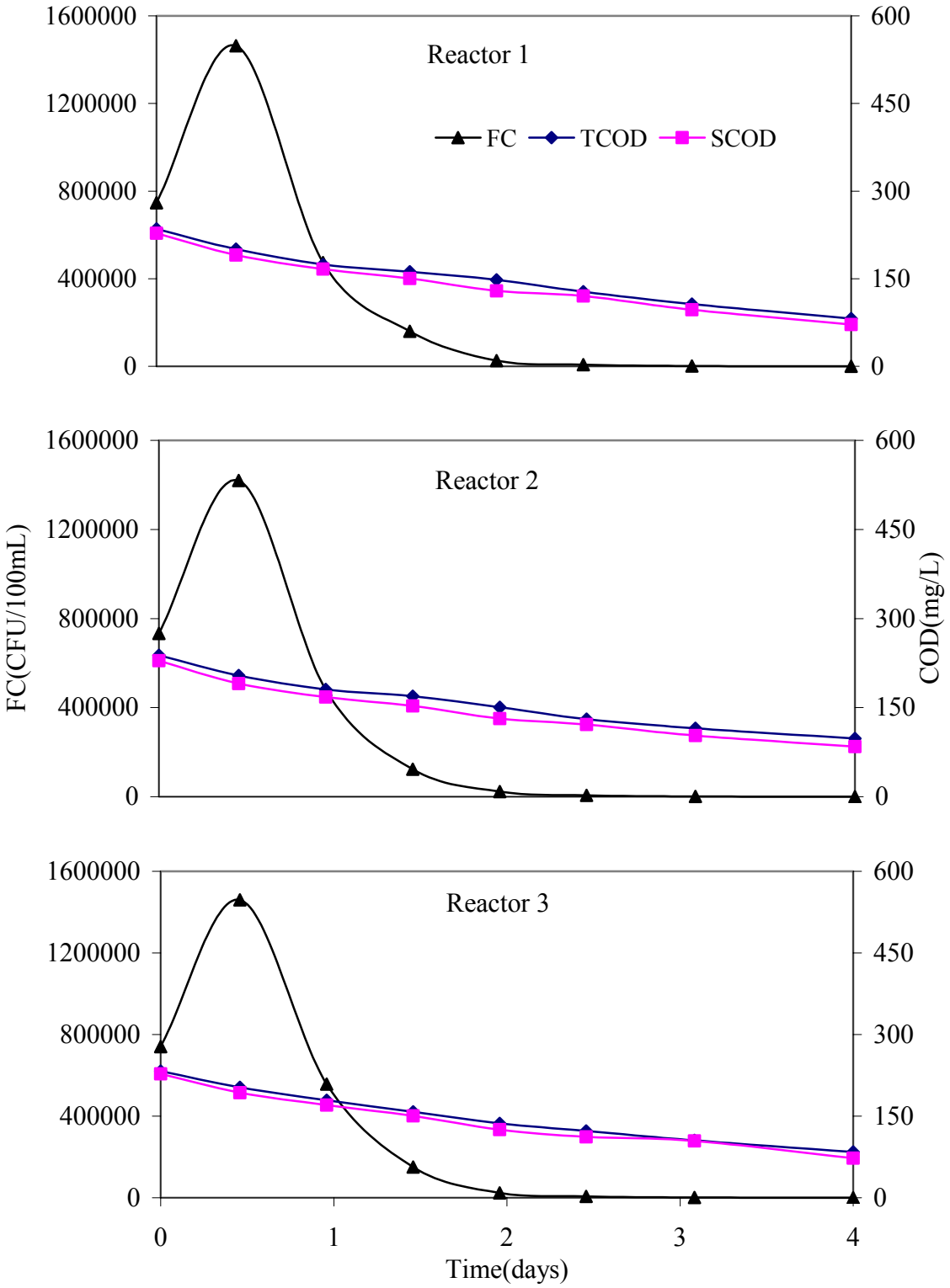


Figure 4.1.2 Batch reactors (Dilution 1/2)

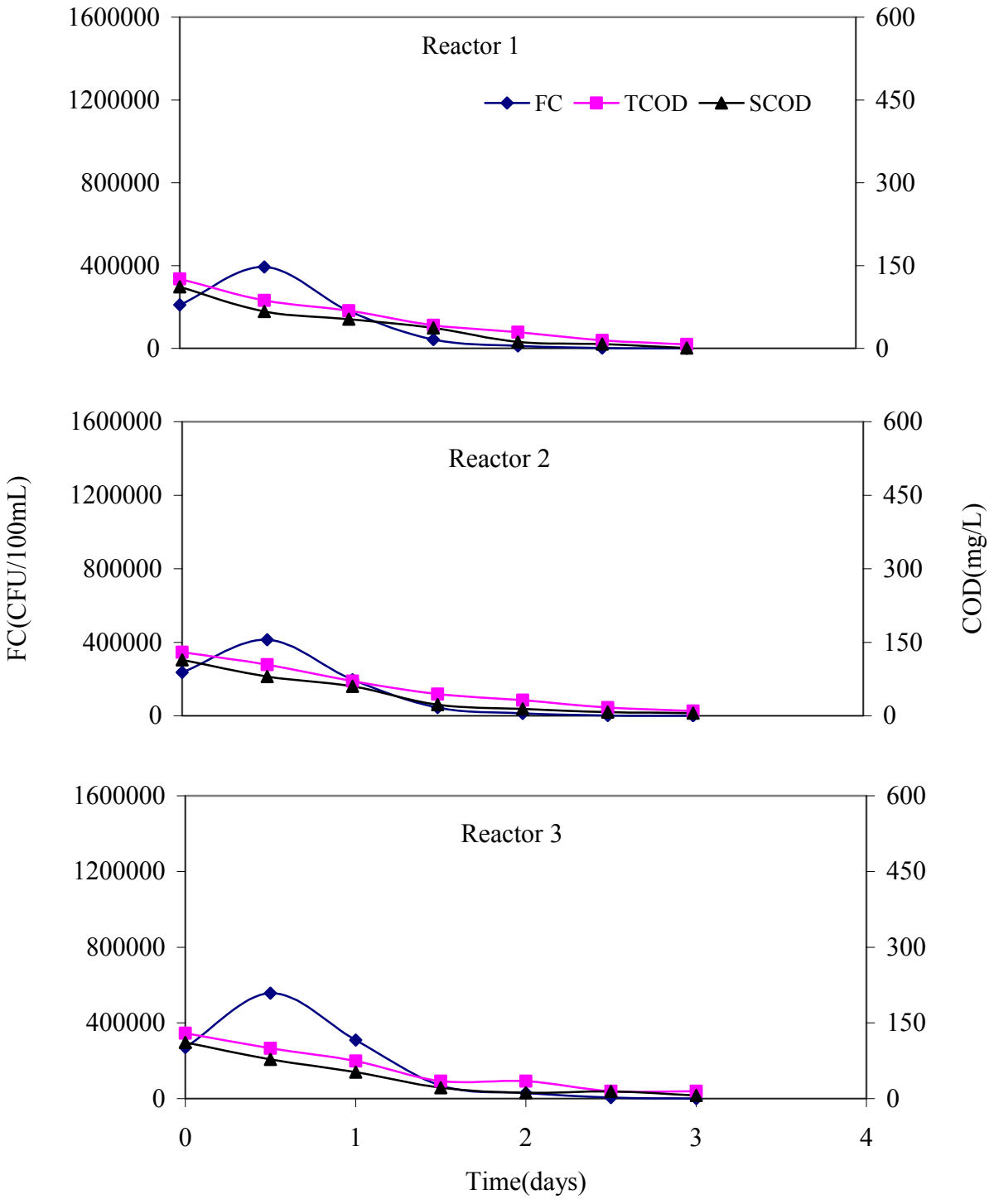


Figure 4.1.3 Batch reactors (Dilution 1/4)

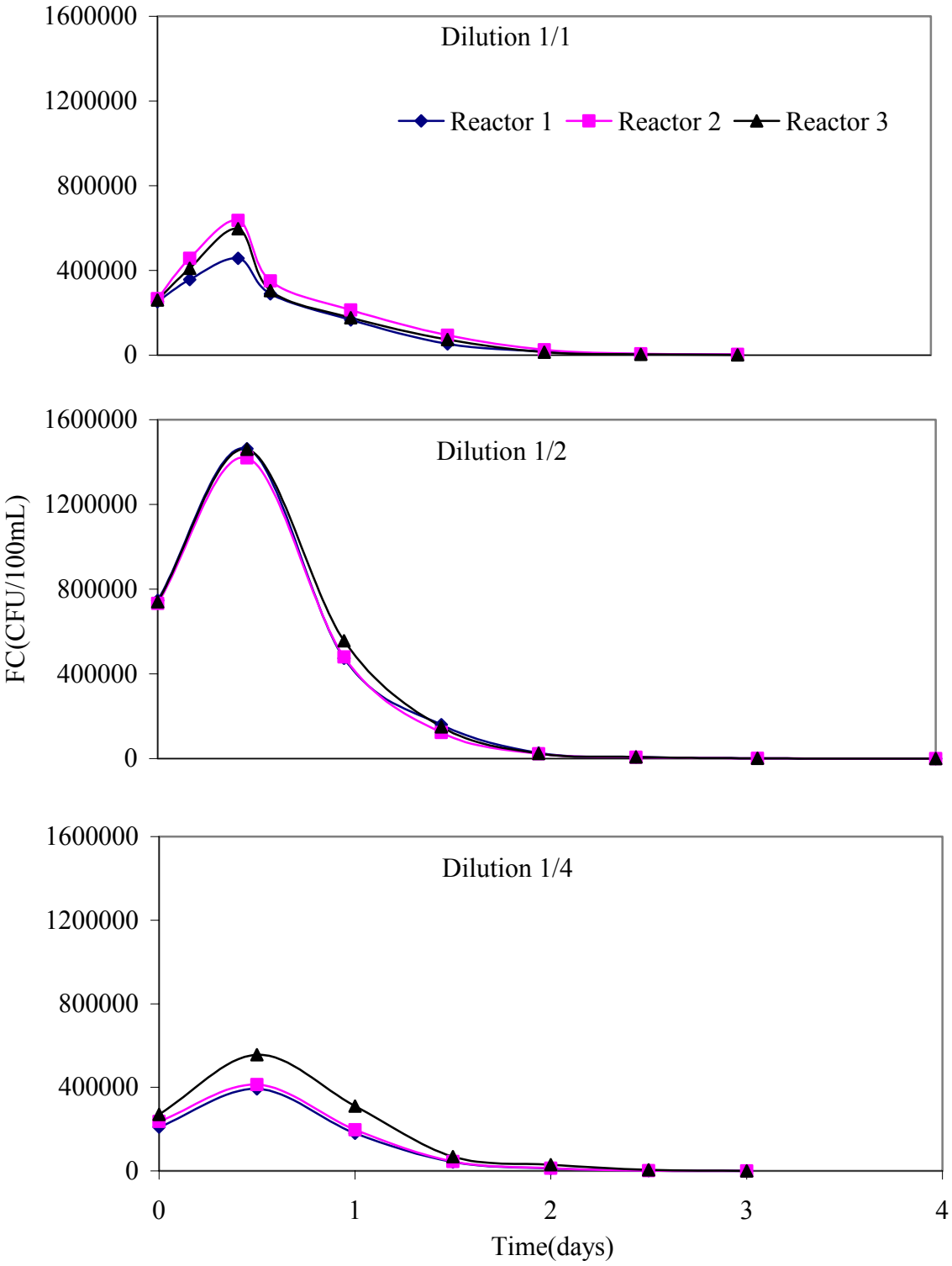


Figure 4.1.4 FC comparison for Different COD Concentrations

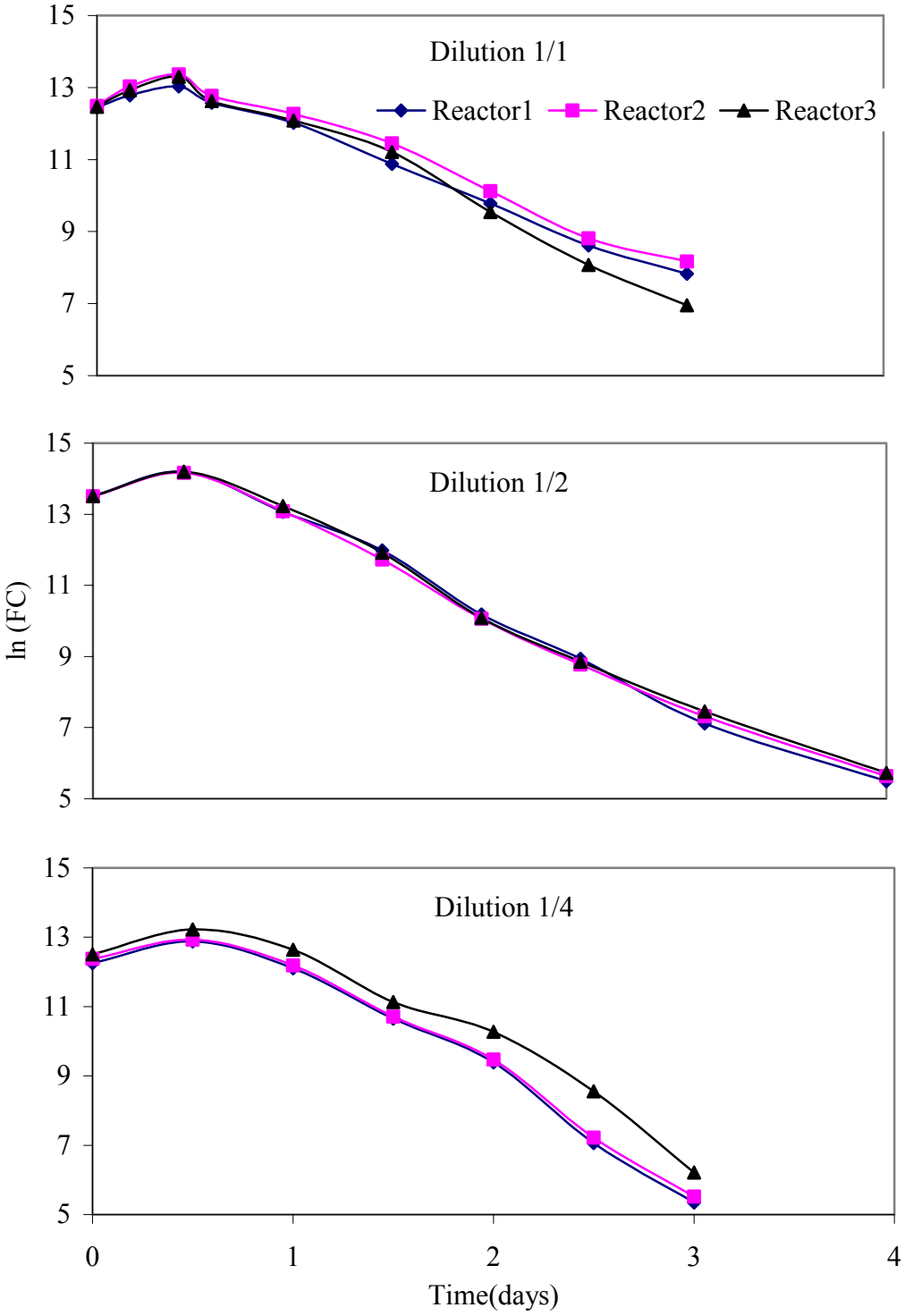


Figure 4.1.5 Comparison of ln(FC) for different COD concentration

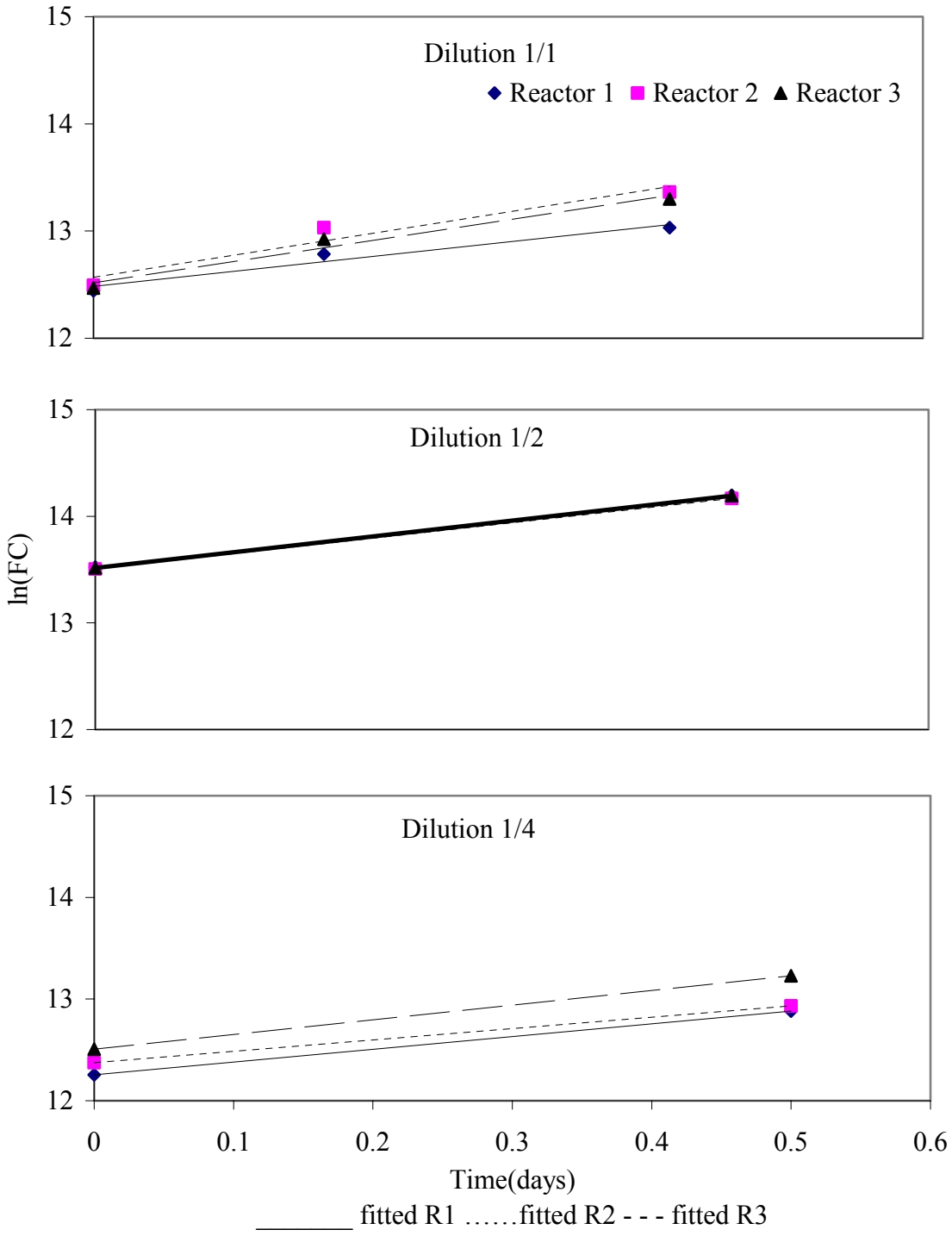


Figure 4.1.6 Regrowth period of batch reactors

Table 4.1.1 Fitted equations for regrowth period

	Reactor 1		Reactor 2		Reactor 3	
	Equation	r^2	Equation	r^2	Equation	r^2
Dilution 1/1	y=1.38x+12.5	0.96	y=2.03x+12.6	0.94	y=1.95x+12.5	0.97
Dilution 1/2	y=1.47x+13.5	NA	y=1.44x+13.5	NA	y=1.48x+13.5	NA
Dilution 1/4	y=1.25x+12.3	NA	y=1.11x+12.4	NA	y=1.45x+12.5	NA

(NA: r^2 is not reported for reactors with 2 data points)

Table 4.1.2 Summary of the regrowth period

Dilution	1/1			1/2			1/4		
Initial COD (mg/L)	470			235			127		
Initial FC (CFU/100mL)	260000			740000			270000		
Reactor	1	2	3	1	2	3	1	2	3
Average COD(mg/L)	438	438	438	218	221	217	106	117	114
$\mu_r(d^{-1})$	1.38	2.03	1.95	1.47	1.44	1.48	1.25	1.11	1.45
Mean μ_r (d^{-1})	1.79			1.46			1.27		
STD of μ_r (d^{-1})	0.35			0.02			0.17		

The r^2 is 0.54. The slope slope k_s/μ_{max} is 31.73, and the intercept$1/\mu_{max}$ is 0.52. The kinetic parameter μ_{max} is 1.92 d^{-1}, and k_s is 60.92mg/L.

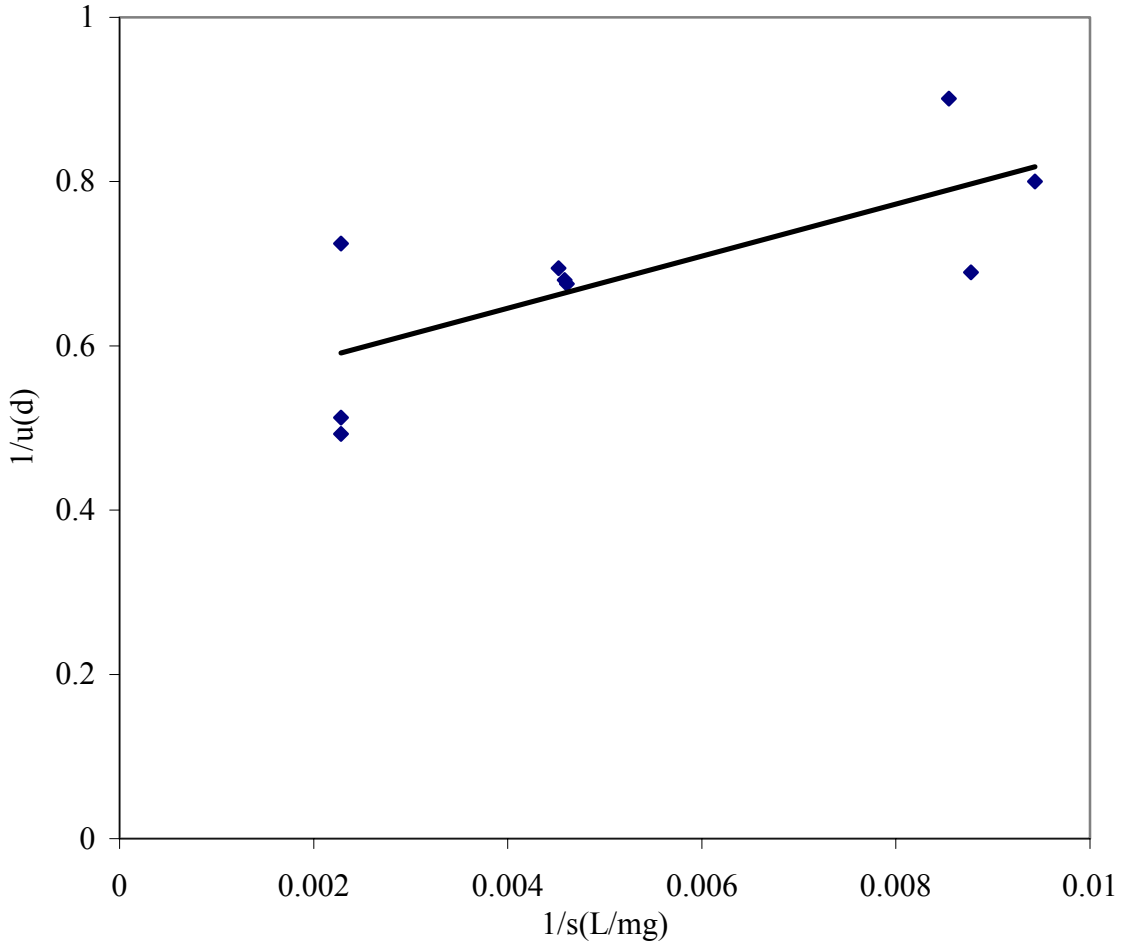


Figure 4.1.7 Lineweaver-Burk Linearization of batch studies

4.1.2 Net Dark Decay Rate Coefficient in Batch Studies at 35^{o}C

The regrowth of FC for the decay period is assumed negligible. Based on equation (4.1.1), the decay rate coefficients can be calculated as the slope of linear regression of natural ln (FC concentration) versus time. Two approaches were used to analyze the data. In Approach one, data for the whole decay period from the maximum FC concentration were fitted (Figure 4.1.8). In Approach two, data from the point after the FC reached a maximum value were fitted (Figure 4.1.9). The comparison was made because both approaches have been used for FC decay rate coefficient determination.

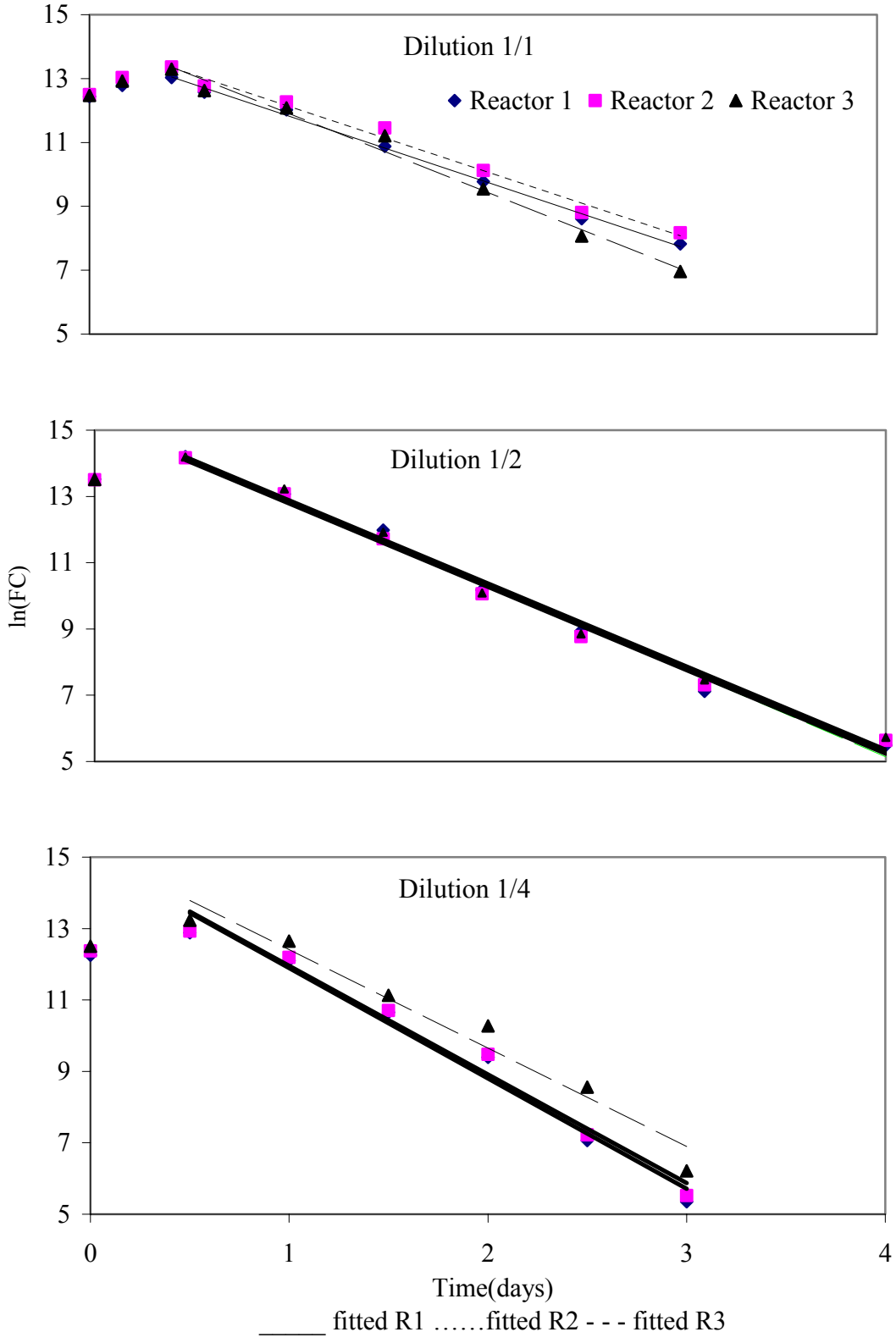


Figure 4.1.8 Plot from the Maximum FC Concentration

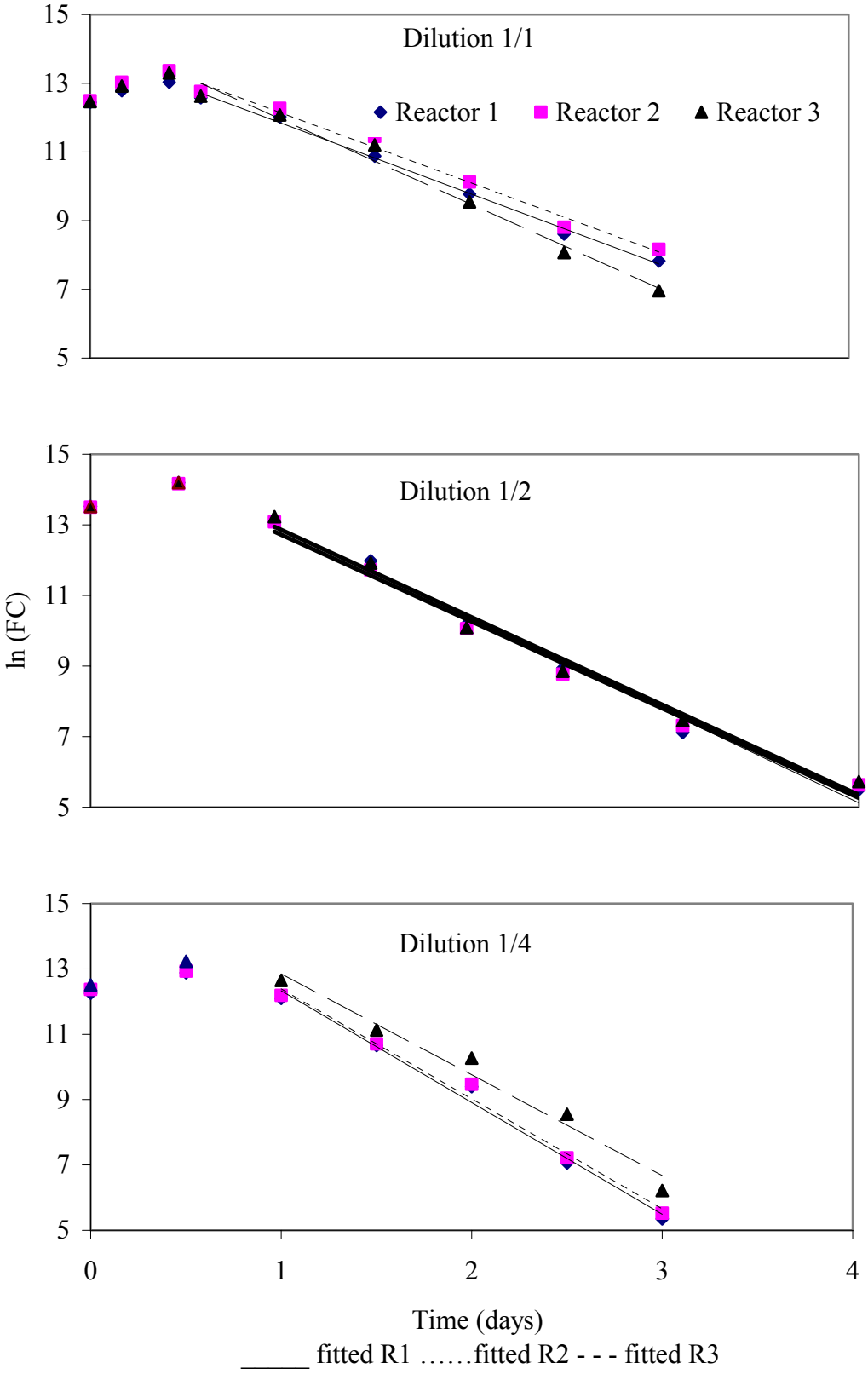


Figure 4.1.9 Plot after the Maximum FC concentration

Table 4.1.3 Fitted equations for plot from the maximum FC concentration

	Reactor 1		Reactor 2		Reactor 3	
	Equation	r^2	Equation	r^2	Equation	r^2
Dilution 1/1	y=-2.05x+13.9	0.99	y=-2.04x+14.2	0.99	y=-2.45x+14.4	0.99
Dilution 1/2	y=-2.57x+15.4	0.99	y=-2.50x+15.2	0.99	y=-2.50x+15.3	0.99
Dilution 1/4	y=-3.09x+14.9	0.98	y=-3.04x+14.9	0.98	y=-2.75x+15.1	0.96

Table 4.1.4 Fitted equations for plot after the maximum FC concentration

	Reactor 1		Reactor 2		Reactor 3	
	Equation	r^2	Equation	r^2	Equation	r^2
Dilution 1/1	y=-2.06x+13.9	0.99	y=-2.04x+14.2	0.98	y=-2.47x+14.4	0.98
Dilution 1/2	y=-2.58x+15.4	0.99	y=-2.48x+15.2	0.99	y=-2.49x+15.3	0.98
Dilution 1/4	y=-3.42x+15.7	0.99	y=-3.36x+15.7	0.99	y=-3.09x+15.9	0.97

Table 4.1.5 Summary of dark decay rate coefficient (k_d)

	Mean k_d (d^{-1}) from Table 4.1.3	STD for mean k_d (d^{-1}) from Table 4.1.3	Mean k_d (d^{-1}) from Table 4.1.4	STD for mean k_d (d^{-1}) from Table 4.1.4
Dilution 1/1	2.18	0.23	2.19	0.24
Dilution 1/2	2.52	0.04	2.52	0.05
Dilution 1/4	2.96	0.18	3.29	0.17

Table 4.1.6 Summary of the decay period from Approach two

Dilution	1/1			1/2			1/4		
Initial COD (mg/L)	470			235			127		
Initial FC (CFU/100mL)	260000			740000			270000		
Reactor	1	2	3	1	2	3	1	2	3
k_d(d^{-1})	2.06	2.04	2.47	2.58	2.48	2.49	3.42	3.36	3.09
Mean k_d (d^{-1})	2.19			2.52			3.29		

From Table 4.1.5, it can be seen that both approaches yield similar results. PROC GLM (SAS, version 8.1) was performed and P-value of 0.221 was obtained. Since P-value is larger than α=0.05, indicating that the two approaches had no big difference.The decay rate coefficient calculated from the point after the maximum FC concentration will be used in this study since Approach two may be more reasonable due to missing the point of the maximum FC concentration . Auer and Niehaus (1993) also calculated the decay rate coefficient of FC over the period when the population was clearly in decline to avoid effects from regrowth during the early stages of incubation. Since there was no light provided in the air bath shaker, the decay rate coefficients calculated in this study were dark decay rate coefficients. After FC concentration reached maximum, FC might still have small amount of regrowth.

Scott (2000) determined k_d values for dairy wastewater by using batch reactors over temperature ranges of 17.6-31.9^{o}C.Using the temperature factor (θ=1.149) and k_d at 20^{o}C (0.133 d^{-1}) obtained in that study, a k_d value of 1.08d^{-1} is predicted for 35^{o}C, which is much lower than the k values of this study. The mean k values at 35^{o}C of this study were 2.19, 2.52 and 3.29 d^{-1}, for the initial COD concentrations of 478, 235 and 127 mg/L, respectively (Table 4.1.6). In Scott's study, the COD concentrations averaged 1160 ± 419 mg/L, because the raw wastewater was filtered only through cotton. However, the COD concentrations in this batch study ranged from 127mg/L to 478mg/L, because the raw wastewater was filtered through 0.2μm filter.

The relationship between FC decay rate coefficient and COD concentration was investigated by using PROC REG (SAS, Version 8.1). The obtained P-value of 0.0004, which is much smaller than α=0.05, implies that the effect of COD concentrations on the FC decay rate coefficient is significant. A linear regression relationship between decay rate coefficient and substrate concentration is

k_d =3.460-0.00497 *S (4.1.4)

Where k_d = dark decay rate coefficient, d^{-1};

S= substrate concentration TCOD, mg/L.

The relationship between COD concentration and decay rate coefficient is supported by adding the data from Scott (2000). Plotting data from this study and data from Scott (k_d =1.08 d^{-1}, COD=1160mg/L), Figure 4.1.10 is obtained. The R^2 for the fitted equation is 0.84. The fitted equation is

k_d =3.248-0.00203 *S (4.1.5)

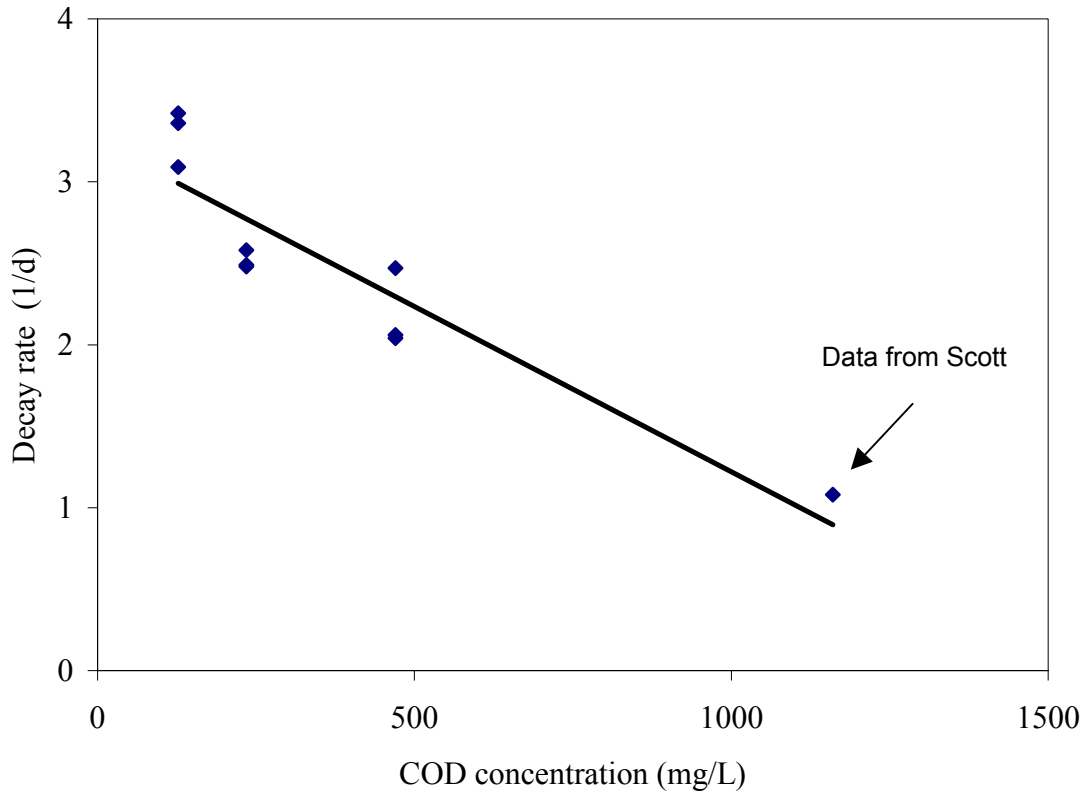


Figure 4.1.10 Relationship between k_d and COD at 35°C

These results suggest that either k_d is a function of organic carbon concentration, or that this batch technique actually measures net decay (decay minus regrowth) rather than true decay. From Figure 4.1.10, it seems that relationship between decay rate and substrate concentration is not linear.

$$k_d(net) = k_d(true) - \mu = k_d - \frac{\mu_{\max} S}{k_S + S} \qquad (4.1.6)$$

PROC NLIN (SAS, Version 8.1) was performed to solve Equation (4.1.6). k_d (true) of 4.076 , μ_{max} of 4.02 , and k_S of 458.6 mg/L were obtained.

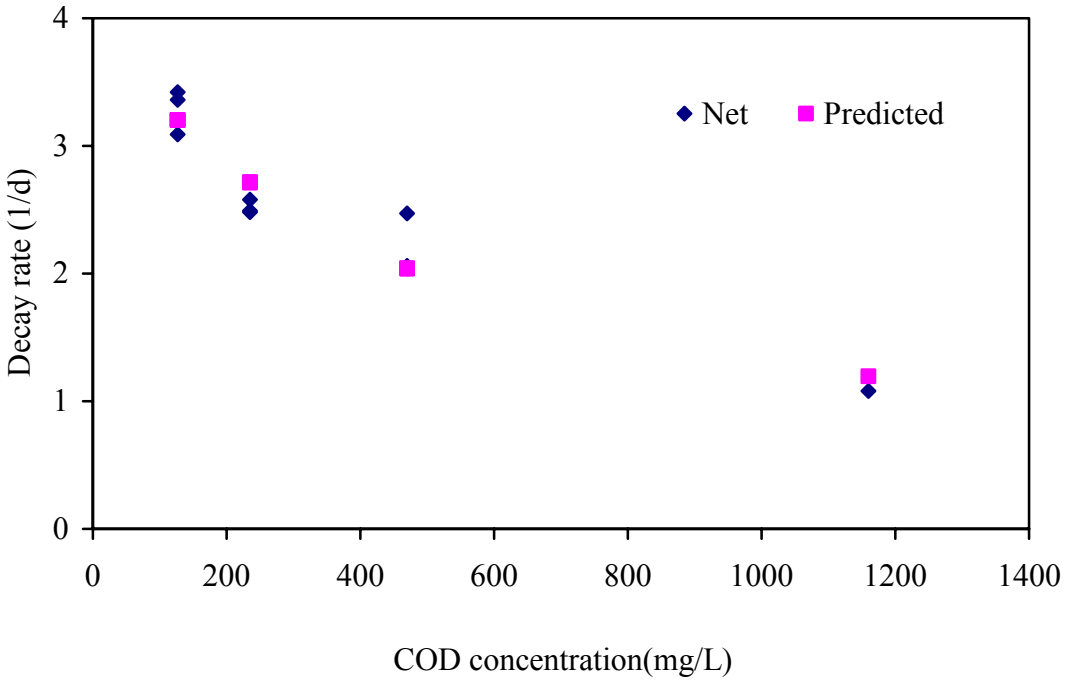


Figure 4.1.11 Comparison of net k_d and predicted net k_d from non-linear regression

PROC REG in SAS (version 8.1) was performed to analyze the linear regression relationship between FC decay rate coefficient and the inoculation. The obtained P-value of 0.522, which is much larger than α= 0.05, indicates that the effect of inoculation on the FC decay rate coefficient is not significant.

4.2 CSTR

4.2.1 CSTR's Operated at 25°C

The results of the three reactors for hydraulic retention times of 1.7, 3.5, and 6.9days at 25°C are shown in Figure 4.2.1-4.2.2. This study was conducted to survey if FC would show regrowth at 25°C; therefore, replicate studies were not conducted. Figure 4.2.1 directly shows the change of FC, Soluble COD, and Total COD with time. Figure 4.2.2 emphasizes the change of ln (FC concentration) with time. From these figures, it can be seen that FC and substrate (Total COD and Soluble COD) decreased continuously with time. It can be concluded that no steady state was achieved in the CSTR at 25°C. Since no light was provided in the experiment, the decay rate coefficient in this study was dark decay rate coefficient k_d.

Since no steady state exists in the CSTR, equation (1.1.4) cannot be used to calculate the decay rate coefficient of FC. However, FC still satisfies the mass balance.

$$\frac{dN}{dt} = \frac{N_{in}}{\tau} + \mu N - k_d N - \frac{N}{\tau} \tag{4.2.1}$$

Where N=effluent FC concentration, CFU/100mL;

N_{in}=influent FC concentration, CFU/100mL;

μ=specific growth rate coefficient, d^{-1};

k_d=decay rate coefficient, d^{-1};

τ=hydraulic retention time (HRT), d.

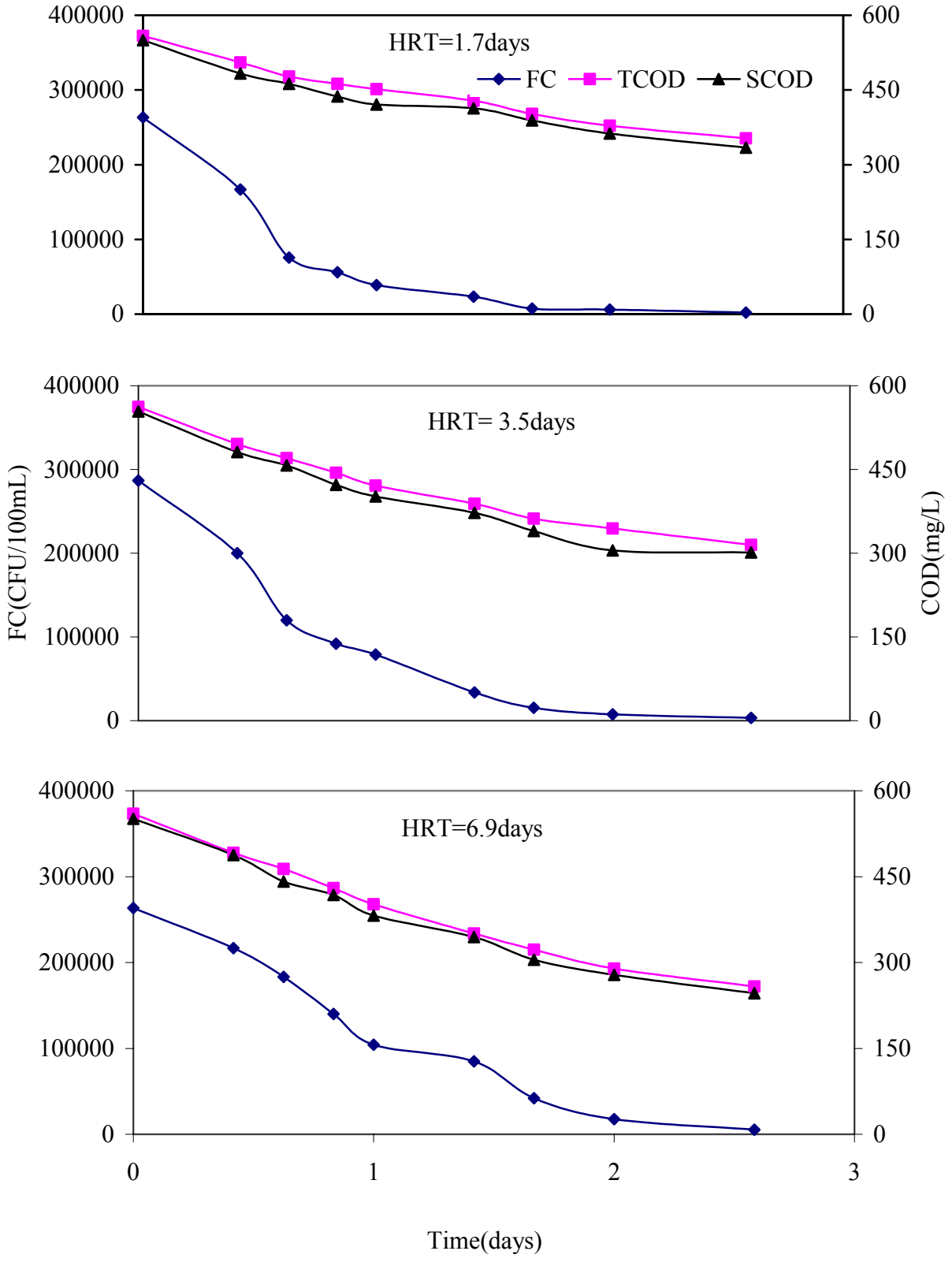


Figure 4.2.1 CSTR study at 25 degree C(plot FC vs time)

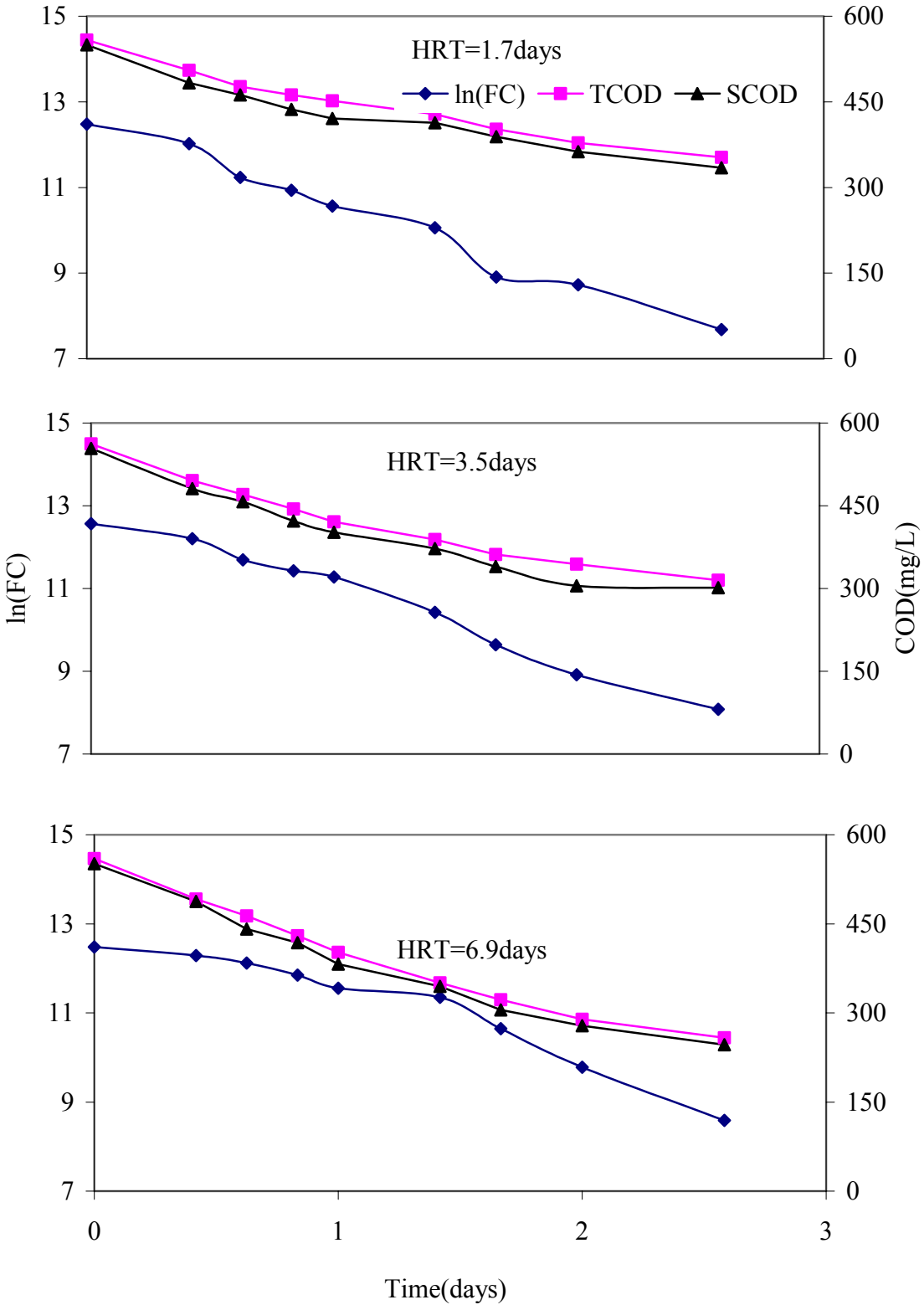


Figure 4.2.2 CSTR study at 25 degree C (plot ln(FC) vs time)

In the CSTRs of this study, the influent ultra-filtered wastewater contained 0-100CFU/100mL FC, which was negligible, compared to the FC concentration in the reactors. So, N_{in} is assumed to be 0 in this study. Equation (4.2.1) is simplified to

$$\frac{dN}{dt} = (\mu - k_d - \frac{1}{\tau})N \qquad (4.2.2)$$

No apparent regrowth was observed during the initial stage of inoculation at 25°C of the three hydraulic retention times. Therefore, μ=0 is assumed. Hence, equation (4.2.2) can be reduced to:

$$\frac{dN}{dt} = (-k_d - \frac{1}{\tau}) * N \qquad (4.2.3)$$

From equation (4.2.3), it can be seen that the slope of linear regression ln (FC concentration) versus time is $(-k_d\text{-}1/\tau)$. The results of ln (FC concentration) versus time for hydraulic retention times of 1.7, 3.5, and 6.9 days at 25°C are shown in Figure 4.2.3. The fitted equations from linear regression are listed in Table 4.2.1.

Table 4.2.1 Fitted equations for FC decay in CSTR at 25°C

	Equation	R^2	$-k_d\text{-}1/\tau$ (d^{-1})	$1/\tau(d^{-1})$	$k_d(d^{-1})$	Mean k_d (d^{-1})
HRT=1.7d	y=-1.93x+12.5	0.98	-1.93	1/1.7	1.34	1.43
HRT=3.5d	y=-1.86x+12.9	0.98	-1.86	1/3.5	1.57	
HRT=6.9d	y=-1.53x+12.9	0.94	-1.53	1/6.9	1.38	

From Figure 4.2.3, it can be seen that loglinear regression fits the data well. The R^2 values reported in Table 4.2.1 are more than 0.90, also indicating the goodness of fit. From Table 4.2.1, it also can be found that the decay rate coefficients of FC for hydraulic retention times of 1.7, 3.5, and 6.9days at 25°C are 1.34, 1.57, and 1.38 d^{-1}, respectively. The mean of decay rate coefficients for these three hydraulic retention times in CSTR is 1.43 d^{-1}. The k values of this study are similar to the values reported for the wastewater environments at similar temperature

(Table 2.3.1), although lower values reported by Scott (2000) for dairy wastewater. Because the substrate concentrations for 1.7, 3.5, and 6.9days of hydraulic retention times had almost the same values of 560 mg/L for total COD, it is reasonable to expect similar k values for other hydraulic retention times within this range.

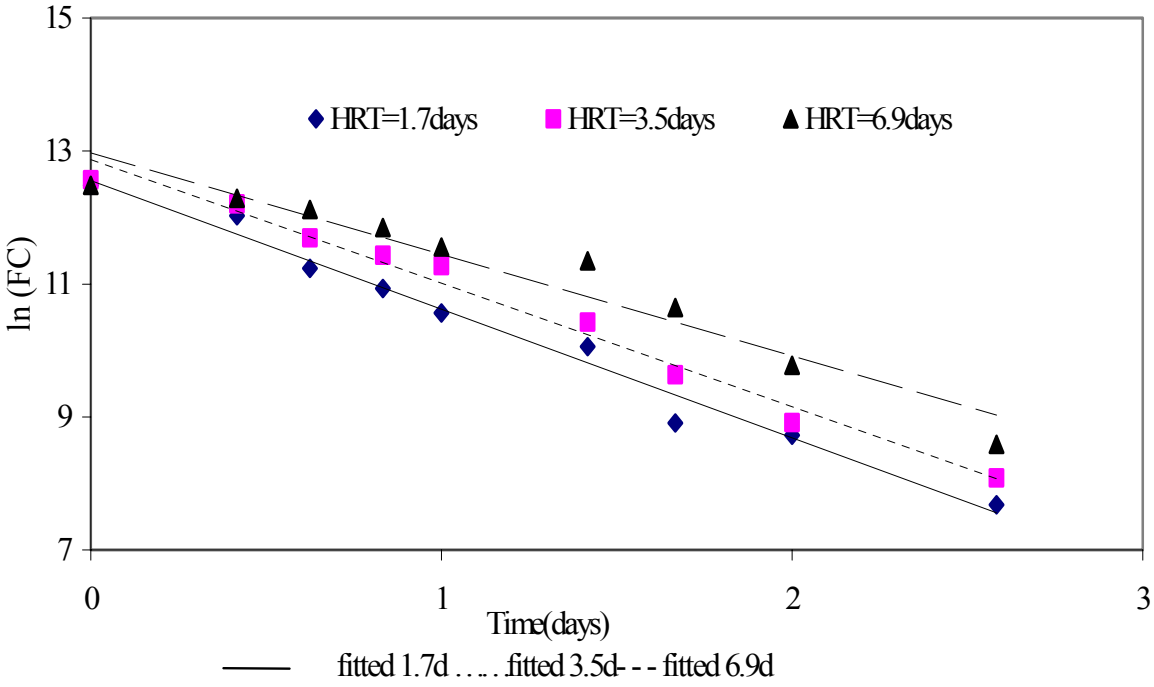


Figure 4.2.3 CSTR study of ln(FC) at 25 degree C

4.2.2 CSTR's Operated at 35°C

Triplicate reactors were conducted for different hydraulic retention times at 35°C. Mean DO values were 0.1mg/L .The results of the CSTR experiments at 35°C are shown in Figures 4.2.4-4.2.8. At 35°C, regrowth of FC occurred to various degrees during the initial stage of inoculation for the three hydraulic retention times of 1.7, 3.5, and 6.9 days. Figure 4.2.9 compares FC concentration change with time for the three hydraulic retention times, while Figure 4.2.10 compares ln (FC concentration) change with time. Because the FC concentration in

the CSTR of hydraulic retention time of 3.5days declined more slowly than those of hydraulic retention time of 1.7days and 6.9days, indicating steady state conditions may be reached, the experiment at hydraulic retention time of 3.5days was conducted three times.

The 1.7 and 3.5 days hydraulic retention time reactors appeared to reach steady state conditions with respect to COD concentration. However, the FC concentration declined rapidly for the 1.7 and 6.9days hydraulic retention times.

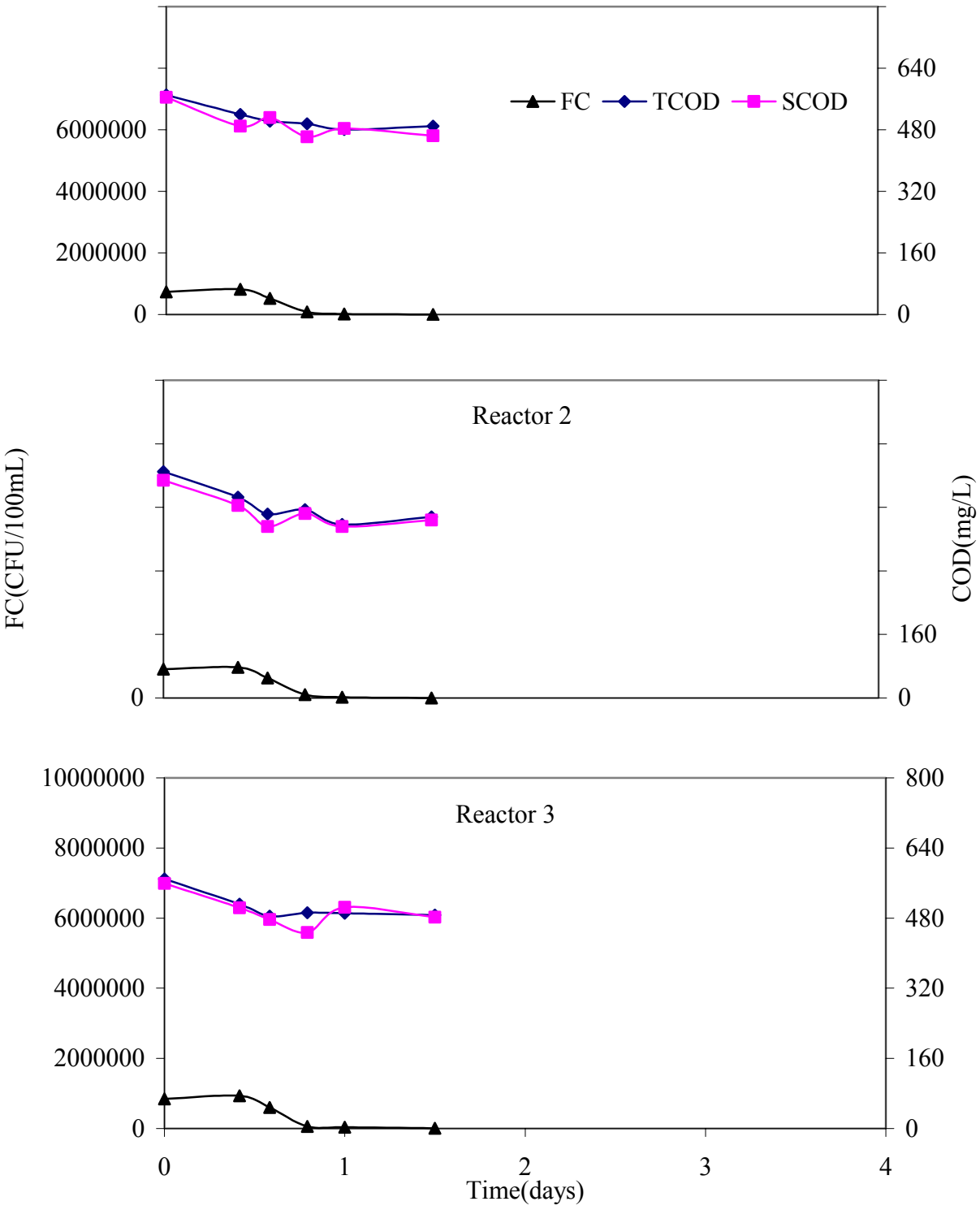
At 35^{o}C, no obvious steady state conditions with respect to FC were achieved, although 3.5 days hydraulic retention times were near steady state. Therefore, equation (1.1.4) cannot be used to separate regrowth rate coefficient and decay rate coefficient. But FC still satisfies mass balance in CSTR at 35^{o}C as given in equation (4.2.1). Because the regrowth of FC is very obvious, the decay for the first stage may be negligible. Assuming N_{in}=0 and k_d =0 for the first stage, equation (4.2.1) is simplified to:

$$\frac{dN}{dt} = (\mu_r - \frac{1}{\tau})N \qquad (4.2.4)$$

Where μ_r = the regrowth rate coefficient of the regrowth period, d^{-1}.

μ_r-1/τ can be calculated by linear regression of ln (FC concentration) versus time. Hendricks (1972) also used this equation to calculate the growth rate coefficient of E.coli in river water in continuous culture system.

The regrowth rate coefficient μ_r is calculated by using the data till the point of maximum FC concentration. The plot of ln (FC concentration) versus time for the first stage is shown in Figure 4.2.11. The fitted equations from linear regression are listed in Table 4.2.2.



Figur 4.2.4 HRT=1.7days and Temp=35degree C

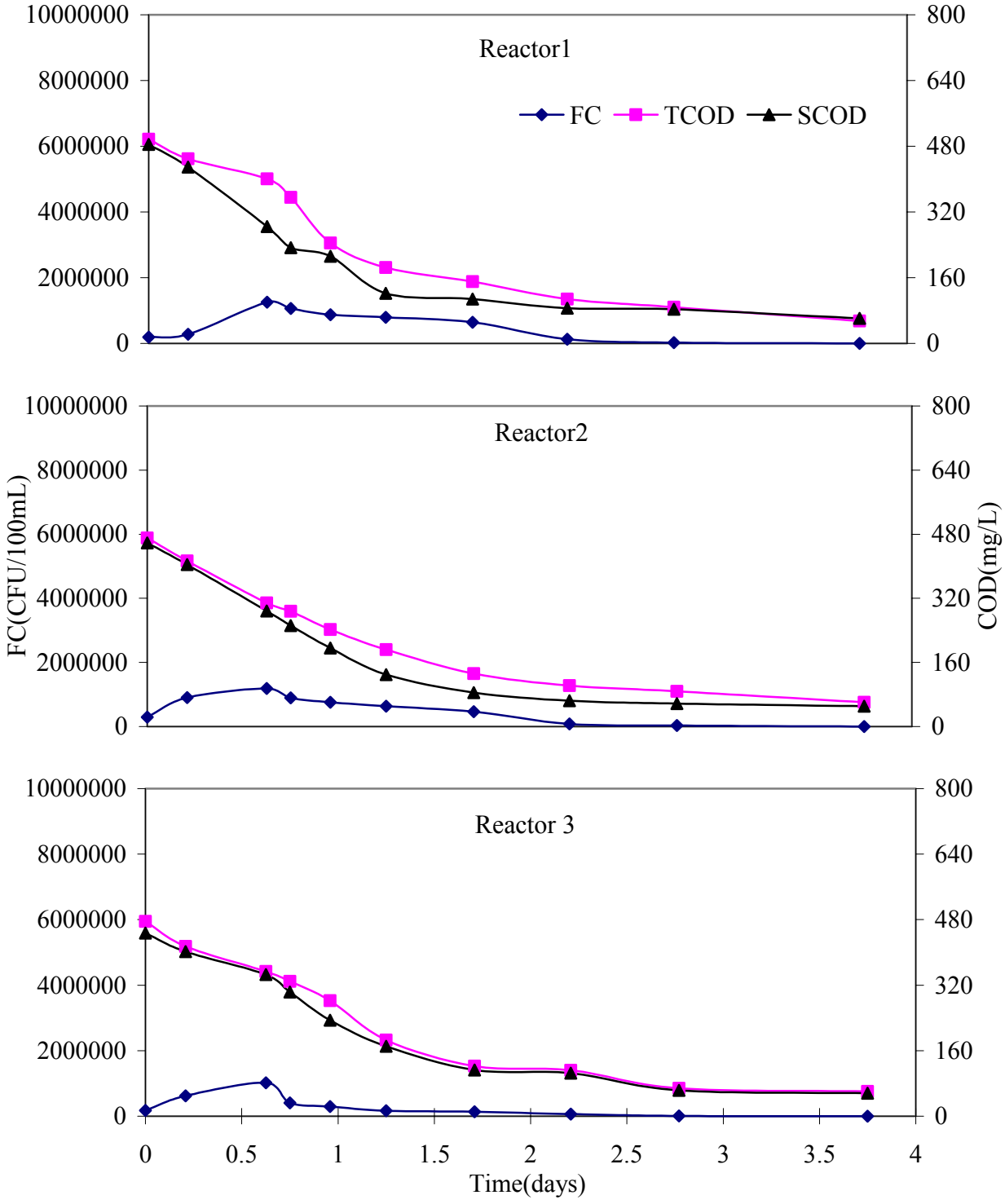


Figure 4.2.5 HRT=3.5d Trial 1 and Temp=35degree C

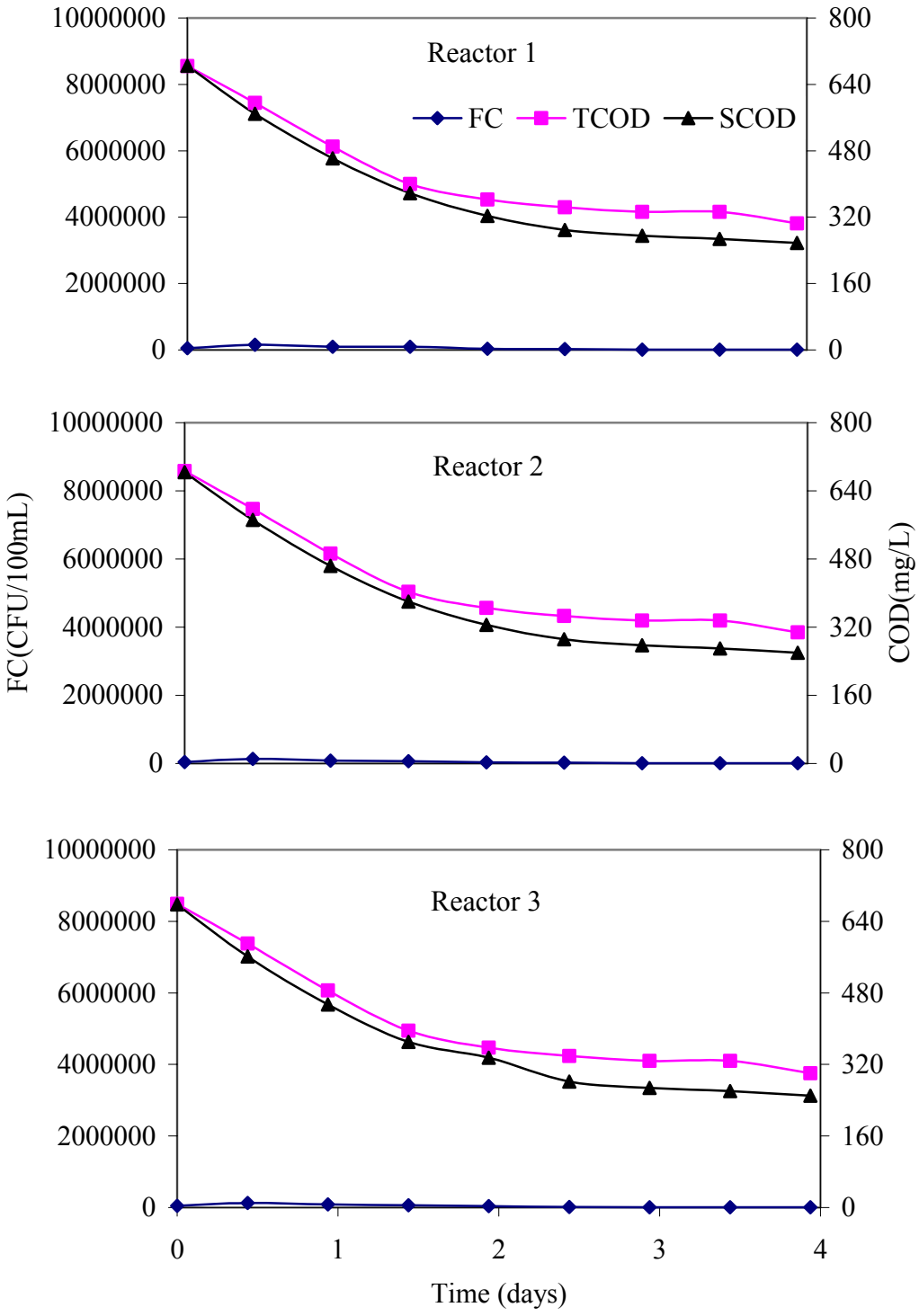


Figure 4.2.6 HRT=3.5d Trial 2 and Temp=35degree C

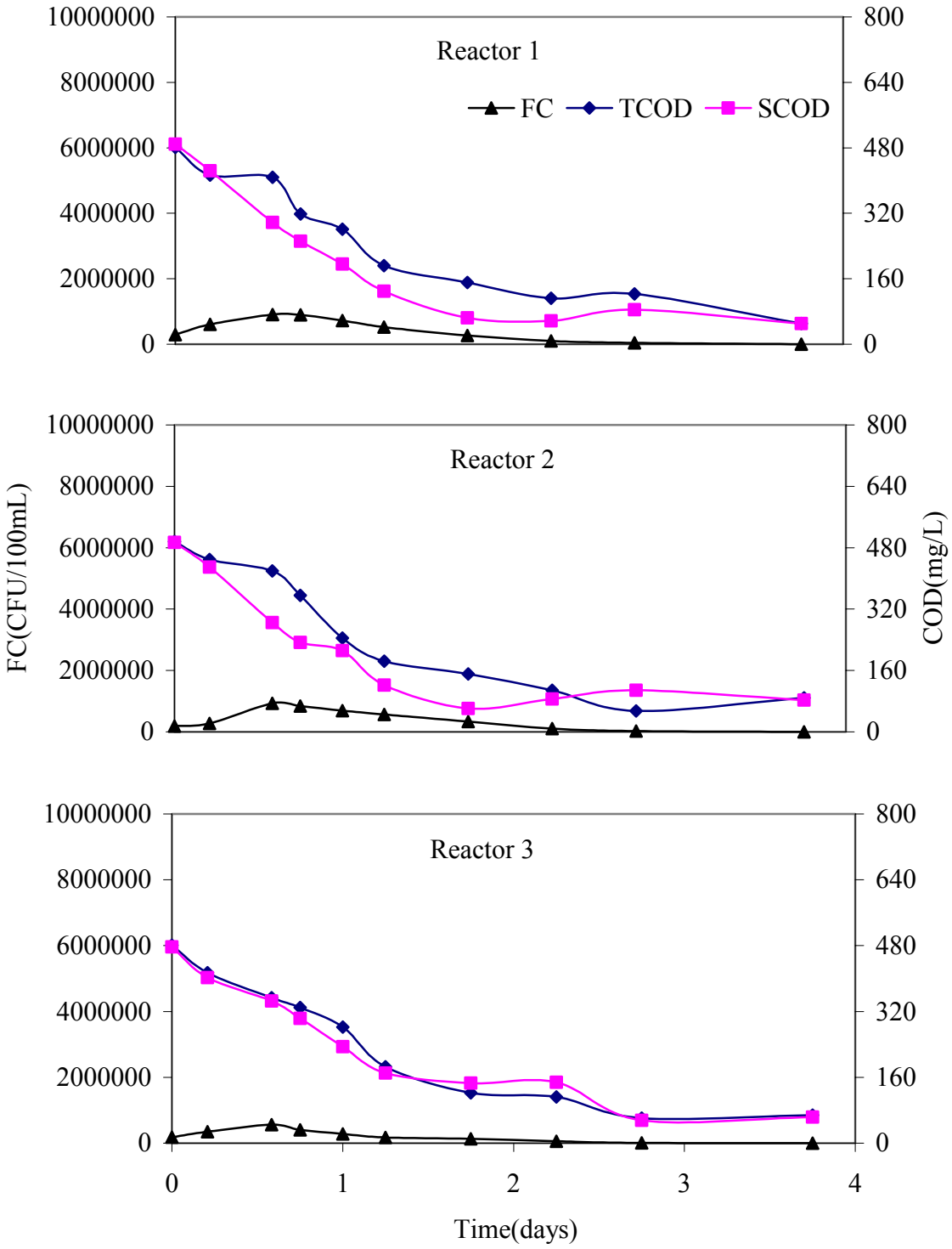


Figure 4.2.7 HRT=3.5days Trial 3 and Temp=35degree C

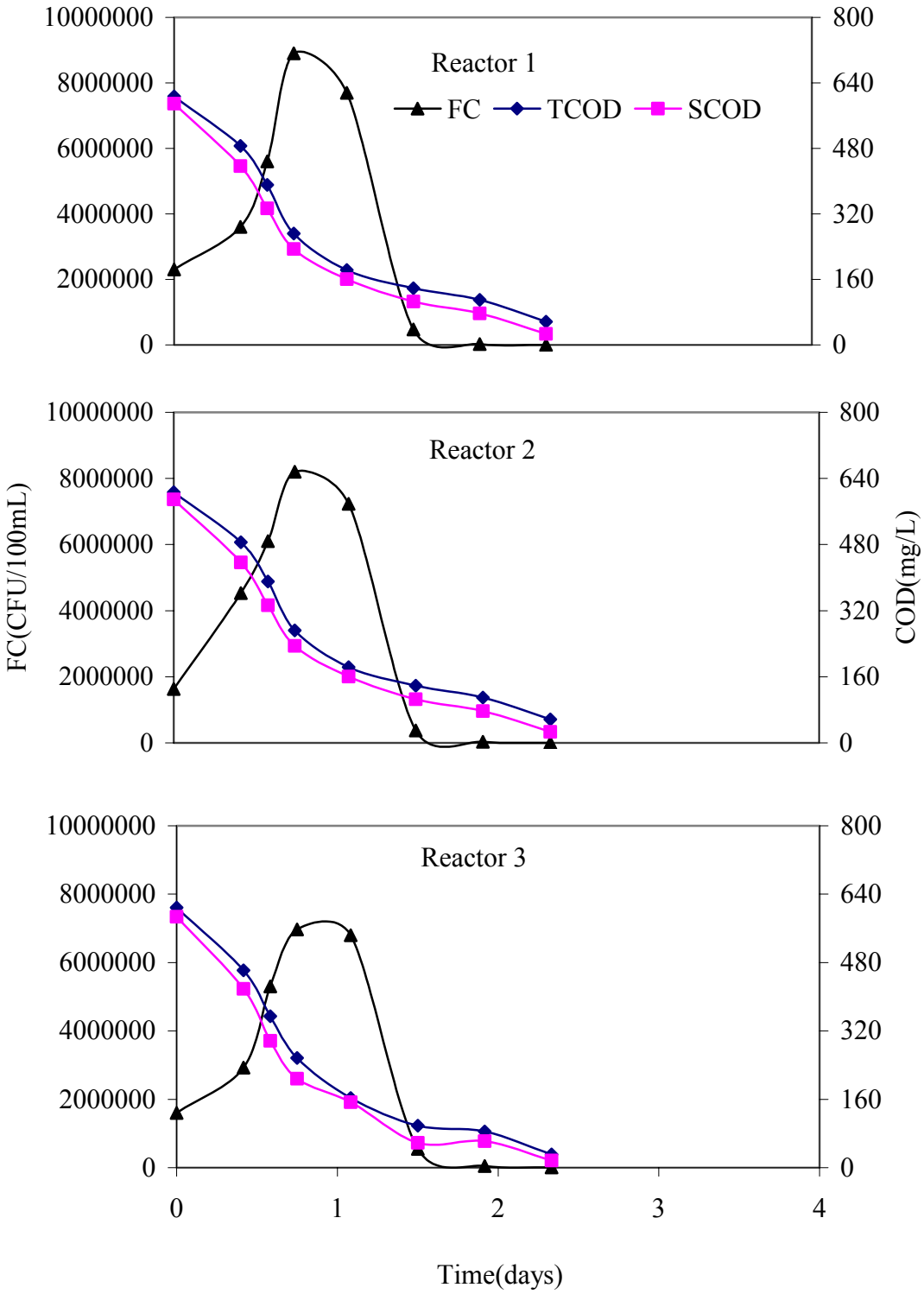


Figure 4.2.8 HRT=6.9days and Temp=35degree C

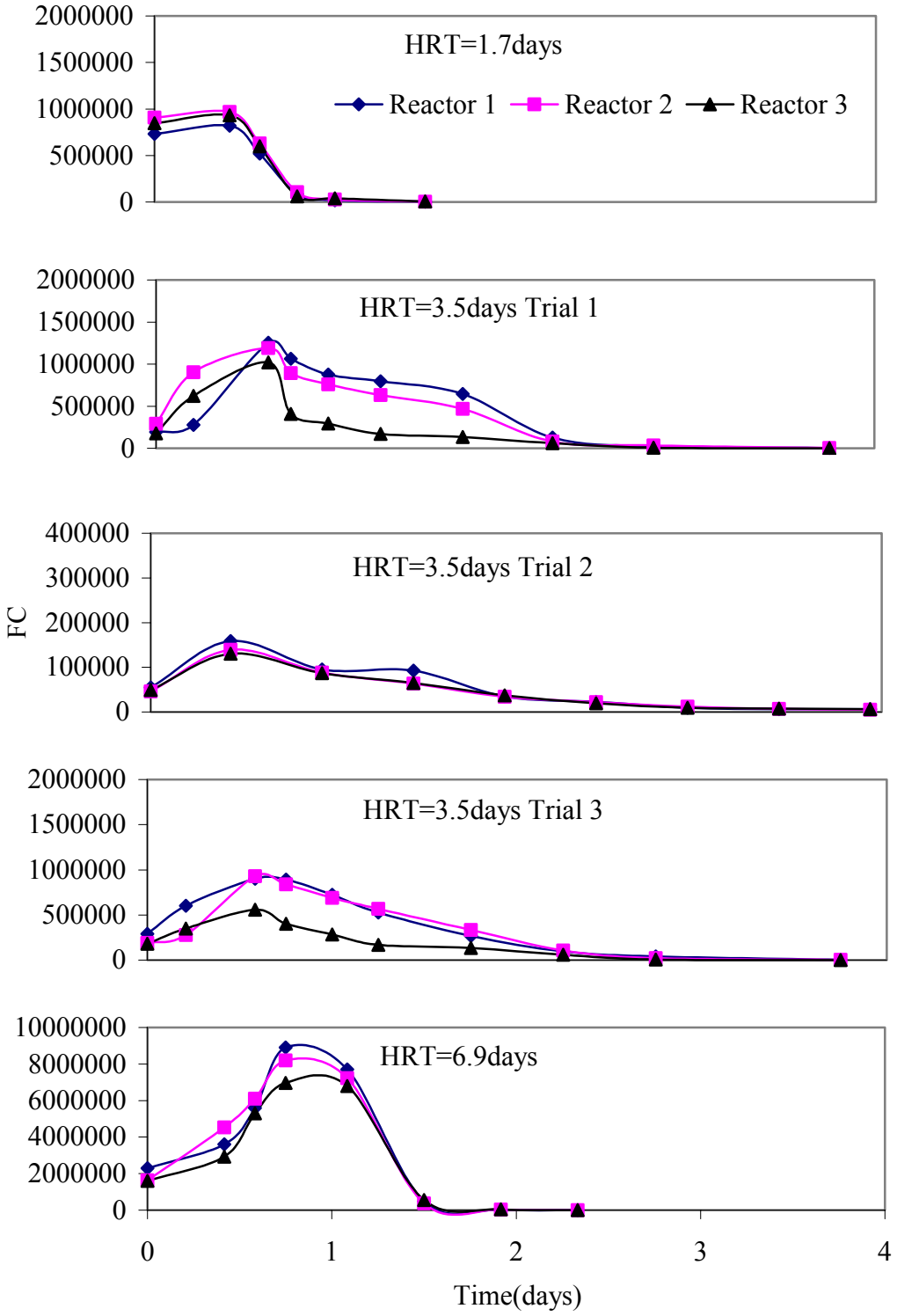


Figure 4.2.9 FC comparison at 35 degree C

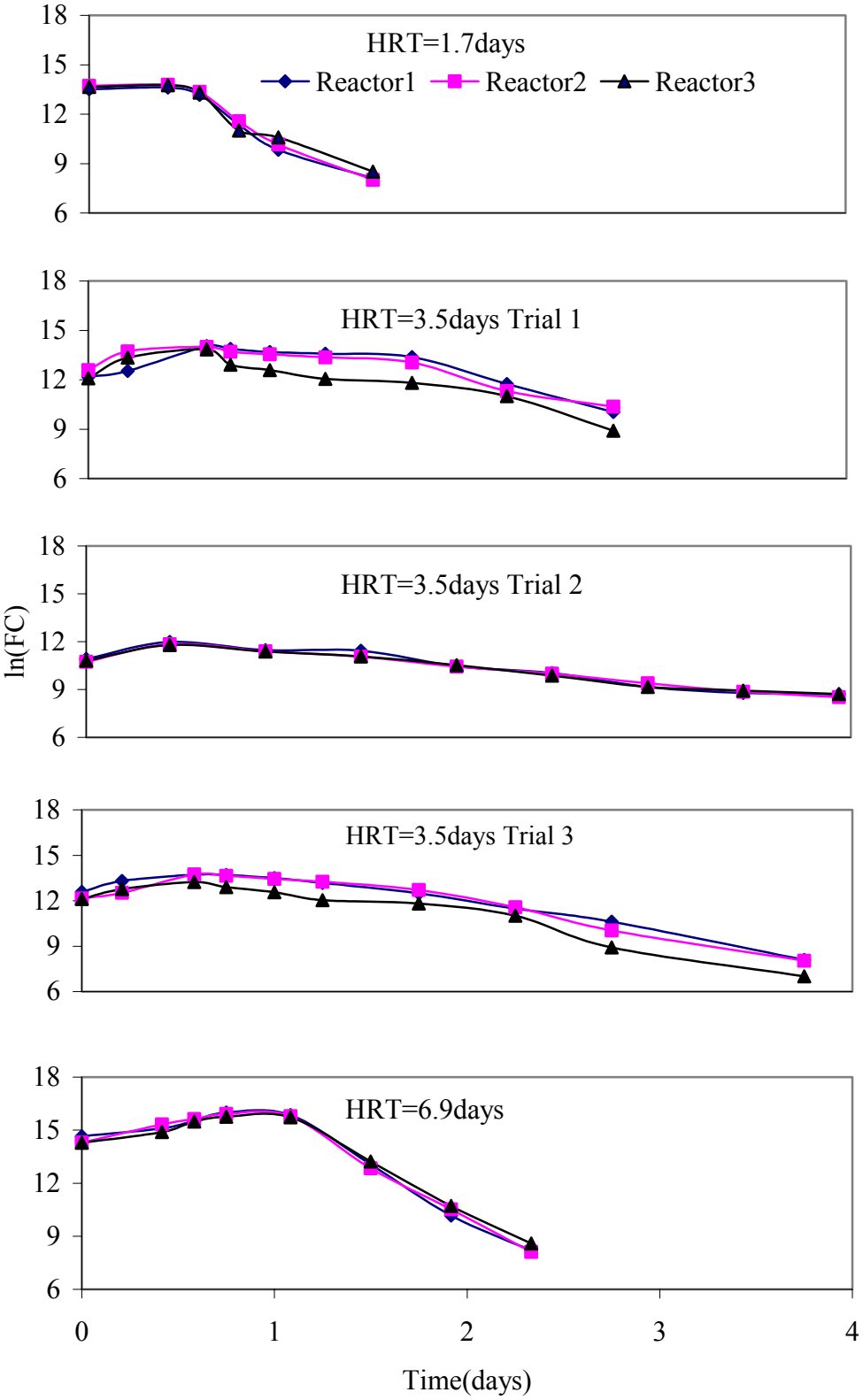


Figure 4.2.10 Comparison of ln(FC) at 35 degree C

Table 4.2.2 Fitted equations for regrowth period of CSTR at 35°C

	Reactor 1		Reactor 2		Reactor 3	
	Equation	r^2	Equation	r^2	Equation	r^2
HRT=1.7days	y=0.27x+13.5	NA	y=0.15x+13.7	NA	y=0.23x+13.6	NA
HRT=3.5days Trial 1	y=3.08x+12.0	0.98	y=2.03x+12.8	0.74	y=2.57x+12.4	0.82
HRT=3.5days Trial 2	y=2.42x+10.9	NA	y=2.53x+10.7	NA	y=2.23x+10.8	NA
HRT=3.5days Trial 3	y=1.70x+12.7	0.90	y=2.56x+12.1	0.98	y=1.73x+12.2	0.94
HRT=6.9days	y=1.75x+14.6	0.94	y=2.17x+14.9	0.99	y=2.00x+14.3	0.97

(NA: R^2 is not reported for reactors with 2 data points)

Table 4.2.3 Summary of the regrowth period of CSTR at 35°C

HRT (day)	μ_r-1/τ (d^{-1})			μ_r(d^{-1})			Mean of μ_r (d^{-1})	STD (d^{-1})
	Reactor1	Reactor2	Reactor3	Reactor1	Reactor2	Reactor3		
1.7	0.27	0.15	0.23	0.86	0.74	0.90	0.83	0.08
3.5	3.08	2.03	2.57	3.37	2.32	2.86	2.85	0.52
3.5	2.42	2.53	2.23	2.71	2.82	2.52	2.68	0.15
3.5	1.70	2.56	1.73	1.99	2.85	2.02	2.29	0.49
6.9	1.75	2.17	2.00	1.89	2.31	2.14	2.11	0.21

The mean regrowth rate coefficients in the CSTR are 0.83, and 2.11 d^{-1} for the hydraulic retention time of 1.7, and 6.9days at 35°C. The mean regrowth rate coefficients in the CSTR are 2.85, 2.68, and 2.29d^{-1} for the hydraulic retention time of 3.5days at 35°C. Hendricks (1972) obtained specific growth rate coefficients of 0.696 d^{-1} for E.coli at 30°C. Compared to the study of Hendricks, the CSTR in this study was operated under higher temperature and higher COD concentration. It is reasonable to have a higher regrowth rate coefficient than Hendrick's.

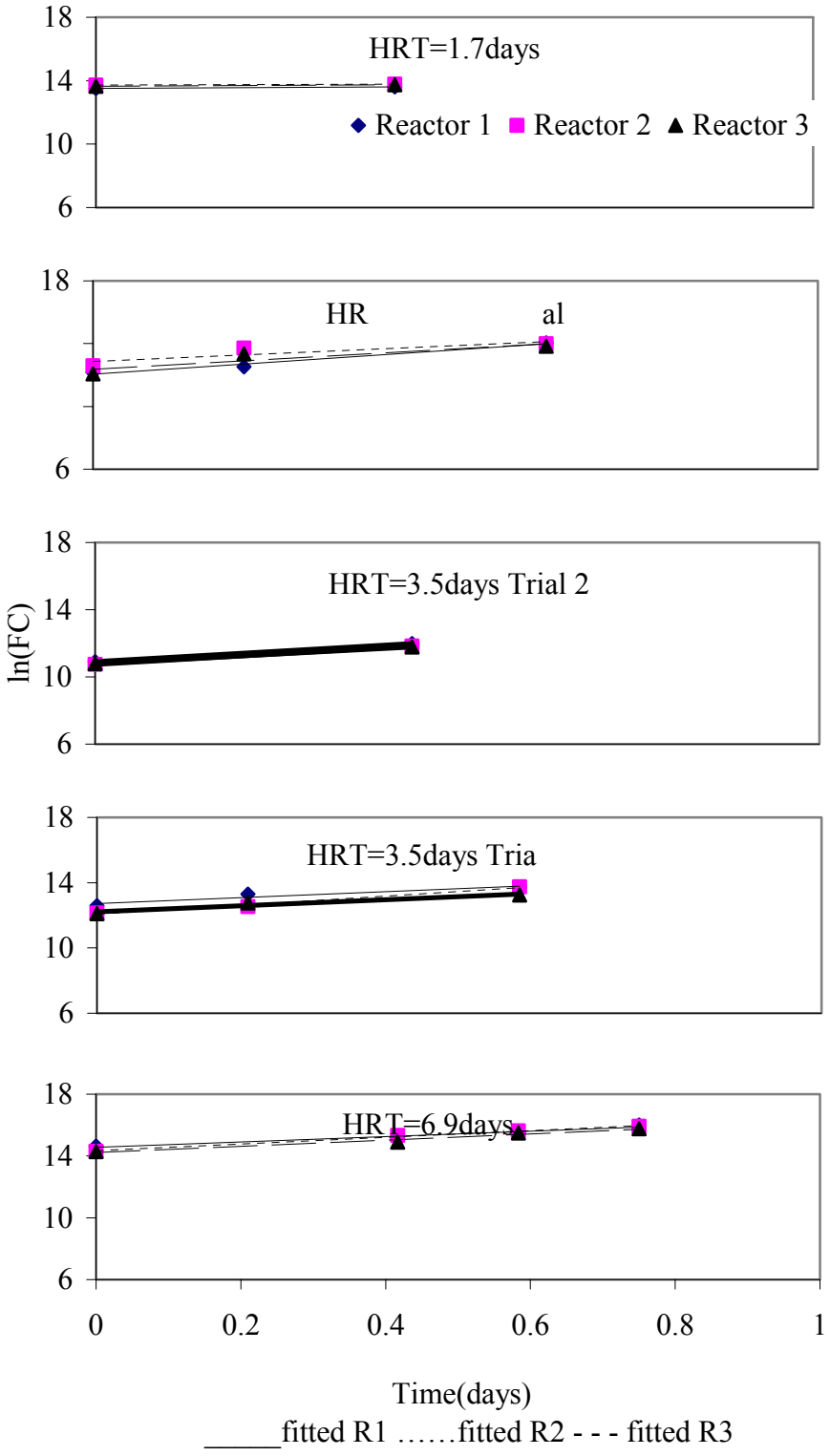


Figure 4.2.11 Regrowth period of CSTR at 35 degree C

The mean regrowth rate coefficients in the CSTR are 0.83, and 2.11 d^{-1} for the hydraulic retention time of 1.7, and 6.9days at 35^{o}C. The mean regrowth rate coefficients in the CSTR are 2.85, 2.68, and 2.29d^{-1} for the hydraulic retention time of 3.5days at 35^{o}C. Hendricks (1972) obtained specific growth rate coefficients of 0.696 d^{-1} for E.coli at 30^{o}C. Compared to the study of Hendricks, the CSTR in this study was operated under higher temperature and higher COD concentration. It is reasonable to have a higher regrowth rate coefficient than Hendrick's.

Table 4.2.4 Summary of μ_r and mean COD concentration of the regowth period.

HRT (d)	μ_r (d^{-1})	S (mg/L)
1.7	0.86	450
	0.74	389
	0.9	414
3.5 Trial 1	3.37	439
	2.32	458
	2.86	414
3.5 Trial 2	1.99	587
	2.86	589
	2.03	582
3.5 Trial 3	2.72	544
	2.82	537
	2.52	540
6.9	1.89	439
	2.31	439
	2.14	432

From Table 4.2.4, it can be seen that the regrowth rate coefficient for the hydraulic retention time of 1.7 days is much lower than the others, even under the similar mean COD concentrations. This may be due to near washout conditions in the reactors of the 1.7 days of hydraulic retention time.

Based on Table 4.2.4, Lineweaver-Burk Linearization method is used to determine the kinetic parameters of k_s and μ_{max} for the CSTR of the regrowth period by using data for 3.5days hydraulic retention times. Plotting $1/\mu_r$ versus 1/S (Figure 4.2.12), to get

$$\frac{1}{\mu_r} = 68.78\frac{1}{S} + 0.25 \qquad (4.2.5)$$

Where S = the mean COD of the regrowth period, mg/L.

The r^2 is 0.11. The slope k_s/μ_{max} is 68.78, and the intercept$1/\mu_{max}$ is 0.25. The kinetic parameter μ_{max} is 4.00d^{-1}, and k_s is 275.12mg/L. This relationship may be of limited usefulness for this small data set.

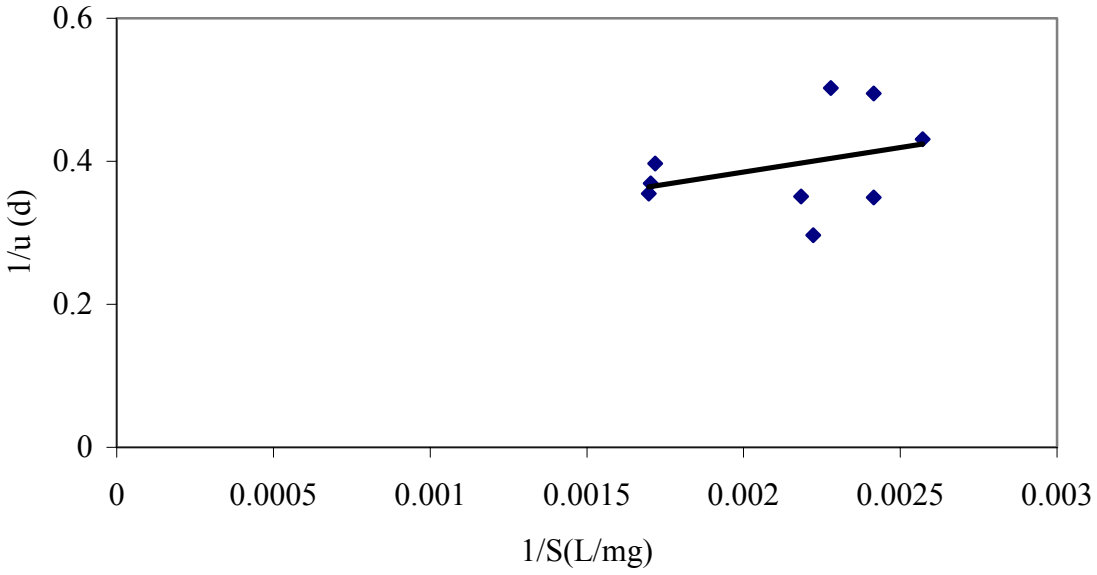


Figure 4.2.12 Lineweaver-Burk Linearization of CSTR

Lineweaver-Burk Linearization method is also used to determine the kinetic parameters of k_s and μ_{max} of data for the batch studies and CSTR for the regrowth period. Plotting $1/\mu_r$ versus 1/S (Figure 4.2.13), to get

$$\frac{1}{\mu_r} = 55.78\frac{1}{S} + 0.33 \qquad (4.2.6)$$

The r^2 is 0.65. The slope k_s/μ_{max} is 55.78, and the intercept$1/\mu_{max}$ is 0.33. The kinetic parameter μ_{max} is 3.03 d^{-1}, and k_s is 169.01mg/L.

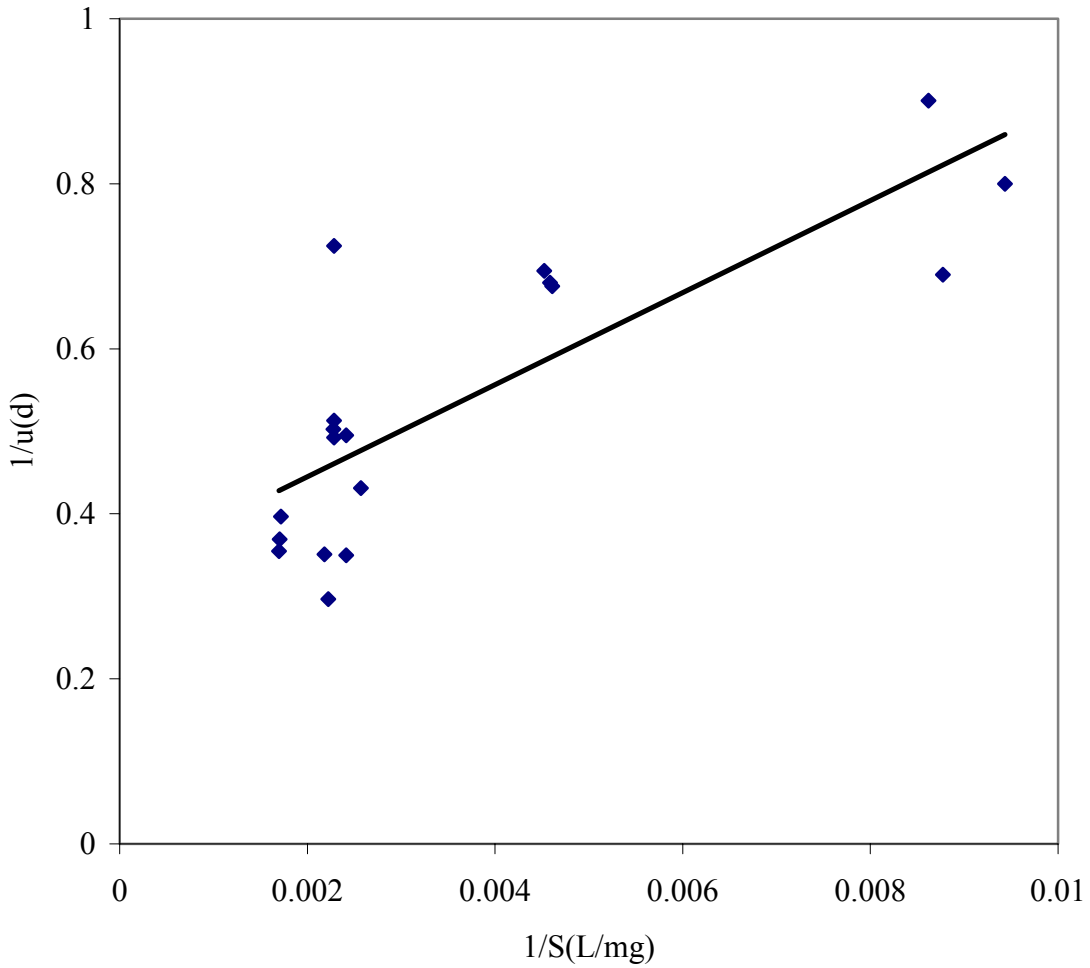


Figure 4.2.13 Lineweaver-Burk Linearization of batch studies and CSTR

Equation (4.2.2) can be used to calculate the net decay rate coefficient k_d-μ_d during the decay period, as reported in the literature (Auer and Niehaus, 1993), with μ_d representing the regrowth rate coefficient during decay period. Therefore, the net decay rate coefficient was calculated by linear regression of ln(FC concentration) versus time. Data from the point after the

maximum of FC concentration were used for net decay rate coefficient calculation. Obvious regrowth of FC occurred during the first 10-20 hours after inoculation. After the point of the maximum FC concentration, a low rate of regrowth μ_d might still occur. The results of ln(FC concentration) versus time for hydraulic retention times of 1.7, 3.5, and 6.9 days at 35°C are shown in Figure 4.2.14. The fitted equations from linear regression are listed in Table 4.2.5.

Table 4.2.5 Fitted equations for the decay period of CSTR at 35°C

	Reactor 1		Reactor 2		Reactor 3	
	Equation	r^2	Equation	r^2	Equation	r^2
HRT=1.7d	y=-5.34+15.8	0.95	y=-5.66+16.3	0.97	y=-4.79x+15.5	0.92
HRT=3.5d	y=-2.10x+16.0	0.94	y=-1.90x+15.6	0.97	y=-2.04x+14.8	0.96
HRT=3.5d Redo 1	y=-1.0x+12.4	0.99	y=-1.06x+12.6	0.96	y=0.97x+12.3	0.97
HRT=3.5d Redo 2	Y=-1.85x+15.4	0.99	Y=-1.93x+15.5	0.97	Y=-1.98+14.7	0.96
HRT=6.9d	y=-6.22x+22.4	0.99	y=-6.08x+22.2	0.99	y=-5.74x+21.8	0.99

Table 4.2.6 Summary of net decay rate coefficient of CSTR at 35°C

HRT (d)	μ_d- k_d-1/τ (d^{-1})			k_d-μ_d(d^{-1})			Mean of k_d-μ_d (d^{-1})	STD (d^{-1})
	Reactor1	Reactor2	Reactor3	Reactor1	Reactor2	Reactor3		
1.7	-5.34	-5.66	-4.79	4.75	5.07	4.20	4.67	0.44
3.5	-2.10	-1.90	-2.04	1.81	1.61	1.75	1.72	0.10
3.5	-1.0	-1.06	-0.97	0.71	0.77	0.68	0.72	0.04
3.5	-1.85	-1.93	-1.98	1.56	1.64	1.69	1.63	0.06
6.9	-6.22	-6.08	-5.74	6.08	5.94	5.60	5.87	0.25

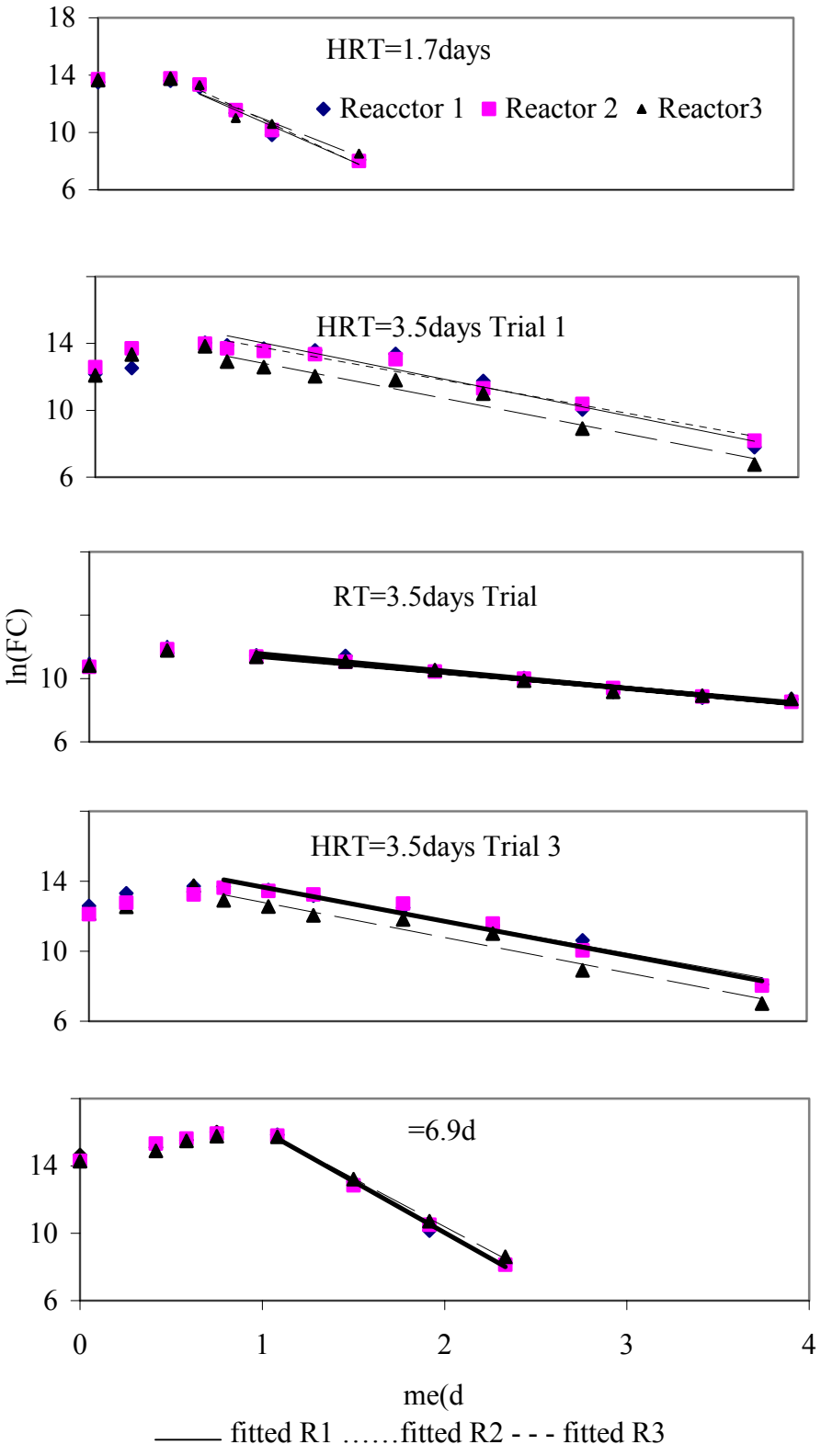


Figure 4.2.14 CSTR study at 35 degree C

Table 4.2.7 Summary of some parameters and measurement

HRT (d)	k_d-μ_d(d^{-1})	Mean effluent Soluble COD (mg/L)	Influent soluble COD (mg/L)
1.7	4.75	492	564
	5.07	457	548
	4.2	488	560
3.5 Trial 1	1.81	169.5	484
	1.61	158	458
	1.75	166	447
3.5 Trial 2	0.71	366	685
	0.77	369	686
	0.68	361	679
3.5 Trial 3	1.56	175	489
	1.64	170	494
	1.69	165	477
6.9	6.08	122	590
	5.94	93	590
	5.6	73	587

The mean values of k_d-μ_d in Table 4.2.6 for hydraulic retention times of 1.7 and 6.9 days are high, 4.67d^{-1} and 5.87d^{-1}, respectively. However, large decay rate coefficient values can also be found in the literature. For example, Scarpino(1962) obtained k value of 6.907 d^{-1}at 28^{o}C in artificial seawater. Parhad (1974) reported k value range of 3.31 to 6.9 d^{-1} at 26-33^{o}C in waste stabilization pond.

The mean values of k_d-μ_d for hydraulic retention time of 3.5 days are 1.72 d^{-1}, 0.72 d^{-1}, and 1.63d^{-1}, which are much lower than those for hydraulic retention times of 1.7 or 6.9 days. These results indicate that the 3.5days hydraulic retention time reactors were near steady state

conditions. It is well known that specific growth rate decreases with hydraulic retention time after the washout point in CSTR. From the high values k_d-μ_d of hydraulic retention time of 1.7days and little difference between influent and effluent soluble COD concentrations, we can estimate that the CSTR was near washed out at hydraulic retention time of 1.7days. The low mean effluent soluble COD at hydraulic retention time of 6.9days showed result in low regrowth rate, which could explain the high k_d-μ_d value. This supports the conclusion that low COD concentration resulted in high net dark decay rate coefficient in the batch study.

CHAPTER 5

CONCLUSIONS

5.1 Summary of Conclusions

5.1.1 Summary of Batch Studies

The relationship between substrate concentration and FC regrowth and decay rate coefficient was studied under batch conditions. There was obvious regrowth of FC at the first stage of incubation at 35^{o}C**.** The mean specific growth rate during the regrowth phase, μ_r, in the batch study were 1.79, 1.46, and 1.27d^{-1} for initial organic carbon concentrations of 478, 235 and 127 mg/L COD, respectively (Table 4.1.2). PROC REG was performed to test the effect of the three COD concentrations on the FC regrowth rate coefficient. The analysis indicated that substrate had a significant impact on the FC regrowth. Lineweaver-Burk Linearization method is used to determine the kinetic parameters of maximum specific growth rate (μ_{max}) and half-saturated coefficient (k_s) in the batch studies for the regrowth period. μ_{max} of 1.92 d^{-1}, and k_s of 60.92mg/L were determined from these data.

The mean dark FC decay rate coefficient, k_d , at 35^{o}C in the batch studies were 2.19, 2.52 and 3.29 d^{-1} for organic carbon concentrations of 478, 235 and 127 mg/L COD, respectively (Table 4.1.6). The k_d values of this study were similar to the values reported for the wastewater environments at similar temperature (Table 2.3.1). PROC REG was performed to analyze the regression relationship between FC decay rate coefficient and the inoculation. The analysis showed that the effect of inoculation on the FC decay rate coefficient was very small. PROC REG was also applied to analyze the regression relationship between FC decay rate coefficient and substrate concentration. The obtained

P-value implied that the effect of substrate concentrations on the FC decay rate coefficient was significant. A simple linear regression equation was obtained for the batch study.

k_d= 3.460-0.00497 *S

The relationship between COD concentration and decay rate coefficient is supported by adding the data form Scott (2000).These results suggest that the batch technique used to measure FC decay rates represents net decay (decay minus regrowth) rather than true decay.

5.1.2 Summary of CSTR Study

In order to separate the decay and regrowth terms, a technique utilizing continues stirred tank reactors (CSTRs) was used. At steady state, decay and regrowth can be determined from as a function of the hydraulic retention time.

No regrowth was observed during the first stage of inoculation at 25^{o}C for the three hydraulic retention times in CSTR. Steady state conditions were not achieved. The decay rate coefficients of FC, determined from non-steady state data, for hydraulic retention times of 1.7, 3.5, and 6.9days at 25^{o}C were 1.34, 1.57, and1.38 d^{-1}, respectively (Table 4.2.1).

There was obvious regrowth of FC at the first stage of the CSTR studies at 35^{o}C. Steady state conditions were not achieved in the study; therefore the decay and regrowth terms couldn't be separated. The mean μ_r values in the CSTR at 35^{o}C were 0.83, 2.85, 2.68, 2.29, 2.11 d^{-1} for the hydraulic retention times of 1.7, 3.5 (Trial 1), 3.5 (Trial 2), 3.5 (Trial 3) and 6.9days, respectively (Table 4.2.3). μ_{max} of 4.00d^{-1}, and k_s of 275.12mg/L were obtained for the CSTR studies. μ_{max} of 3.03 d^{-1}, and k_s of 169.01mg/L was obtained for the combined data from batch and CSTR studies.

The decay rate coefficients calculated in Table 4.2.6 were not real decay rate

coefficients, which included the regrowth rates during the decay period. The mean k_d-μ_d values for at hydraulic retention times of 1.7, 3.5 (Trial 1), 3.5(Trial 2), 3.5 (Trial 3), and 6.9days at 35^{o}C were 4.67, 1.72, 0.72, 1.63, and 5.87d^{-1}, respectively (Table 4.2.6). These results indicate that the 3.5days hydraulic retention time reactors were near steady state.

5.2 Recommendations for Future Research

1 CSTR with hydraulic retention time of 3.5days was near steady state conditions. In the future, the hydraulic retention time around 3.5 days can be applied to CSTR at 35^{o}C.

2. CSTR and Batch Reactor under aerobic conditions can be studied to compare with those under anaerobic conditions.

3. In this study, the inoculum still had some other heterotrophic bacteria. The FC colonies from the petri dish may be used as inoculum.

4. The wastewater collected from the dairy farm was affected by many factors, such as the ages and quantity of cows, the flush times, the rain water, and so on. The unstable wastewater concentration affected the experiment results to great extent. In the future study, a small number of dairy cows should be isolated for waste collection for use in lab research.

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APPENDIX A

BATCH REACTOR STUDY DATA

Table A.1 Batch reactor study data at 35°C (1/1 dilution)

	Reactor 1			Reactor 2			Reactor3		
Time (hrs)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)
0	474.25	478	253333	480	476	266667	474.25	478	260000
4	443	443	356667	445	440	456667	443	433	410000
10	398	396.75	456667	389	381	636667	398	389.25	596667
14	359.25	351.75	290000	351	345.75	350000	359.25	351.75	305000
24	309.25	289.25	166667	309.25	289.25	213333	309.25	289.25	176667
36	271.75	266.75	53000	270.15	256	94200	271.75	256.25	73667
48	243	239.25	17666	238	229.25	25000	243	231.25	14000
60	191.75	174.25	5500	192.25	174.25	6700	191.75	174.25	3200
72	148	145.5	2500	153.25	143.25	3533	148	140.5	1050

Table A.2 Batch reactor study data at 35°C (dilution 1/2).

	Reactor 1			Reactor 2			Reactor 3		
Time (hrs)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)
0	235.4	227.9	746666.7	237.9	229.15	733333	232.9	227.9	740000
11	200.4	190.4	1463333	204.15	190.4	1420000	202.9	192.9	1460000
23	174.15	166.65	473333.3	180.4	167.9	480000	179.15	170.4	556667
35	161.65	150.4	160000	169.15	152.9	123333	157.9	150.4	150000
47	147.9	129.15	26333	150.4	131.65	23333	136.65	125.4	24000
59	127.9	120.4	7600	130.4	121.65	6433	122.9	111.65	7000
74	106.65	96.65	1233	115.4	102.9	1500	105.4	104.4	1733
96	81.65	71.65	243	97.9	84.15	280	84.15	72.9	307

Table A.3Batch reactor study data at 35^{o}C (dilution1/4)

	Reactor 1			Reactor 2			Reactor 3		
Time (hrs)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)
0	125.75	111.5	210000	129.5	114	236666	129.5	111.5	270000
12	87	66.5	393000	104.5	80.25	413333	99.5	77.75	556666
24	68.25	52.75	180000	70.75	60.25	196666	74.5	52.75	310000
36	42	37	42333	44.5	22.75	44666	34.5	21.5	68000
48	29.5	11.5	12000	32	14	13000	34.5	11.5	29000
60	14.5	7.75	1167	17	7.75	1366	14.5	14	5200
72	7	0.25	210	9.5	5.25	250	14.5	6.5	500

APPENDIX B

CSTR STUDY DATA

Table B.1CSTR at 25^{o}C

	HRT=1.7days			HRT=3.5days			HRT=6.9days		
Time (hrs)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)
0	558.25	550	263333	562.25	554	286667	560	551.25	263333
10	505.25	483.25	166667	495.75	481.25	200000	491.75	487.5	216667
15	476.75	462.25	75666	470.5	457.25	120000	463.25	441.25	183333
20	462.25	436.75	56000	444.25	422.25	92000	430	418	140000
24	451.75	421	38666	421	401.75	79000	401.75	382	104333
34	428.25	413.25	23333	388.75	372.5	33666	350.75	344.5	85000
40	402	389	7400	361.8	339.75	15333	322.25	304.75	42000
48	378.25	362.75	6166	344.25	305	7466	289.25	278.25	17667
62	352.75	334.25	2166	315	301.25	3233	258.25	246.25	5367

Table B.2 CSTR at 35^{o}C (Hydraulic Retention Time =1.7days)

	Reactor 1			Reactor 2			Reactor 3		
Time (hrs)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)
0	570.75	564.5	733000	569.5	548.25	907000	569.5	559.5	847000
10	519.5	589.5	820000	505.75	484.5	967000	512	503.25	933000
14	502	512	520000	463.25	432	630000	484.5	477	600000
19	495.75	462	86000	474.5	464.5	105000	492	447	60000
24	480.75	483.25	18700	437	432	26000	490.75	504.5	40000
36	489.5	464.5	3300	455.75	448.25	3000	487	482	5000

Table B.3 CSTR at 35°C Trial 1 (Hydraulic Retention Time=3.5 days)

	Reactor 1			Reactor 2			Reactor 3		
Time (hrs)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)
0	497	484.5	193300	470.75	458.25	290000	475.75	447	176700
5	449.5	429.5	276700	413.25	404.5	903300	414.5	402	623300
15	400.5	284.5	1253300	308.25	288.25	1190000	353.25	345.75	1020000
18	355.75	233.23	1063300	288	252	893300	329.5	303.25	406700
23	244.5	212	876700	242	195.75	760000	282	234.5	293300
30	184.5	122	796700	192	129.5	633000	185.75	170.75	170000
41	150.75	108.25	646700	132	84.5	467000	122	113	134700
53	108.25	85.75	126700	102.25	64.5	81700	112	105	60000
66.5	88.25	83.25	23000	88	57	32000	68.25	63.25	7400
90	54.5	60.75	2430	60.75	50.75	3600	60.75	55.75	860

Table B.4 CSTR at 35°C Trial 2 (Hydraulic Retention Time=3.5 days)

	Reactor 1			Reactor 2			Reactor 3		
Time (hrs)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)
0	684	685.25	55000	686.5	684	46000	679	677.75	49333.3
10	595.25	569	159000	597.75	571.5	139000	590.25	561.5	130667
22	490.25	461.5	95000	492.75	464	88333.3	485.25	454	86666.7
34	400.25	377.75	92333.3	402.75	380.25	63333.3	395.25	370.25	64666.7
46	362.75	322.75	34666.7	365.25	325.25	34000	357.75	335.25	37333.3
58	344	289	22666.7	346.5	291.5	22000	339	281.5	19333.3
70	332.75	275.25	9466.67	335.25	277.75	12000	327.75	267.75	9466.67
82	332.75	267.75	6500	335.25	270.25	7000	327.75	260.25	7500
94	305.25	257.75	6000	307.75	260.25	5000	300.25	250.25	6066.67

Table B.5 CSTR at 35°C Trial 3 (Hydraulic Retention Time=3.5 days)

	Reactor 1			Reactor 2			Reactor 3		
Time (hrs)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)
0	480.75	489	291000	496	494	189000	480.75	477	182000
5	413.25	424	603000	449.5	429.5	276700	414.5	402	350000
14	408.25	298	903000	419.5	284.5	930000	353.25	345.75	560000
18	318.25	252	893300	355.75	233.25	837777	329.5	303.25	402700
24	280.75	195.75	723000	244.5	212	690000	282	234.5	283300
30	192	129.5	527000	184.5	122	567000	185.75	170.75	170000
42	150.75	64.5	267000	150.75	60.75	333000	122	145.75	127000
54	112	57	97000	108.25	85.75	106700	112	148.25	60000
66	123.25	84.5	41000	54.5	108.25	23000	60.75	55.75	7377
90	50.75	50.75	3300	88.25	83.25	3100	68.25	63.25	1100

Table B.6 CSTR at 35°C (Hydraulic Retention Time=6.9days)

	Reactor 1			Reactor 2			Reactor 3		
Time (hrs)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TC OD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)	TCOD (mg/L)	SCOD (mg/L)	FC (CFU/ 100mL)
0	607	589.5	2300000	607	589.5	1630000	608.25	587	1600000
10	485.75	437	3600000	485.75	437	4530000	462	418.25	2930000
14	390.75	333.25	5600000	390.75	333.25	6100000	354.5	297	5300000
18	272	234.5	8900000	272	234.5	8200000	257	208.25	6970000
26	183.25	160.75	7700000	183.25	160.75	7230000	163.25	153.25	6800000
36	138.25	105.75	470000	138.25	105.75	377000	98.25	58.25	550000
46	109.8	77	26000	109.5	77	36700	84.5	62	45000
56	57	27	3600	57	27	3370	30.75	17	5400

VITA

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