APPLICABILITY OF INHIBITORY KINETICS ON BIOLOGICAL SYSTEMS USING PHENOLIC WASTES

By

S. N. VITHAL REDDY

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Osmania University

Hyderabad, India

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Dr. D. F. Kincannon

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Thesis Approved:

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CHAPTER I

INTRODUCTION

After the industrial and technological revolutions during the last two centuries, a large number of new chemicals and other alien substances have been introduced into the biosphere. While these innovations have helped the world to leap forward, nevertheless they have had an adverse effect onour ecosystem. The indiscriminate discharge of various chemical compounds into the environment has led to severe air and water pollution problems. The magnitude of this problem has affected all living species.

Phenol is one of the compounds that is present in various industrial effluents such as coke plants, oil refineries, chemical plants, etc. These industrial operations are the backbone of any civilized society.

The system, widely used for treating industrial effluents, is the activated sludge system. The inhibitory effect of phenols on the biomass is very important for understanding the control mechanisms and design aspects of the treatment process. This investigational work was undertaken in order to study the applicability of kinetic relationships to biological treatment of phenolic waste and the inhibitory effect of phenol on the biomass.

CHAPTER II

LITERATURE-REVIEW

A. Phenolic Waste Treatment

Biological treatment of wastes containing more than 25 mg/ℓ of phenol was considered impossible until relatively recent times. Research at the Dow Chemical Company (2) in Midland, Michigan, has shown that phenol can serve as bacterial food without serious toxic effects at levels as high as 500 mg/ℓ. It was also recognized that some of the species of microorganisms have appreciable tolerance for phenol and can be effectively utilized to degrade the phenolic compounds by breaking the aromatic ring (3) under certain optimum concentrations.

McKinney and Tomlinson (6) were able to treat a waste water containing 500 mg/ℓ phenol by developing an acclimated, heterogeneous culture of aerobic microorganisms. They used a batch treatment process. Radhakrishna and Ray (5) studied the kinetics of phenol biodegradation with a pure culture. Coe (1) achieved above 90 percent efficiency with influent B.O.D. valves of about 1100 p.p.m. and phenol content of 100 p.p.m. He also found that by increasing the phenol concentration from 100 to 600 p.p.m. caused a temporary disturbance of the system, but the system eventually recovered. Adams (10) reduced phenol concentrations from 3200 mg/ℓ to 0.5 mg/ℓ by biological treatment. Reid (12) employed the trickling filter and activated sludge process to treat phenol wastes. In these studies the influent phenol concentration was 100

p.p.m. He found that the trickling filter removed 80 percent of the phenol whereas the activated sludge removed 99 percent of the phenol. Reid (13) also achieved 80-90 percent phenolic removal by using a continuous rotating biological contactor. Reynold (11) employed continuous flow stirred tank reactor kinetics and enzyme inhibition kinetics to describe the effects of temperature on phenol toxicity to the Alga sele nastrum capric ornutum. Lowe (4) achieved above 90 percent efficiency when using a laboratory activated sludge. The effect of sludge age (θ_c) on the effluent quality was insignificant over the normal range of operation. Discontinuous kinetics were observed between 7.1 and 9.8 days sludge age (θ_c). He observed two separate and unique curves when plotting Y_{obs} versus θ_c due to discontinuous growth kinetics. He observed high Y_{obs} (0.956).

B. Phenolic Inhibition

Most of the industrial wastes contain very high concentrations of phenols which can inhibit the microbial growth; hence the efficiency of biodegradation may be reduced to minimum. However, very little has been reported regarding the kinetics of biodegradation of waste waters containing phenol. A kinetic model for substrate inhibition of enzymatic reaction was derived by Haldane (9). This is a case of competitive inhibition by the substrate itself. By analogy, substrate inhibition of microbial growth by the same mechanism has been expressed as

$$\mu = \mu_{max} \frac{S}{K_s + S + S^2/K_i}$$

where

μ = sp. growth rate (Hr^{-1});

μ_{max} = max. sp. growth rate (Hr^{-1});

S = substrate concentrate (mg/ℓ);

K_s = saturate const.; and

K_i = inhibition const.

Using eight sets of experimental data, Edward (7) tested five kinetic models to describe the dependence of growth rate on an inhibitory substrate and found that the Haldane expression is the best one. The possible type of inhibitory action has been summarized by Edward (7) as follows:

1. Modification of chemical potential of substrate.
2. Change in cell's permeation.
3. Change in activity of enzyme.
4. Dissociation of enzyme.
5. Prevention of enzyme synthesis.
6. Change in functional activity of cell.

Yang and Humphrey (8) concluded, based on their experimental studies, that pure cultures P. Putide and T. Cutaneum exhibit substrate inhibition at phenol concentration above 100 mg/ℓ.

CHAPTER III

MATERIALS AND METHODS

In order to study the growth kinetics of a biomass utilizing a phenolic wastewater, a bench scale activated sludge system was operated continuously under controlled conditions for approximately eight months.

Description of the laboratory apparatus, composition of feed solution, initial acclimation and startup, daily procedural schedule, analytical procedures, and methods of analyzing the data are as follows.

A. Laboratory Apparatus

A schematic diagram of the laboratory setup used in this investigation is shown in Figure 1. The biological activated sludge reactor employed was a rectangular plexiglass unit divided by an adjustable baffle into aeration chamber and clarifier. The volumes of aeration chamber and settling basin were 5.6 liters and 2.4 liters, respectively. A synthetic phenolic wastewater was pumped continuously to the aeration tank by a Milton Roy pump (Model DC-2-117R, mini pump). The feed rate was 16.92 liters/day; the feed rate was checked daily. The feed line was cleaned by pumping 1% clorox solution. Compressed air was supplied to the aeration tank through four porous diffuser stones. The air flow was maintained at 4 liters/minute (measured by a Gelman flow meter). The compressed air provided not only mixing and oxygen supply to the biological solids, but also provided suction to recycle the solids from

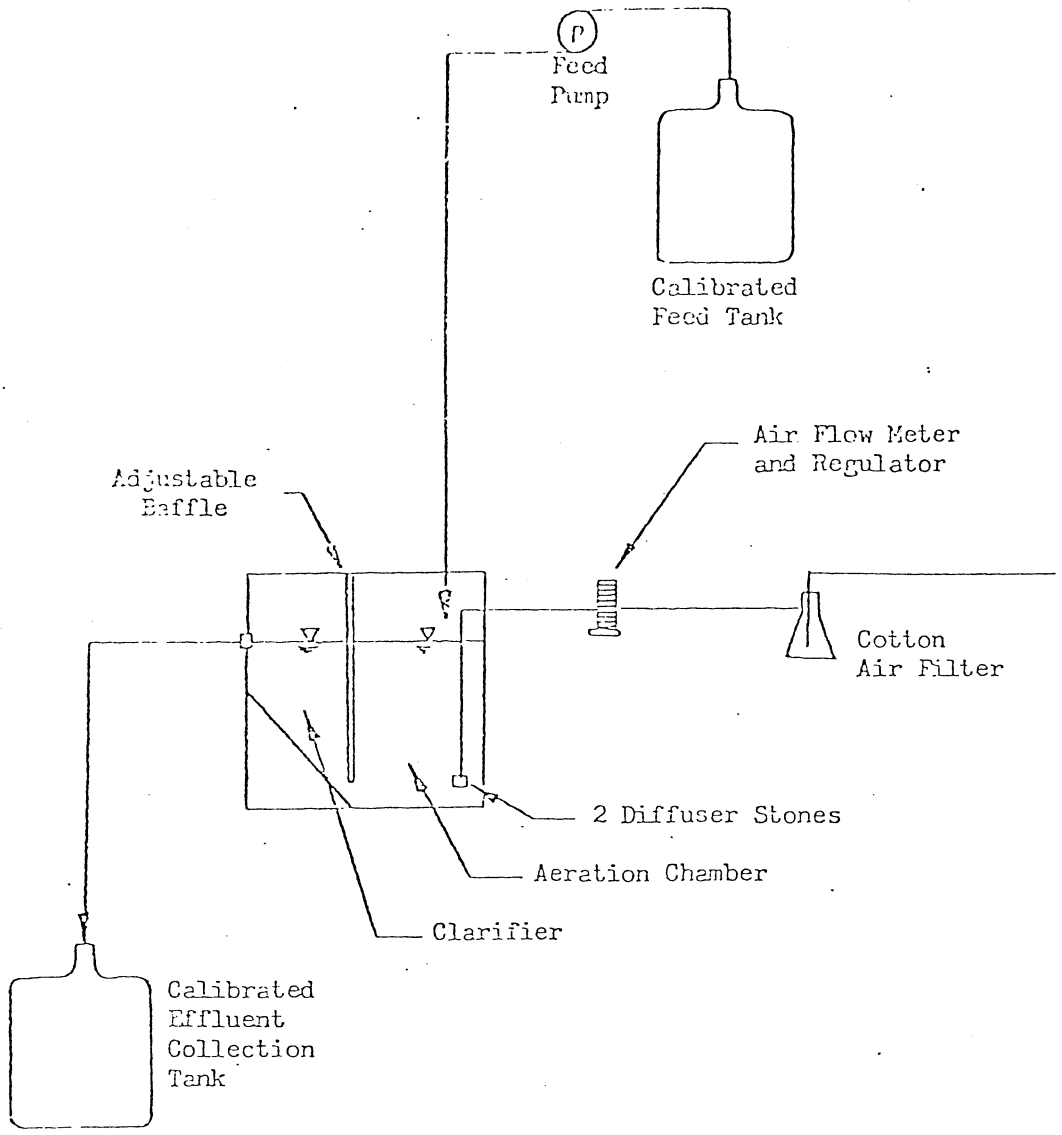

Figure 1. Experimental Activated Sludge Unit With Internal Recycle

the settling chamber. A glass cotton filter was placed just before the air flow meter to prevent oil and water in the air lines from entering the unit.

The pH of the system was checked daily by using a pH meter (Beckman, expandomatic SS-2, Model 76) and was adjusted to 7.2 as needed by adding drops of potassium hydroxide solution. Before using, the pH meter was standardized to a pH of 4.0, using standard buffer solution and checked with another standard of pH 11.0. The temperature was monitored with a thermometer. The temperature in the aeration tank was maintained at $23 \pm 2^{\circ}C$.

B. Feed Solution

The synthetic feed was prepared using four stock solutions of phenol, ammonium sulphate, salts, and phosphate buffer. Composition and concentration of these solutions are given in Table I. The stock solutions were prepared in 2-liter batches as need warranted. Twenty liters of standard feed was prepared daily in a calibrated 20-liter feed bottle. Table II gives volumes of various stock solutions used, and final concentration of each constituent in the feed solution. The feed concentration remains the same throughout the experiment. The feed concentrations were designed to allow a feed COD of 205 mg/ℓ. The phenol was the only carbon source. The other nutrients were added in proportion to the phenol. The feed was made in a 20-liter bottle. Nine or ten liters of tap water were placed in the feed bottle, then the correct amounts (according to Table II) of each stock solution were mixed thoroughly with the tap water in the feed bottle. Finally, tap water was added to the 20-liter mark.

TABLE I

STOCK SOLUTIONS

Constituent	Grams/2 Liters
Phenol	
C_6H_5OH	84.00
Ammonia Sulphate	
$(NH_4)_2SO_4$	500.00
Salts	
Calcium Chloride $CaCl_2$	7.30
Ferric Chloride $FeCl_3 \cdot 6H_2O$	0.50
Magnesium Sulfate $MgSO_4 \cdot 7H_2O$	100.00
Manganous Sulfate $MnSO_4 \cdot H_2O$	10.00
Phosphate Buffer	
KH_2PO_4	105.40
K_2HPO_4	214.00

TABLE II

STANDARD FEED

Serial No.	Constituent	Quantity Stock Solution (Per 20 Liters)	Final Concentration (Per 20 Liters)
1	Phenol	50 ml	250.00 mg/ℓ
	C_6H_5OH		
2	Ammonia Sulphate		
	$(NH_4)_2SO_4$	12 ml	150.00 mg/ℓ
3	Salts	10 ml	
	$CaCl_2$		2.00 mg/ℓ
	$FeCl_3 \cdot 6H_2O$		0.15 mg/ℓ
	$MgSO_4 \cdot 7H_2O$		30.00 mg/ℓ
	$MnSO_4 \cdot H_2O$		3.00 mg/ℓ
4	Buffer	125 ml	
	KH_2PO_4		
	$KHPO_4$		
5	Tap Water	20 liters - 1,2,3,4	

C. Initial Acclimation and Startup

The original seed of microorganisms came from the effluent of the primary settling basin at the municipal wastewater treatment plant in Stillwater, Oklahoma. The original seed was placed into an 8-liter batch unit. Glucose along with other nutrients were used as the initial substrate. The glucose feed of 500 mg/ℓ was used as the major carbon source. Nutrients and buffer were added in corresponding amounts. After the eighth day of batch operation, phenol was introduced along with the glucose feed. The phenol concentration was increased by 25 mg/ℓ each day until a concentration of 250 mg/ℓ was reached. At this point the glucose concentration was reduced each day by 100 mg/ℓ. After six days the wastewater contained no glucose. Microorganisms were acclimated to phenol by this time. Phenol now became the sole carbon source for the microorganisms. The nutrients and buffer concentration remained the same. Phenol-acclimated microorganisms used for this study were fed phenol with a COD of 250 mg/ℓ. Once the phenol-acclimated solids reached the desired concentration, the unit was switched to a continuous flow system.

D. Daily Experimental Protocol

A daily operating procedure was developed to obtain efficient and accurate data.

D.1 Feed Solution

a. COD.

b. pH level.

c. Flow rate of feed.

D.2 Effluent

a. pH level.

b. S.S. concentration.

c. COD filtered.

D.3 Biological Reactor

a. pH level.

b. Total system MLSS.

c. Temperature.

D.4 Feed Solution

A 20-liter synthetic feed solution was prepared daily according to the proportions shown in Table II. From this solution a 20 ml sample was taken for feed COD analysis. pH of the feed was checked periodically.

The feed flow rate was checked four times daily by means of a graduate cylinder.

D.5 Effluent

Approximately 50 ml effluent was collected in a beaker. Twenty-five ml of effluent was filtered through preweighed 45 m millipore filter paper. A 20 ml filtrate sample was taken for COD analysis. The effluent pH, which ranged between 7.2 to 7.6, was checked daily.

D.6 Biological Reactor

The baffle from the reactor was removed and the biomass in the reactor was mixed completely. A 25 ml sample was filtered through

preweighed 45mm millipore filter paper for determination of total system MLSS concentration. The pH of the system was checked daily by using a pH meter (Beckman Expandomatic SS-2, Model 76) and was adjusted to 7.2 as needed by adding a few drops of potassium hydroxide solution. The temperature was monitored with a thermometer. The temperature in the aeration tank was maintained at 23 $\pm$ 2°C.

E. Analytical Procedures

The methods adopted to measure the chemical oxygen demand, biological solids concentration in the reactor and in the effluent, pH, and temperature during this investigation are given below.

E.1 Chemical Oxygen Demand

Chemical oxygen demand (COD) was measured using the procedure given in standard methods (15). Mercuric sulfate and silver sulfate were used in all determinations.

E.2 Biological Solids

The suspended solid concentrations were determined as described in standard methods (15) by filters (0.45 mm, Type HA, Millipore Filter Corp., Bedford, Massachusetts). The filters were weighed on a balance (Metter Instrument Corp.).

E.3 pH and Temperature

The pH was determined using a Beckman Expandomatic SS-2 (Model 76) pH meter, and the temperature was measured with a Sargent-Welch thermometer.

F. Methods of Analyzing the Data

F.1 COD Removal Efficiency Equation

$$E = \frac{(S_i - S_e)}{S_i} (100) \qquad (F.1)$$

where

S_i = influent substrate concentration, mg/ℓ;

S_e = effluent substrate concentration, mg/ℓ; and

E = COD removal efficiency, percent.

F.2 Mean Cell Residence Time (or Sludge Age) (θ_c) Equation

$$\theta_c = \frac{VX}{F_w x + (F - F_w) X_e} \qquad (F.2)$$

where

θ_c = mean cell residence time, days;

V = volume of total reactor, liters;

X = observed total reactor MLSS concentration, mg/ℓ;

X_e = observed effluent suspended solids concentration, mg/ℓ;

F_w = waste MLSS (from total reactor), liters/day; and

F = flow rate, liters/day.

F.3 Observed Yield Coefficient (Y_{obs}) Equation

$$Y_{obs} = \frac{F_w X + (F - F_w) X_e}{F(S_i - S_e)} \qquad (F.3)$$

where

Y_{obs} = observed yield coefficient;

F_w = wasted MLSS (from total reactor), liters/day;

F = flow rate, liters/day;

X = observed total reactor MLSS concentration, mg/ℓ;

X_e = observed effluent suspended solids concentration, mg/ℓ;

S_i = influent substrate concentration, mg/ℓ; and

S_e = effluent substrate concentration, mg/ℓ.

F.4 Food to Microorganism Ratio Equation

$$F/M = \frac{S_i F}{XV} \tag{F.4}$$

where

F/M = food to microorganism ratio, day^{-1};

S_i = influent substrate concentration, mg/ℓ;

X = observed total reactor MLSS, mg/ℓ;

V = volume of reactor, liters; and

F = flow rate, liters/day.

F.5 Specific Utilization (U) Equation

$$U = \frac{(S_i - S_e)\ F}{VX} \tag{F.5}$$

where

U = specific utilization, day^{-1};

S_i = influent substrate concentration, mg/ℓ;

S_e = effluent substrate concentration, mg/ℓ;

F = flow rate, liters/day;

V = volume of reactor, liters; and

X = observed total reactor MLSS, mg/ℓ.

F.6 Rate of Substrate Utilization ($\Delta S/\Delta T$) Equation

$$\frac{\Delta S}{\Delta T} = \frac{(S_i - S_e)\ F}{V} \qquad (F.6)$$

where

$\frac{\Delta S}{\Delta T}$ = substrate utilization rate, mg COD utilized/liters/day;

S_i = influent substrate concentration, mg/ℓ COD;

S_e = effluent substrate concentration, mg/ℓ COD;

F = flow rate, liters/day; and

V = volume of reactor, liters.

F.7 Amount of Cell Production ($\Delta X/\Delta T$) Equation

$$\frac{\Delta X}{\Delta T} = \frac{X}{\theta_c} \qquad (F.7)$$

where

$\frac{\Delta X}{\Delta T}$ = amount of cell production/liters/day;

X = observed total reactor MLSS concentration, mg/ℓ; and

θ_c = mean cell residence time, days.

F.8 Calculated Total Reactor Microorganism Concentration (X) Equation

$$X = \frac{Y_t\ \theta_c\ F\ (S_i - S_e)}{(1 + K_d\ \theta_c)\ V} \qquad (F.8)$$

where

X = calculated total reactor MLSS concentration, mg/ℓ;

Y_t = microorganism constant-yield coefficient, mg/mg;

S_i = influent substrate concentration, mg/ℓ COD;

S_e = effluent substrate concentration, mg/ℓ COD;

θ_c = mean cell residence time, days;

F = flow rate, liters/day;

K_d = microorganism maintenance energy coefficient, day^{-1}; and

V = volume of reactor, liters.

F.9 Sludge Production (X_w) Equation

$$X_w = \frac{V\bar{X}}{\theta_c} \tag{F.9}$$

where

X_w = sludge production, mg/day;

V = volume of reactor, liters;

$\bar{X}$ = calculated total reactor MLSS concentration, mg/ℓ; and

θ_c = mean cell residence time, days.

F.10 Haldane's Equation

$$\mu = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}} \tag{F.10}$$

where

μ = specific growth rate, day^{-1};

μ_{max} = maximum specific growth rate, day^{-1};

S = substrate concentration, mg/ℓ COD;

K_s = saturation constant; and

K_i = inhibition constant.

G. Batch Growth Studies

Batch growth experiments were conducted to determine kinetic constants μ_{max} and K_s. Batch growth experiments were conducted for four steady state continuous flows only.

The cells were grown in Erlen Meyer flasks with phenol concentrations of 50, 100, 200, 400, 600, 800, and 1000 mg/ℓ. The initial inoculum was taken from the continuous flow unit. A volume of 1.5 ml was used in all flasks, and the initial optical density was approximately 0.036 (percent transmission = 92%). Total volume in each flask was 40 ml. These flasks were placed on an oscillating shaker (Eberbach) which was adjusted to 100-110 oscillations per minute. The growth curve was obtained by measuring optical density at frequent intervals. Optical density was measured by using Bausch and Lomb Spectronic-20 (at 560 mm). The μ_{max} and K_s were calculated by plotting the data obtained from batch growth experiments.

CHAPTER IV

RESULTS

The laboratory activated sludge unit was operated for a period of eight months. The influent C.O.D. was maintained at approximately 250 mg/ℓ. The pH in the reactor was maintained at 7.0 $\pm$2. The mean cell residence time was used as an operating parameter. The reactor was operated at ten different mean cell residence times ranging from 3.0 days to 26.23 days. A summary of the steady state data for these ten different mean cell residence times (θ_c) are presented in Table III. Batch growth studies were conducted, with cells harvested from the unit when operating at mean cell residence times of 12.69, 7.93, 4.15, and 3.0 days. The results are summarized in Table IV.

A. Operating Data

1. C.O.D. Removal Efficiencies

Figure 2 shows the C.O.D. removal efficiencies of the laboratory activated sludge unit. The C.O.D. removal efficiency was found to be greatest when the mean cell residence time was between 8-10 days with an efficiency of approximately 93 percent. The average C.O.D. removal efficiency for the various mean cell residence time was 85 percent.

Figure 3 shows a plot of the effluent C.O.D. versus sludge age (θ_c). The lowest effluent C.O.D. was obtained when the mean cell residence time was between 8-10 days. For the mean cell residence time (θ_c) values

TABLE III

SUMMARY OF STEADY STATE DATA

	26.23	12.69	9.95	9.58	7.22	9.70	7.93	4.15	9.37	3.00
1. Feed C.O.D., mg/ℓ (S_i)	228	269	241	235	242	269	245	261	241	263
2. Effluent C.O.D., mg/ℓ (S_e)	39	54	16	18	39	33	21	49	16	68
3. Removal efficiency, percent	83	80	94	92	84	88	91	81	94	74
4. Observed total reactor, MLSS, mg/ℓ, $\bar{X}$	2752	2656	2510	2277	2153	2781	2479	1815	2040	1116
5. Effluent, MLSS, mg/ℓ, $\overline{X_e}$	14	37	46	33	59	25	32	34	56	12
6. Solids wasted per day, mg	605	1062	1255	1366	1421	1947	1983	2723	816	2790
7. Observed sludge production, mg/day, Px	839	1674	2010	1905	2380	2352	2499	3247	1741	2963
8. Y_{obs}, mg/mg	0.260	0.460	0.530	0.520	0.690	0.590	0.660	0.840	0.450	0.900
9. $1/Y_{obs}$, mg/mg	3.846	2.174	1.890	1.930	1.445	1.700	1.515	1.190	2.220	1.110
10. F/M, day^{-1}	1.175	0.214	0.203	0.218	0.237	0.204	0.209	0.304	0.249	0.498
11. Specific utilization, day^{-1}, U	0.145	0.169	0.188	0.200	0.198	0.157	0.190	0.300	0.233	0.360
12. μs, mg C.O.D./l/day	400	455	476	459	429	499	474	448	476	412
13. μx, mg/l/day	105	209	252	238	298	287	313	437	218	372
14. $1/\theta_c$ day^{-1}	0.038	0.079	0.101	0.104	0.138	0.103	0.126	0.240	0.106	0.333
15. Calculated total reactor, MLSS, mg/ℓ, $\bar{X}$	2990	2706	2553	2420	1965	2647	2277	1469	2483	1076
16. Calculated sludge production, mg/d, $\overline{Px}$	912	1706	2053	2021	2177	2183	2297	2832	2120	2869
17. μ, day^{-1}	0.158	0.198	0.220	0.224	0.258	0.223	0.246	0.360	0.226	0.451
18. U_n	0.038	0.079	0.101	0.104	0.138	0.103	0.126	0.240	0.106	0.333

TABLE IV

BATCH GROWTH STUDY RESULTS

Sample No.	θ_c Days	Specific Growth Rate 50 mg/ℓ	100 mg/ℓ	200 mg/ℓ	400 mg/ℓ	600 mg/ℓ	800 mg/ℓ	1000 mg/ℓ
1	12.97	0.080	0.123	0.093	0.060	0.070	0.066	0.046
2	7.93	0.077	0.119	0.092	0.073	0.088	0.068	---
3	4.15	0.078	0.128	0.091	0.061	0.072	0.065	0.045
4	3.00	0.077	0.123	0.088	0.059	0.069	0.063	0.043

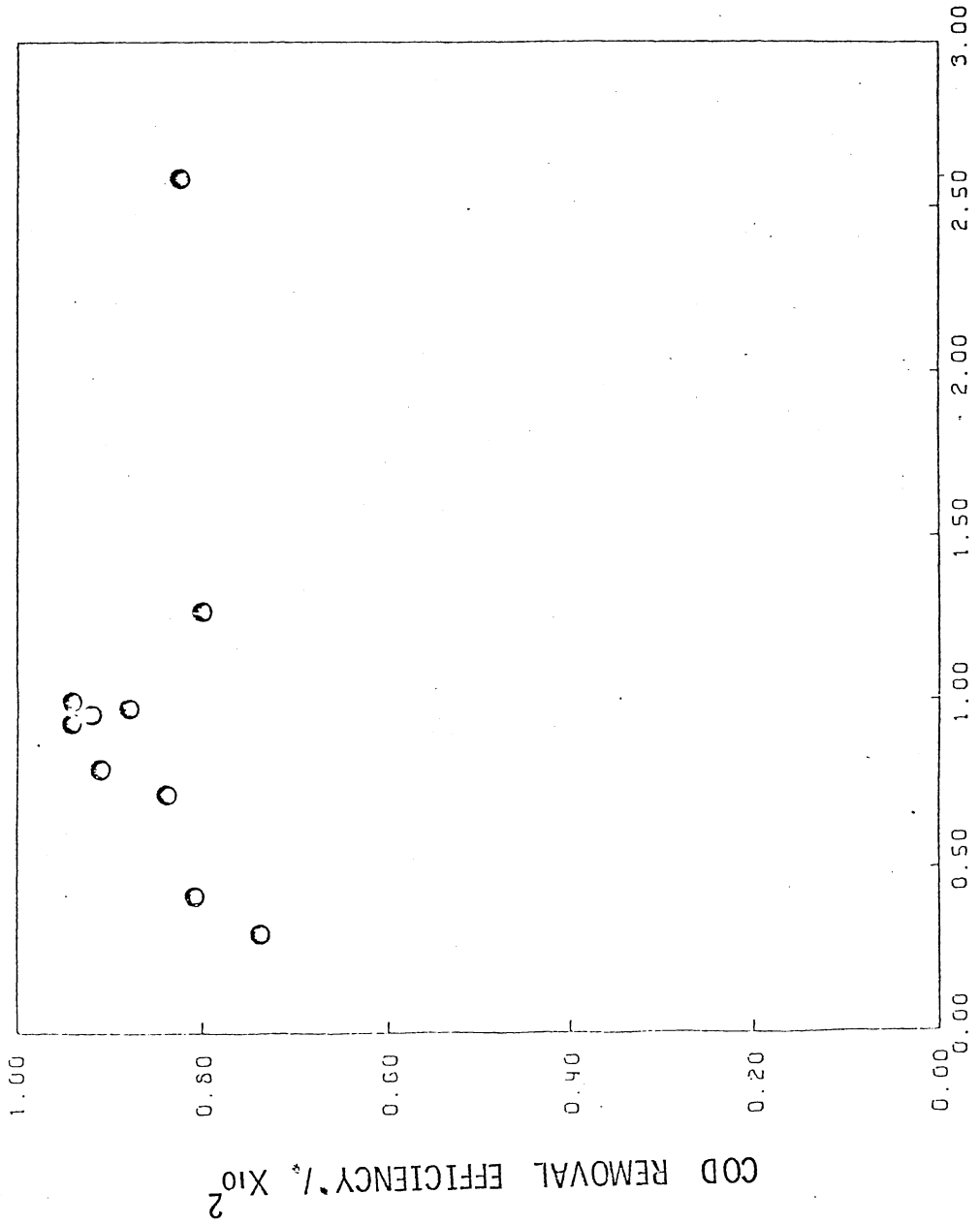

Figure 2. Treatment Efficiency Versus θ_c

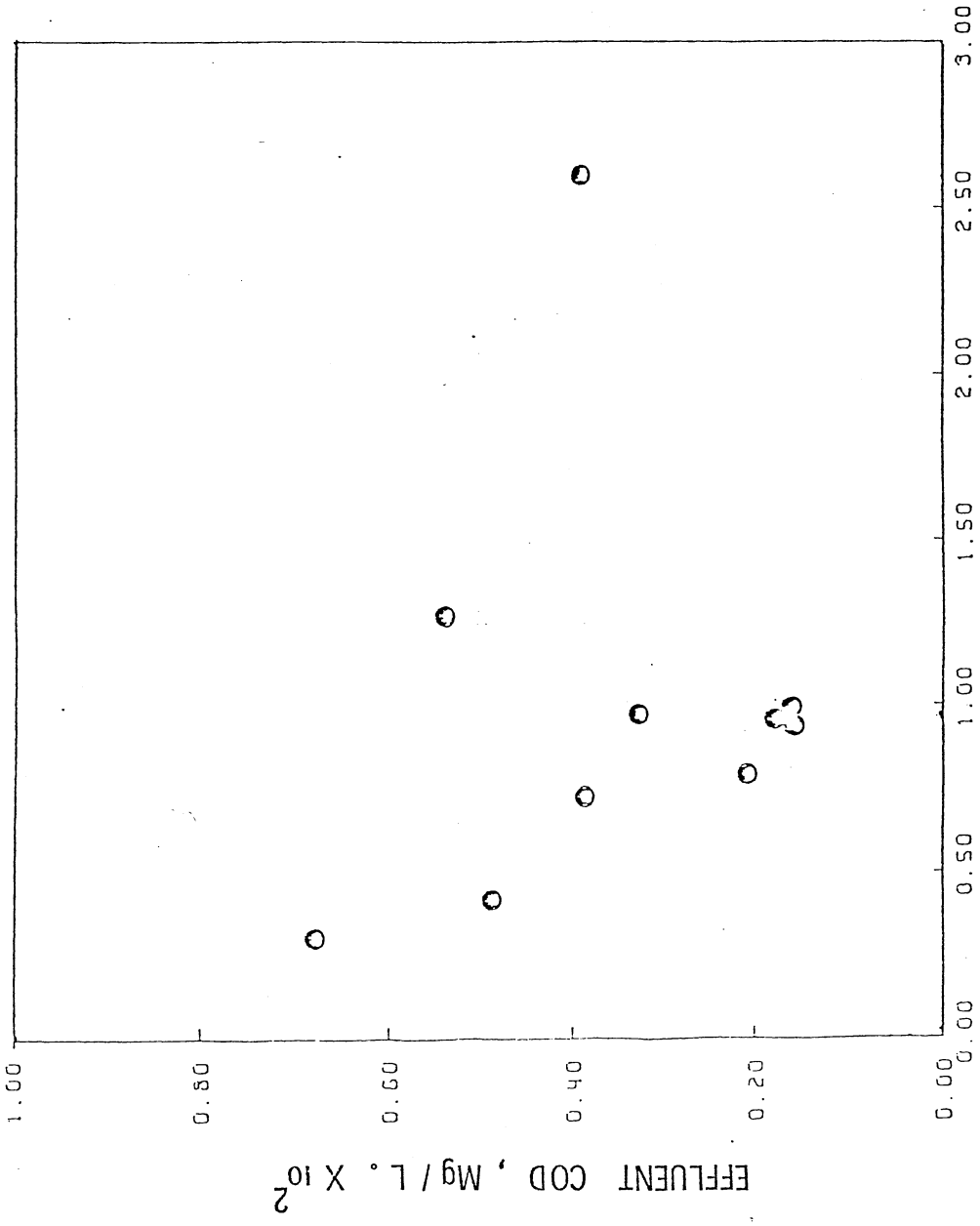

Figure 3. Effluent COD Versus θ_c

above and below this range, the effluent COD was comparatively higher and it ranged between 30 mg/ℓ to 70 mg/ℓ.

Figure 4 shows the sludge age (θ_c) versus observed yield (Y_{obs}). The observed yield increases as sludge age (θ_c) decreases. The observed yield (Y_{obs}) values range from 0.26 to 0.9.

The true yield (Y_t) and decay coefficient (K_d) values were calculated by two different methods. Figure 5 shows the plot of specific substrate utilization versus net growth rate (μ_n). From this graph a value of 1.19 was obtained for true yield (Y_t) and 0.12 day^{-1} was obtained for the decay coefficient (K_d). Figure 6 shows the plot of the reciprocal of the observed yield (Y_{obs}) versus sludge age (θ_c). From this graph the calculated value of Y_t is 1.19 and K_d is 0.12 day^{-1}. Values obtained for Y_t and K_d by these two different ways of plotting are reasonably close.

Figure 7 shows the plot of specific substrate utilization rate (U) versus sludge age (θ_c). The specific substrate utilization rate (U) ranges between 0.145 to 0.360. The specific substrate utilization rate increases as the sludge age (θ_c) decreases.

The food to microorganism ratio (F/M) is shown in Figure 8 as a function of sludge age (θ_c). Food to microorganism ratio (F/M) increases while the sludge age (θ_c) decreases (see Figure 8).

Total reactor microorganism concentration (observed) values as a function of sludge age is shown in Figure 9. The predicted MLSS values, according to Equation (F.8), are also shown in Figure 9. It can be seen that the observed MLSS values closely follow the predicted values.

The observed sludge production and predicted sludge production are plotted function of θ_c in Figure 10. The predicted and observed sludge

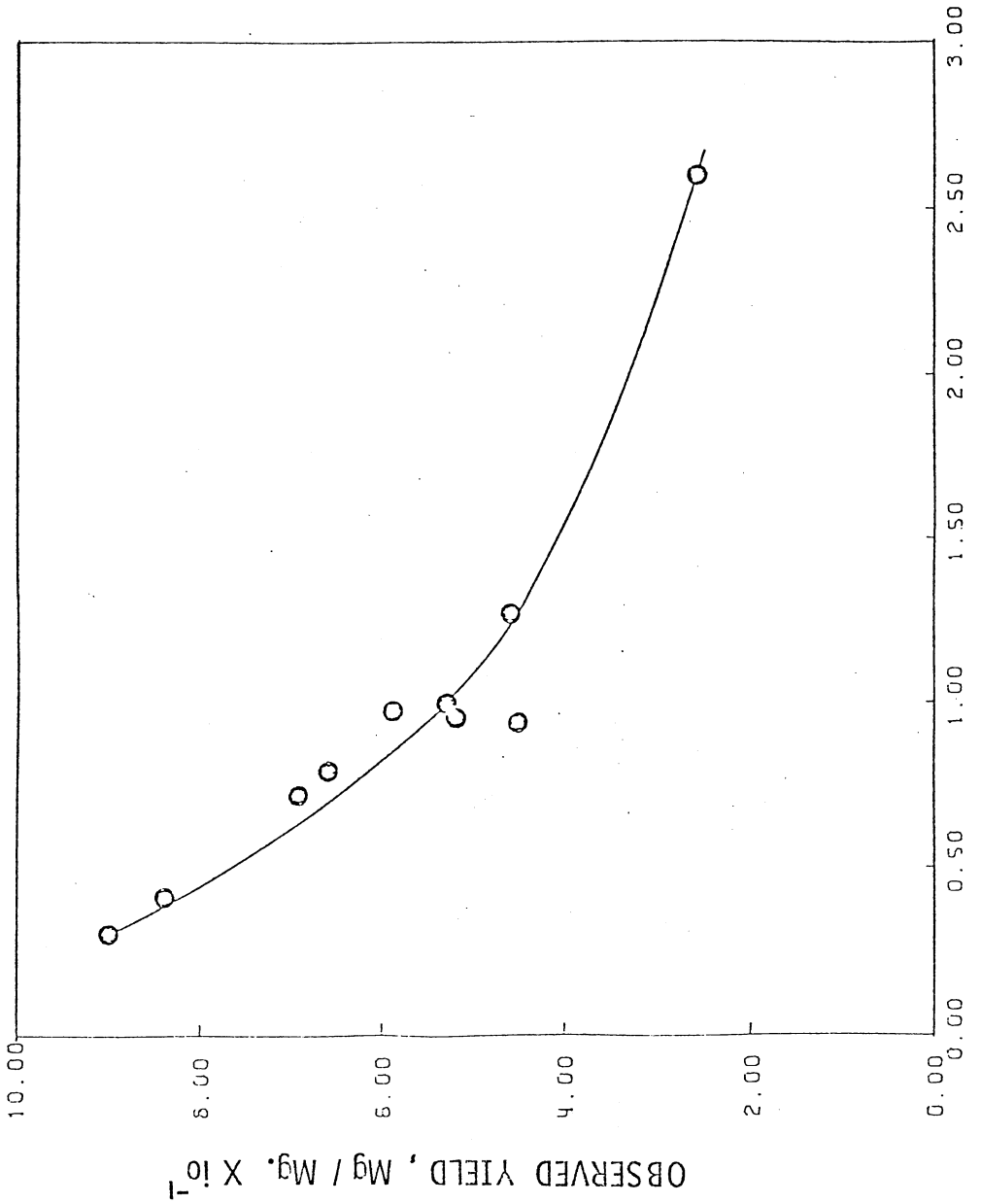

Figure 4. Observed Yield Versus θ_c

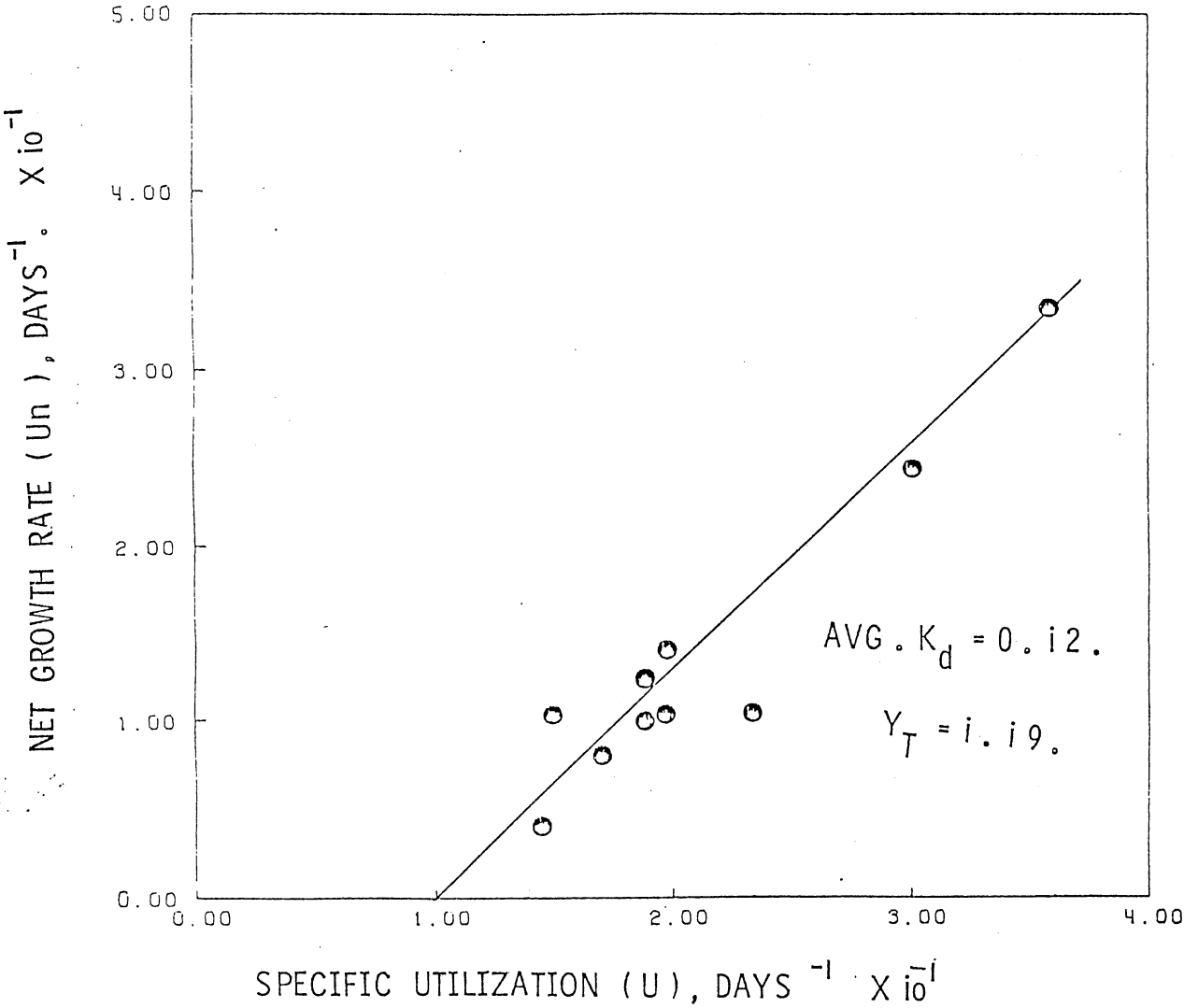

Figure 5. Specific Growth Rate Versus Specific Utilization Rate

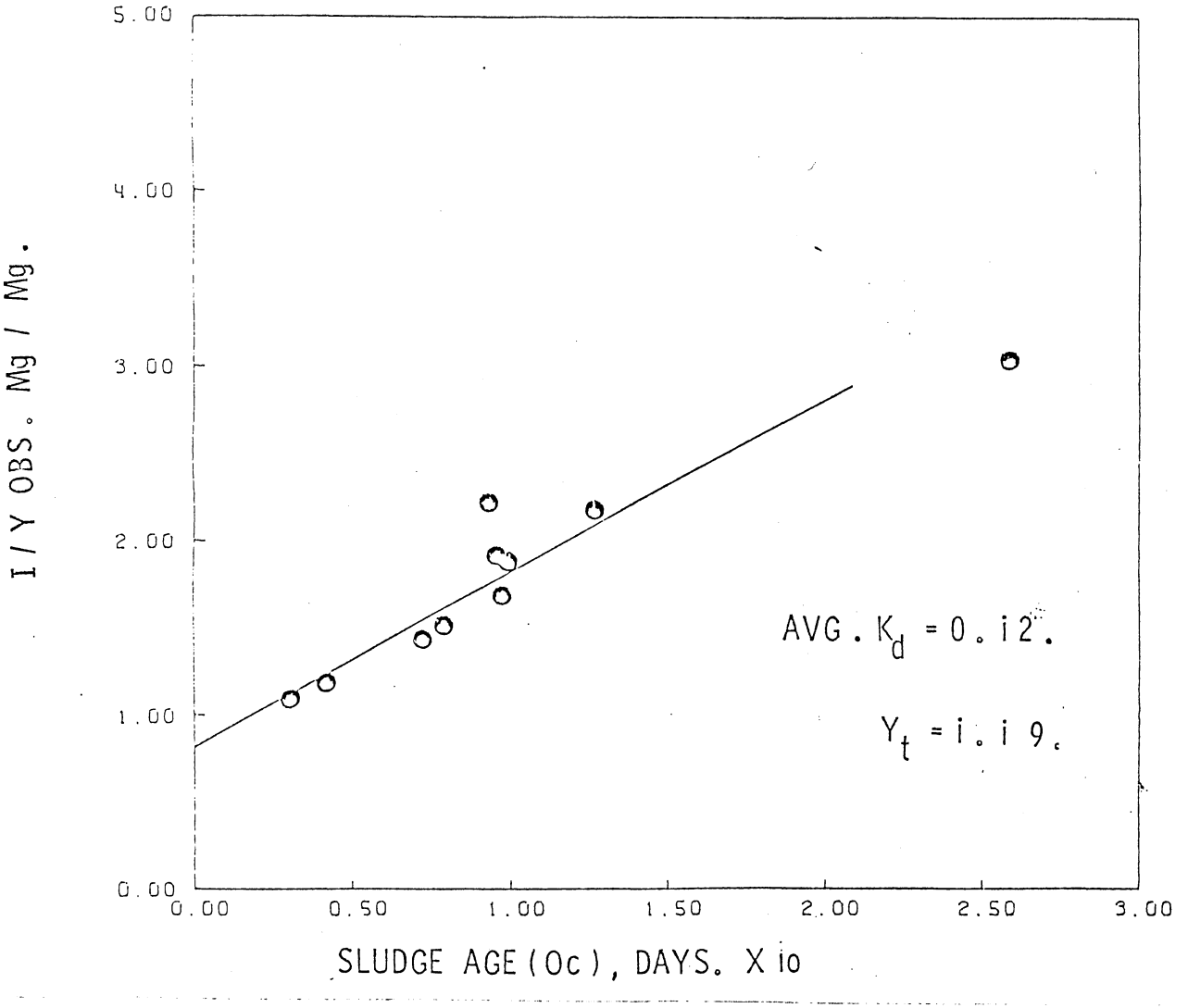

Figure 6. Reciprocal of Observed Yield Versus θ_c

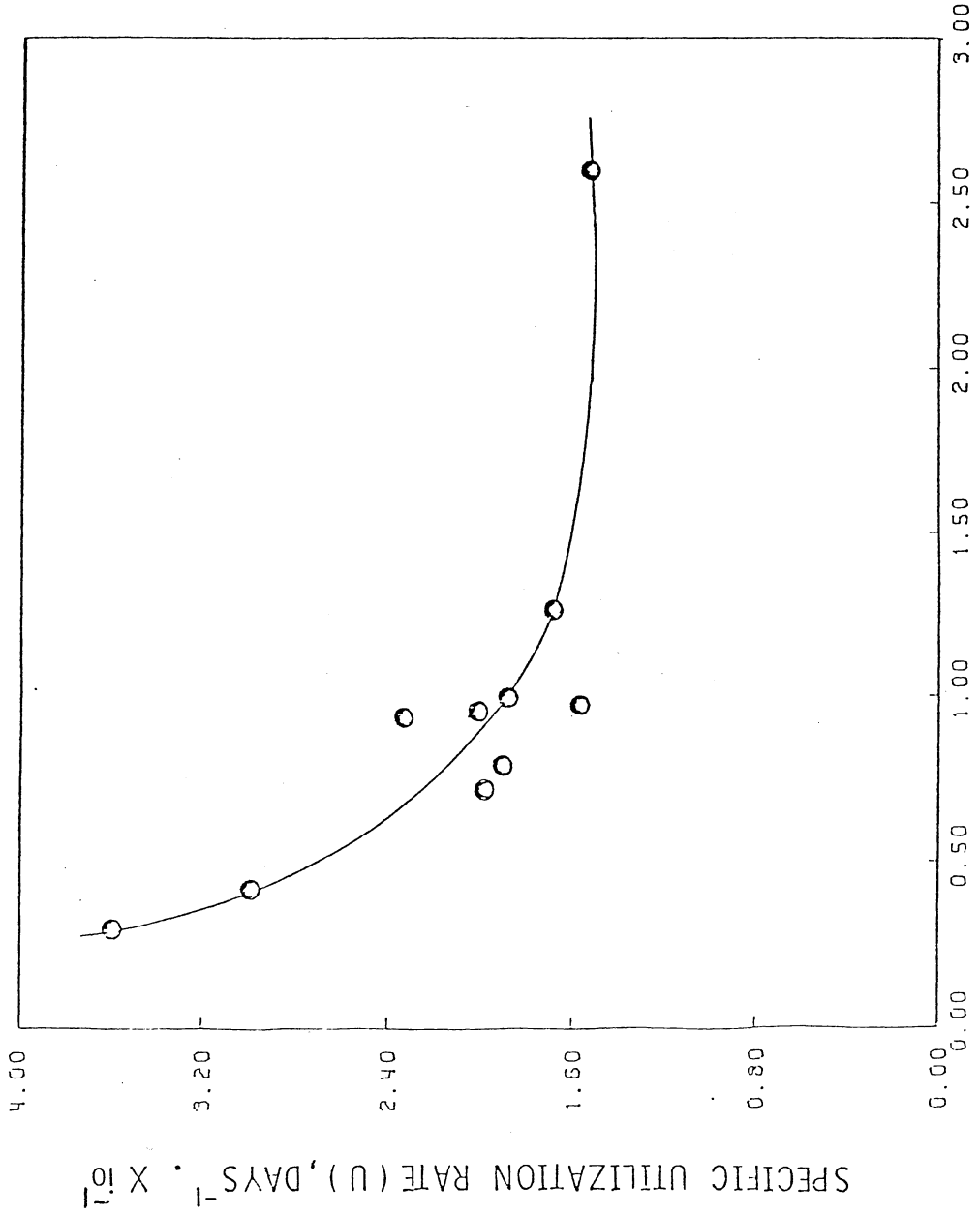

Figure 7. Specific Utilization Rate Versus θ_c

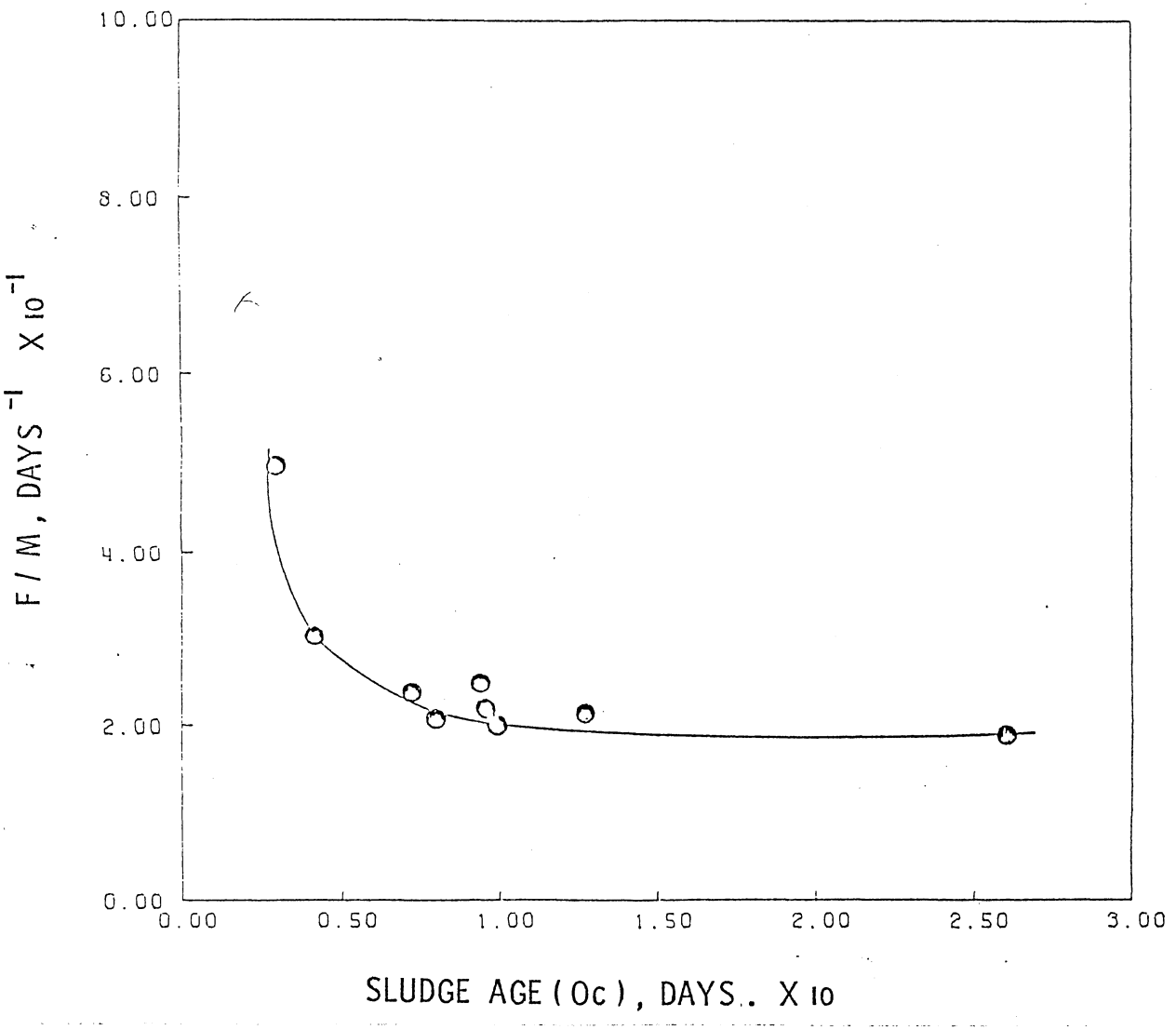

Figure 8. Food to Microorganism Ratio Versus θ_c

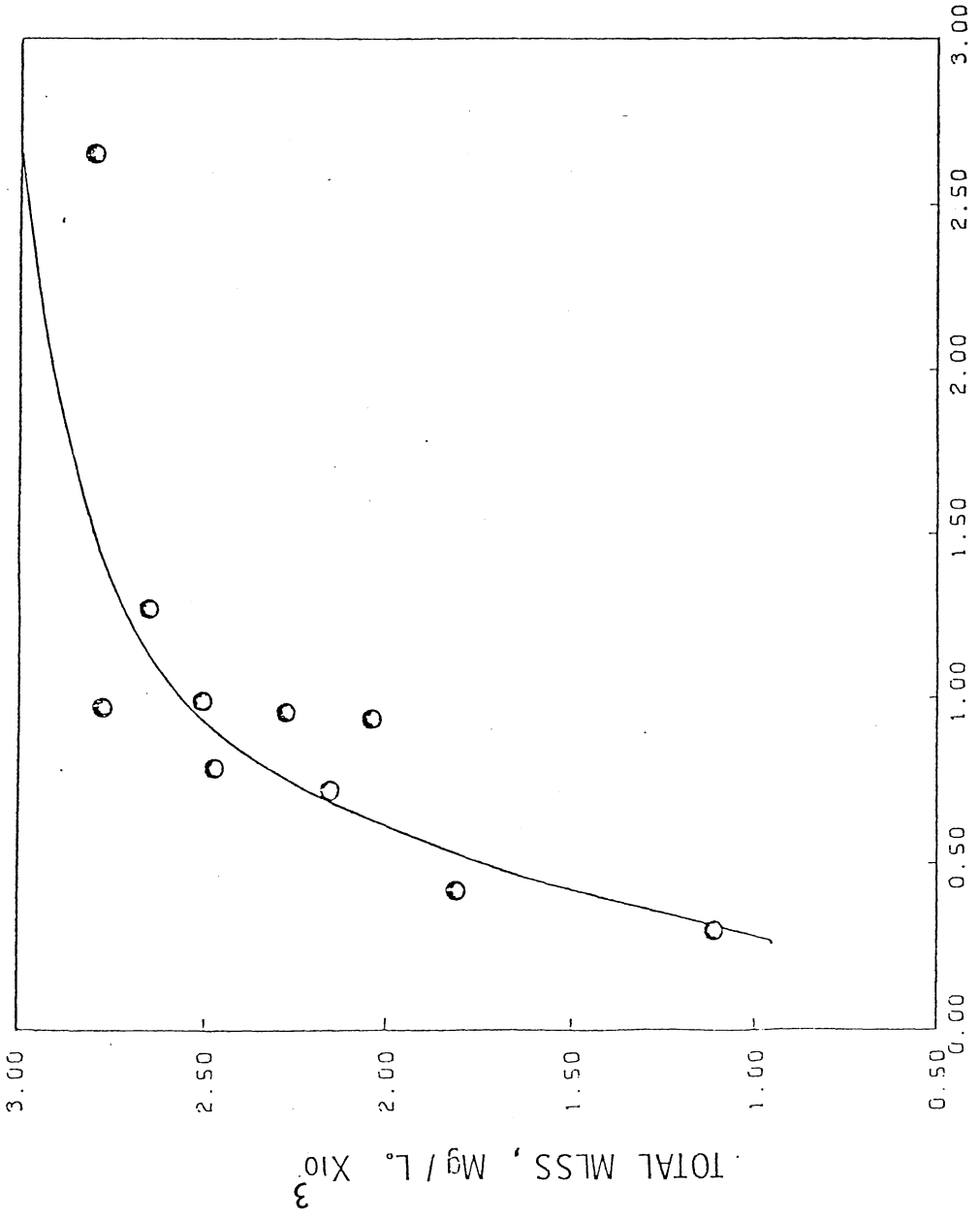

Figure 9. Total MLSS Versus θ_c

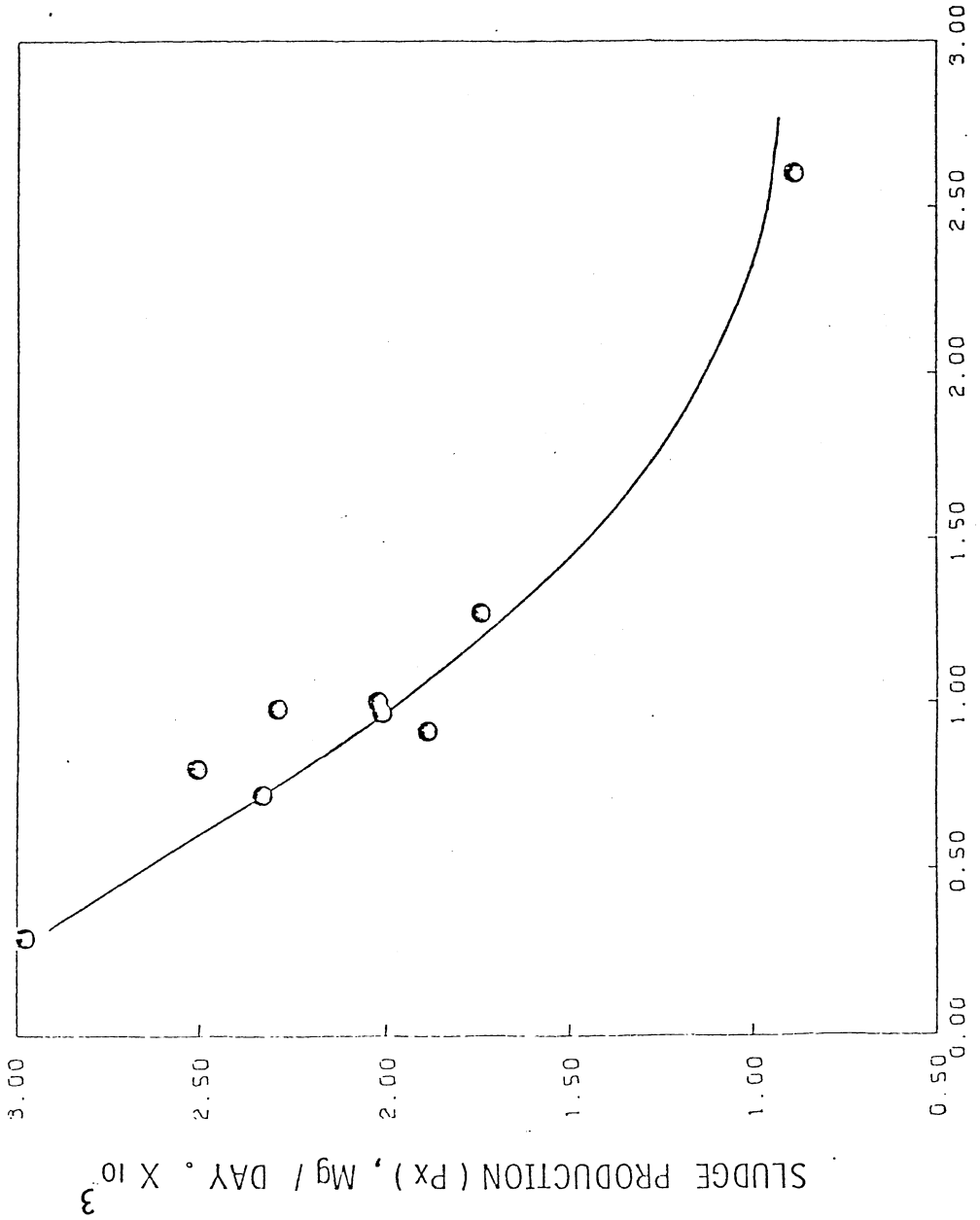

Figure 10. Sludge Production Versus θ_c

production are in close agreement. The sludge production decreases with increasing θ_c as expected.

B. Batch Growth Study Results

When the continuous unit reached steady state, cells were harvested from the unit for batch growth studies. Sludge age (θ_c) of 12.69, 7.92, 4.15, and 3.0 days were selected for batch studies. With phenol as a growth-limiting nutrient and with the concentration of phenol ranging from 50 mg/ℓ to 1000 mg/ℓ, the growth study as described earlier was conducted.

The results of the batch growth studies are shown in Figures 11 through 14, where the specific growth rate is plotted versus the initial substrate concentration. The dotted circles show the actual batch data whereas the solid line represents the relationship developed by Haldane, that is:

$$\mu = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}}$$

It is seen in Figures 11 through 14 that the data does not follow the Monod relationship, where the specific growth rates increase with substrate concentration and approach a maximum specific growth rate. In these studies the specific growth rate increases with increased substrate concentration until the substrate concentration reaches a value of approximately 100 mg/ℓ and then the specific growth rate decreases with increasing substrate concentration. The data does follow the relationship developed by Haldane.

The Haldane relationship is difficult to evaluate in that three unknowns exist in the equation: μ_{max}, K_s, and K_i. It has been shown that

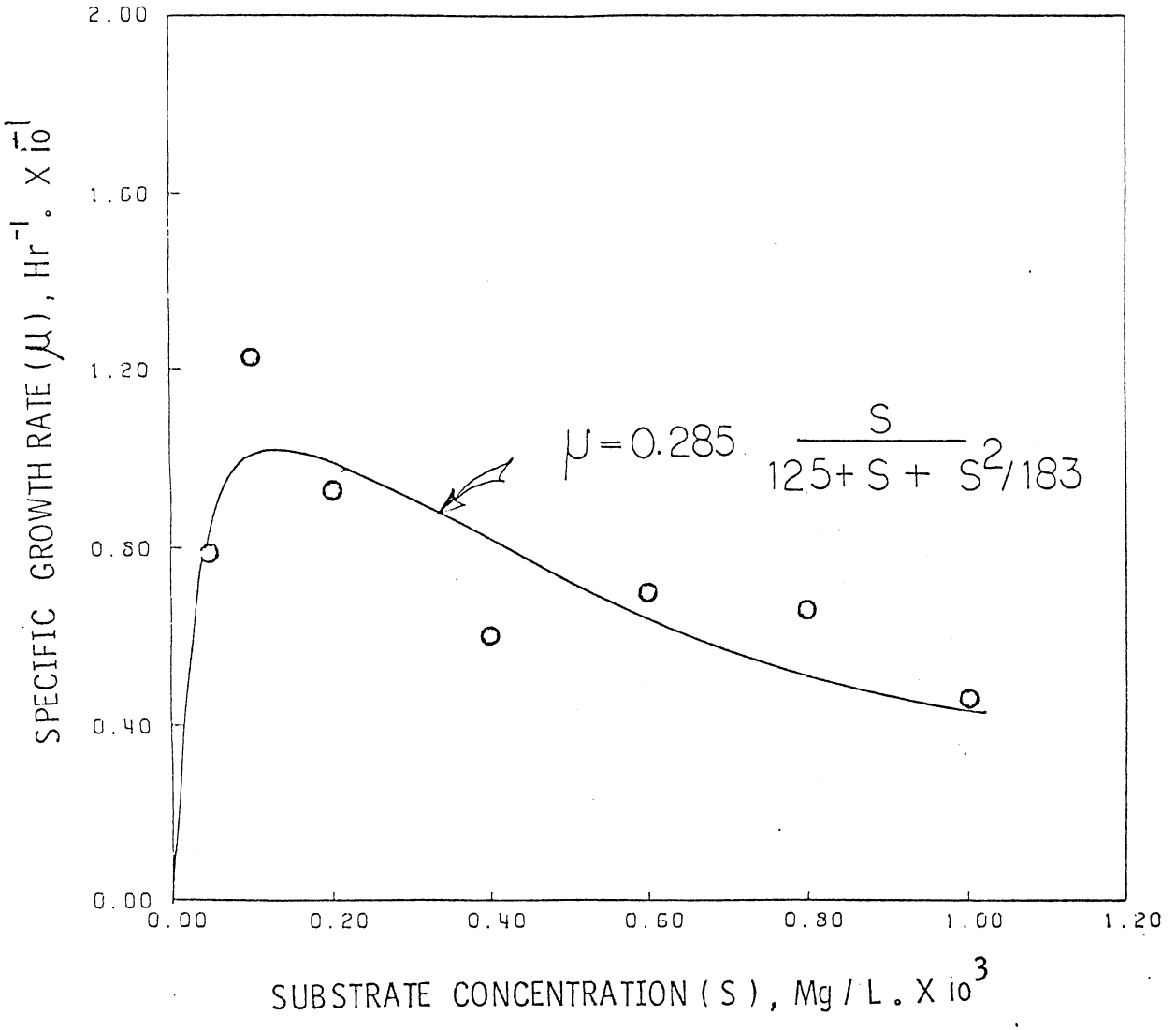

Figure 11. Specific Growth Rate (μ) Versus Substrate Concentration(s) for θ_c = 12.69 Days (Batch Unit)

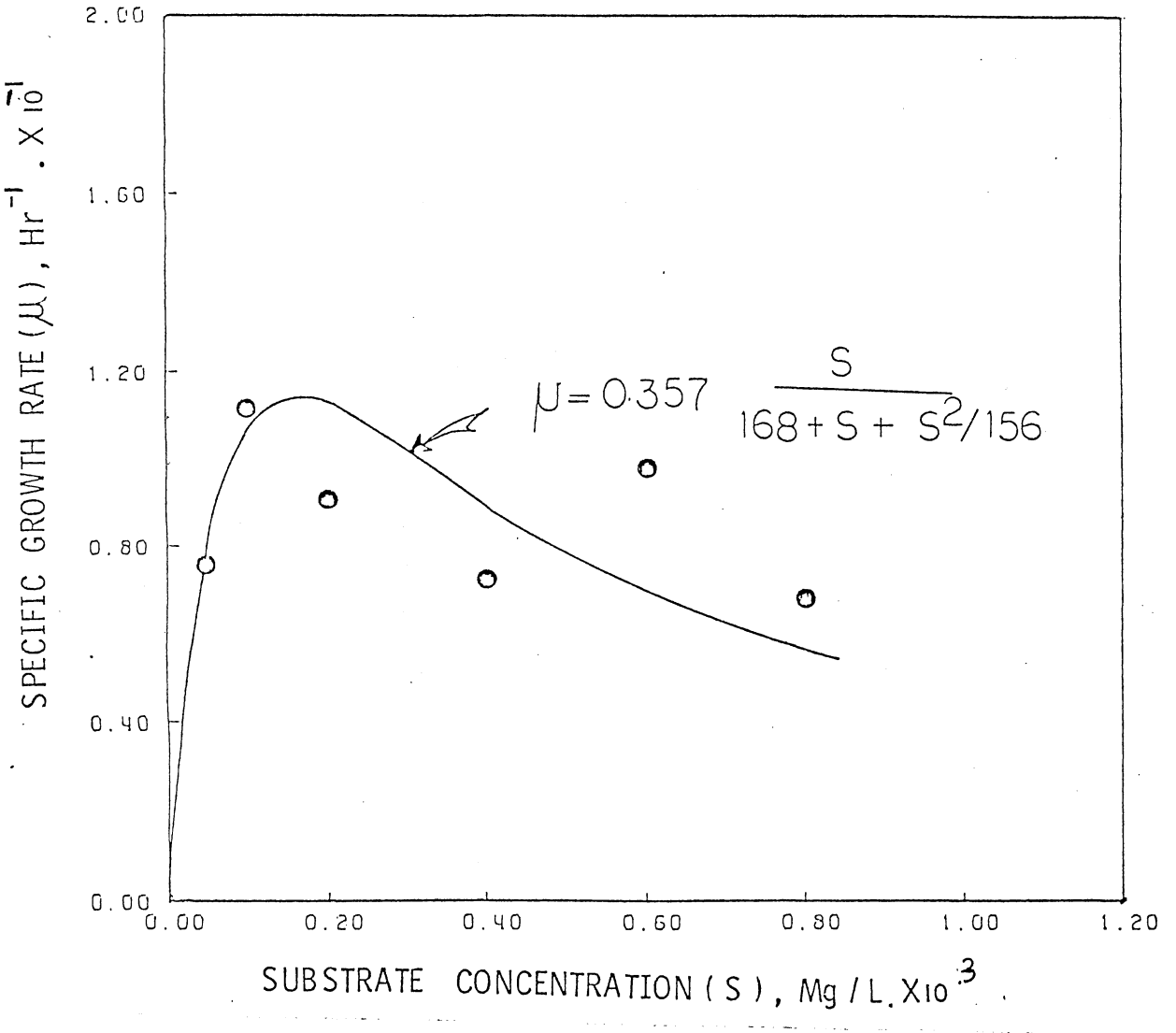

Figure 12. Specific Growth Rate (μ) Versus Substrate Concentration(s) for θ_c = 7.93 Days (Batch Unit)

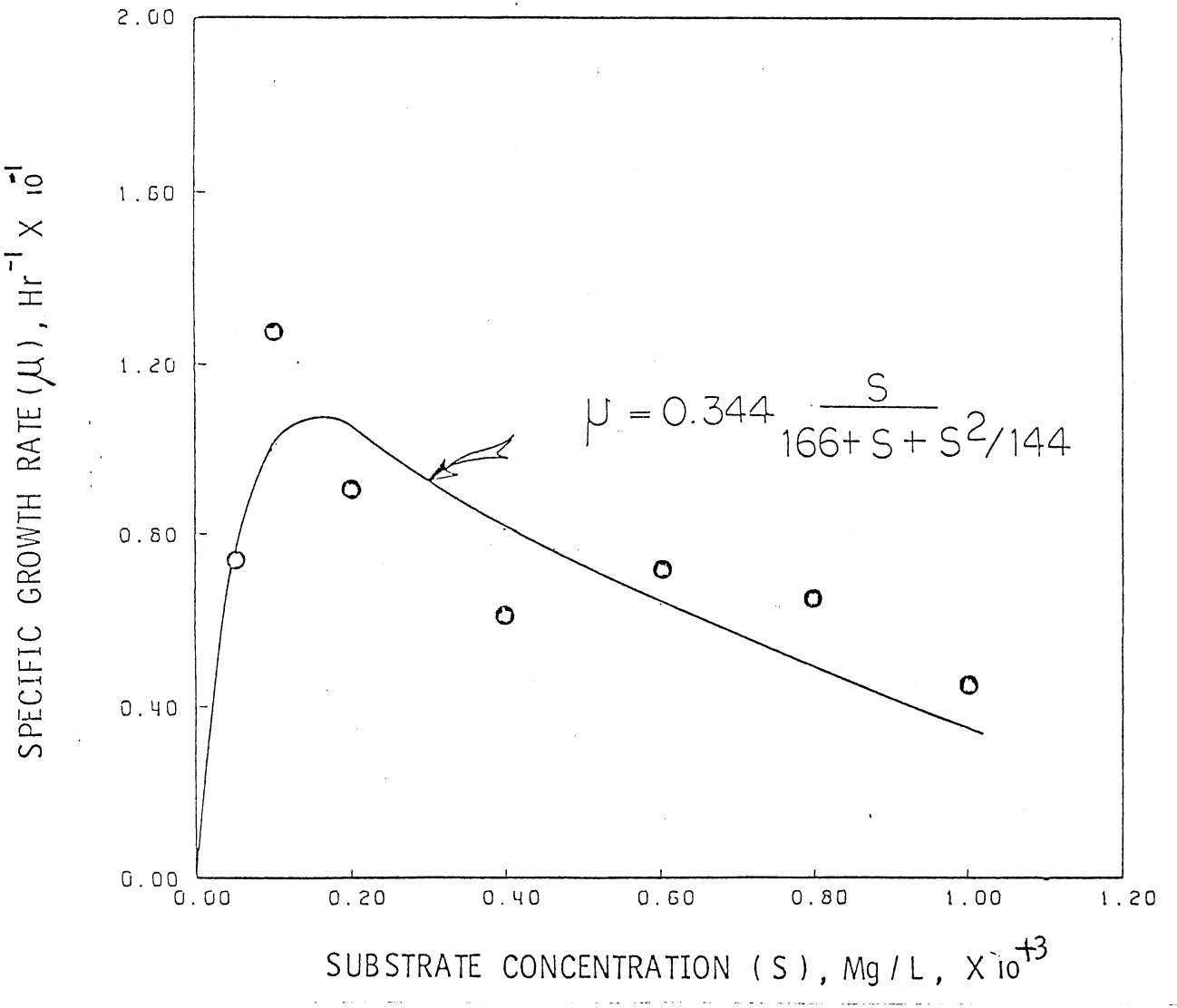

Figure 13. Specific Growth Rate (μ) Versus Substrate Concentration(s) for θ_c = 4.15 Days (Batch Unit)

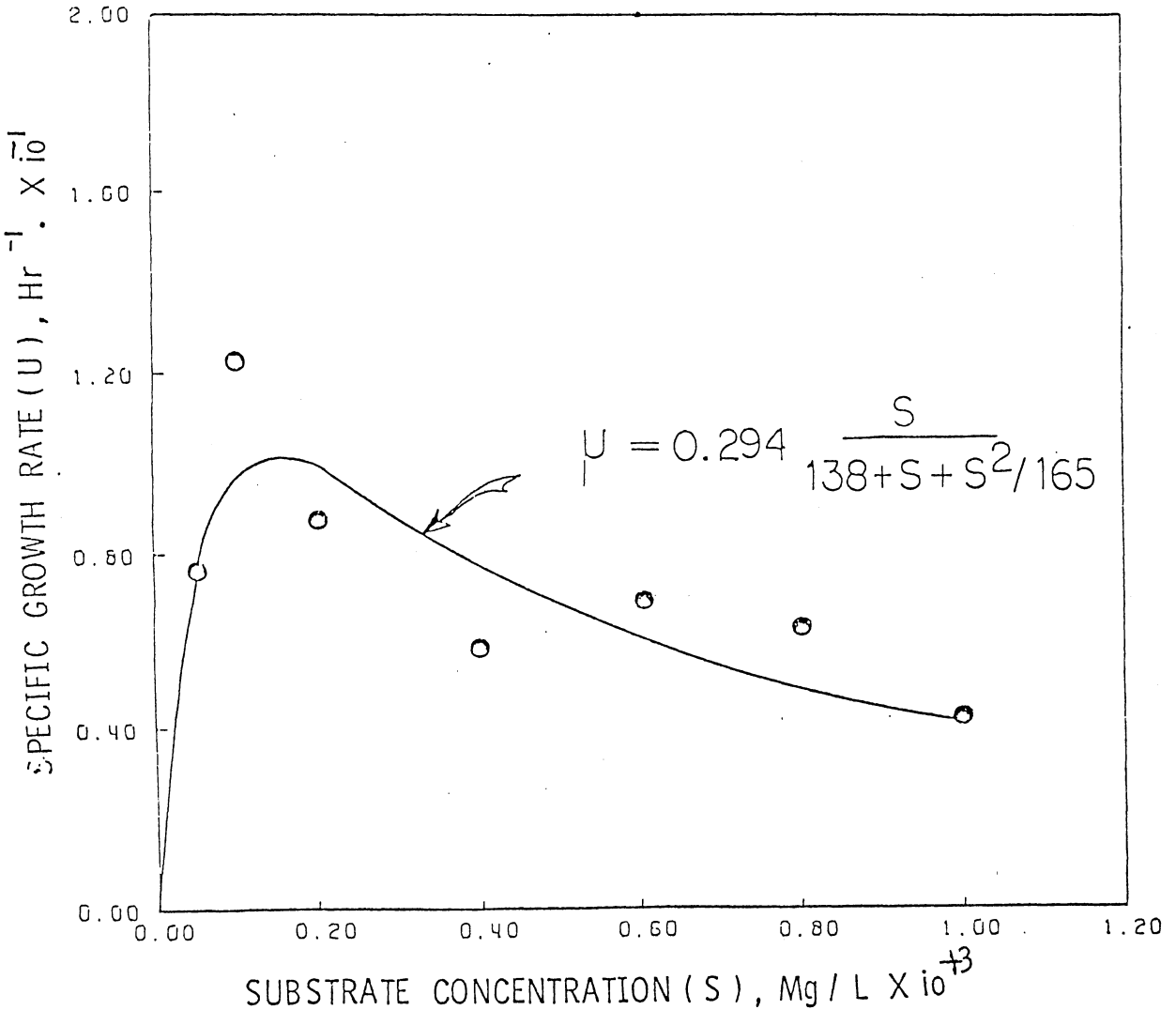

Figure 14. Specific Growth Rate Versus Substrate Concentration at Sludge Age of 3.0 Days for Batch Growth Study Unit

no toxic effects from phenol are observed at low phenol concentrations. Yang and Humphrey (8) have shown that pure cultures did not exhibit a toxic effect from phenol at concentrations below 100 mg/ℓ. Therefore, by plotting $1/\mu$ versus $1/s$ for substrate concentrations below 100 mg/ℓ, μ_{max} and K_s can be evaluated. The determination of μ_{max} and K_s for the four growth studies conducted are shown in Figures 15 through 18. The values for μ_{max} and K_s for each mean cell residence time (θ_c) are shown in Table V. It is seen that the values for μ_{max} and K_s are fairly close for all mean cell residence times (θ_c) studied. Using the μ_{max} and K_s values so obtained, K_i for each substrate concentration of batch growth study was calculated using the Haldane equation. An average of all the K_i values corresponding to s = 50 and 100 mg/ℓ was taken as the K_i value for the cells harvested from the reactor at that sludge age (θ_c) of operation. Average K_i values for different sludge age (θ_c) are shown in Table V. A model calculation is given in Appendix B.

C. Continuous Flow Unit Data

For a continuous flow unit it is not possible to determine or calculate the biological constants (μ_{max}, K_s, K_i) of biomass in the system for any particular cell residence time of operation. Biological constants (μ_{max}, K_s, K_i) for biomass in the continuous flow unit is assumed to be the one obtained from the batch growth study. Using Haldane's Equation (F.10) and substituting s values of 10, 20, and 30 mg/ℓ, and using the biological constants obtained from batch study, the μ values were predicted for a continuous unit. A plot of predicted μ versus s is shown in Figures 19 through 22. Observed μ for a continuous unit is calculated using the following equation:

TABLE V

μ_{max}, K_s, AND AVERAGE K_i FOR BATCH GROWTH STUDY

θ_c	μ_{max}	K_s	K_i
3.00	0.294	138	165
4.15	0.344	166	144
7.93	0.357	168	156
12.69	0.285	125	183

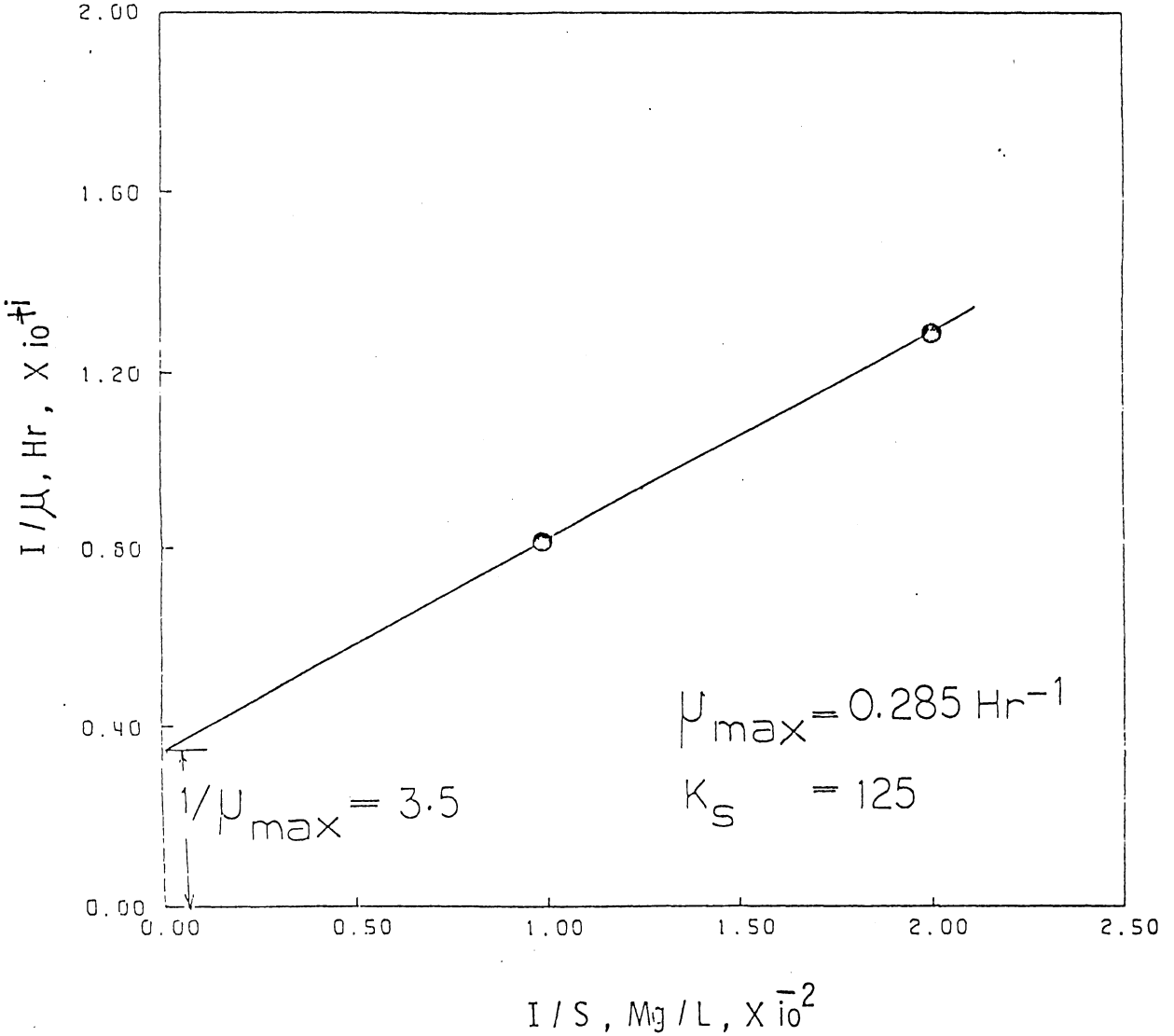

Figure 15. Reciprocal Specific Growth Rate Versus Reciprocal Substrate Concentration at Sludge Age of 12.69 Days for Batch Growth Study

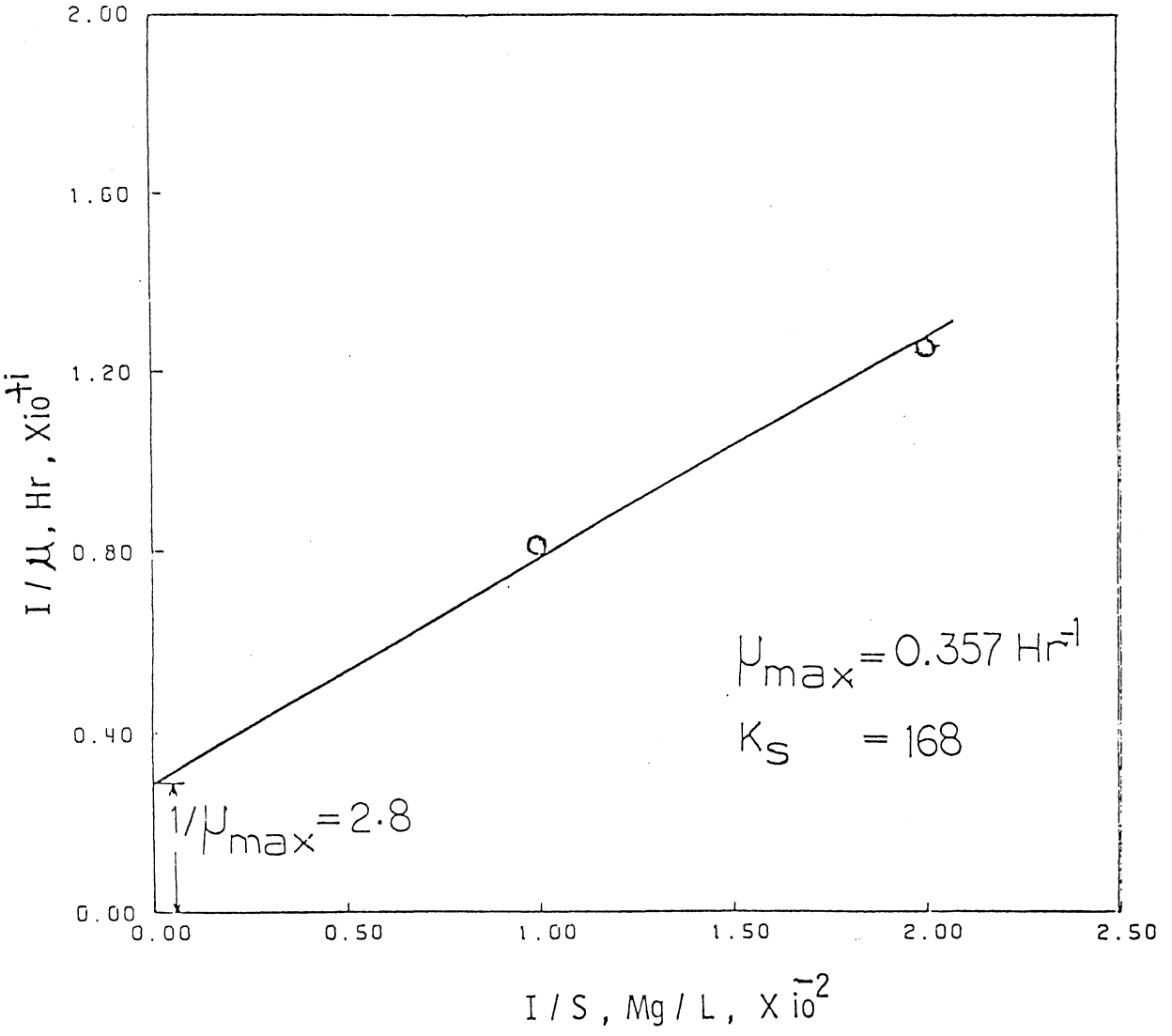

Figure 16. Reciprocal Specific Growth Rate Versus Reciprocal Substrate Concentration at Sludge Age of 7.93 Days for Batch Growth Study

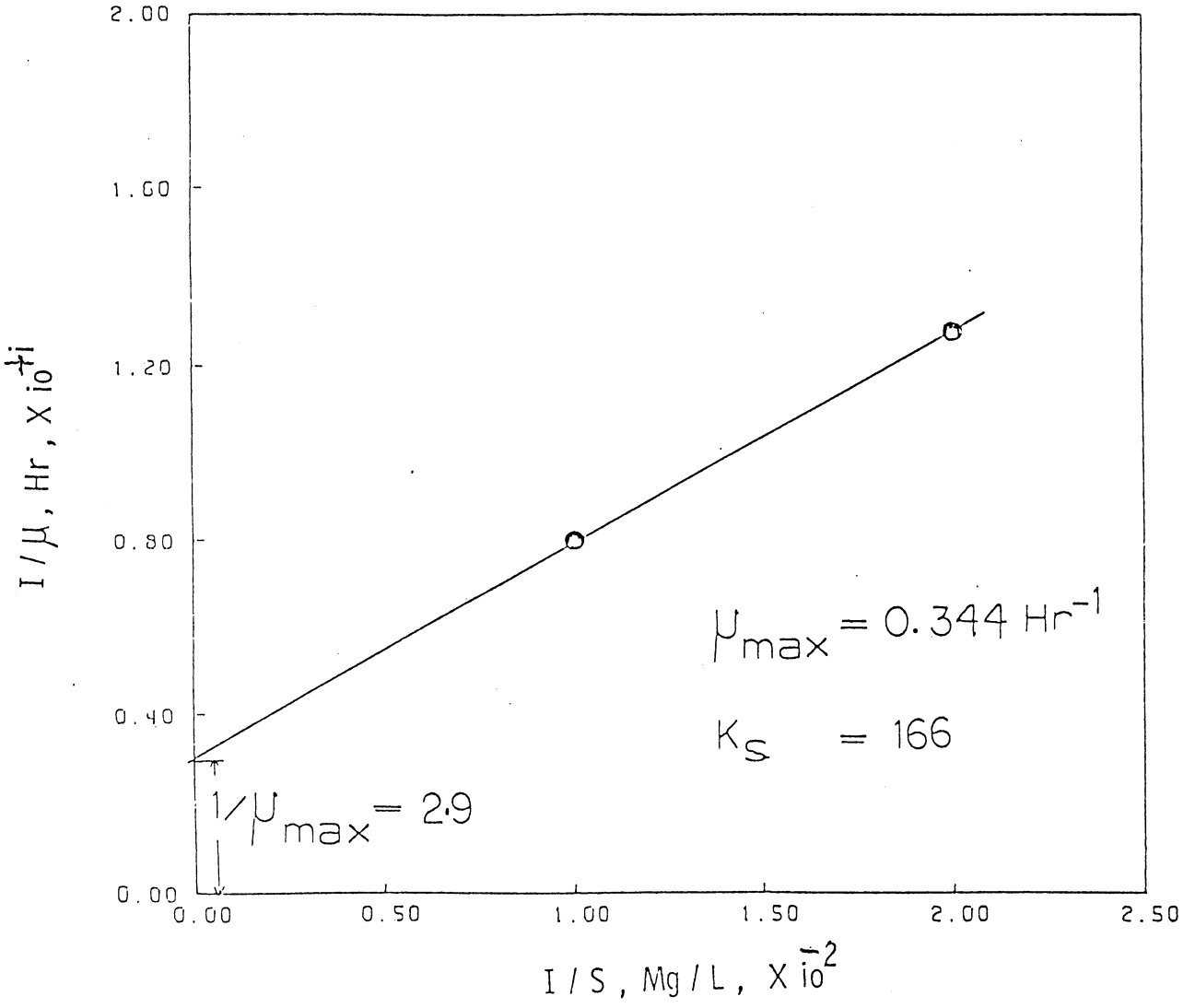

Figure 17. Reciprocal Specific Growth Rate Versus Reciprocal Substrate Concentration at Sludge Age of 4.15 Days for Batch Growth Study

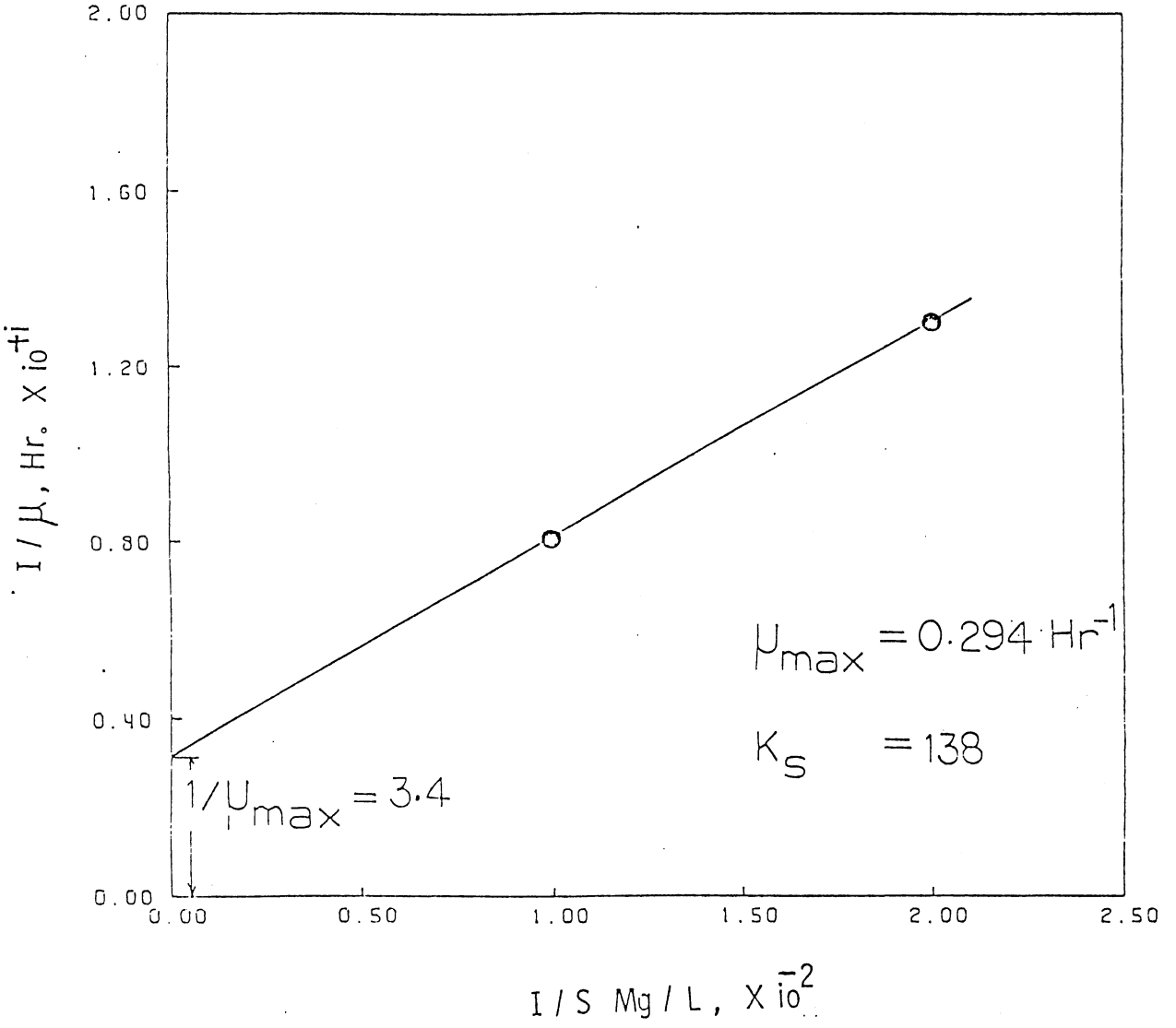

Figure 18. Reciprocal Specific Growth Rate Versus Reciprocal Substrate Concentration at Sludge Age of 3.0 Days for Batch Growth Study

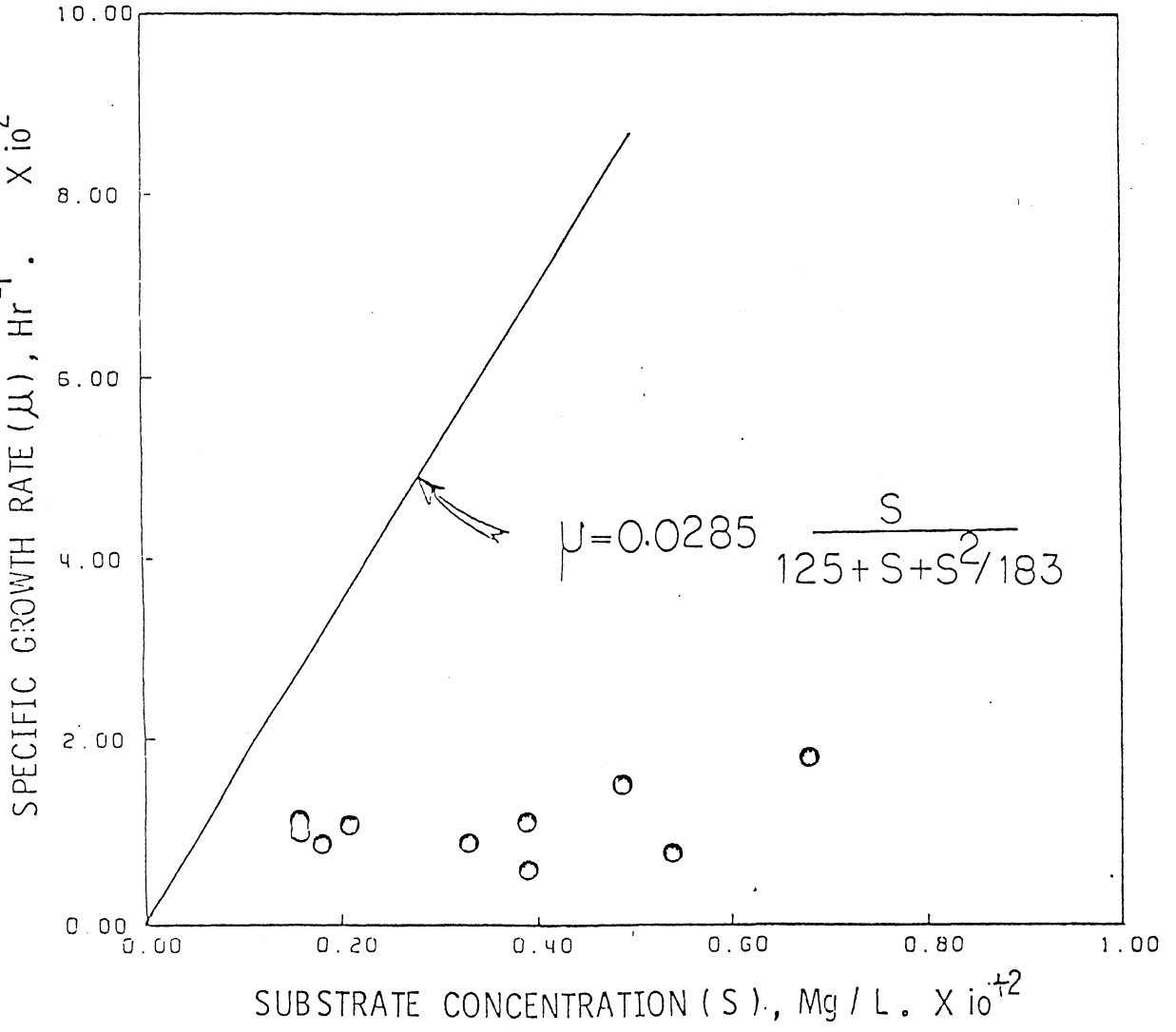

Figure 19. Specific Growth Rate Versus Substrate Concentration at Sludge Age of 12.69 Days for Continuous Flow Unit

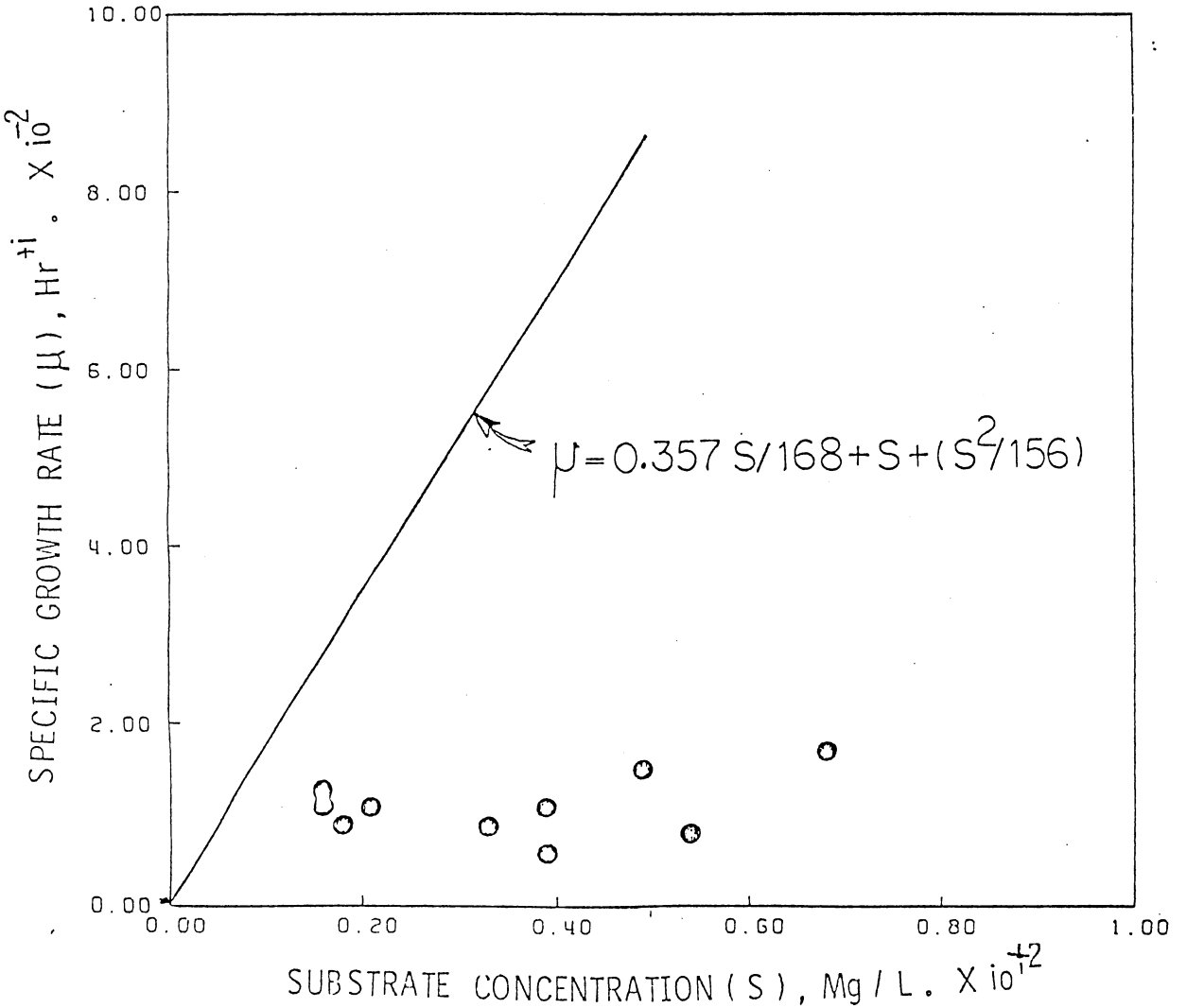

Figure 20. Specific Growth Rate Versus Substrate Concentration at Sludge Age of 7.93 Days for Continuous Flow Unit

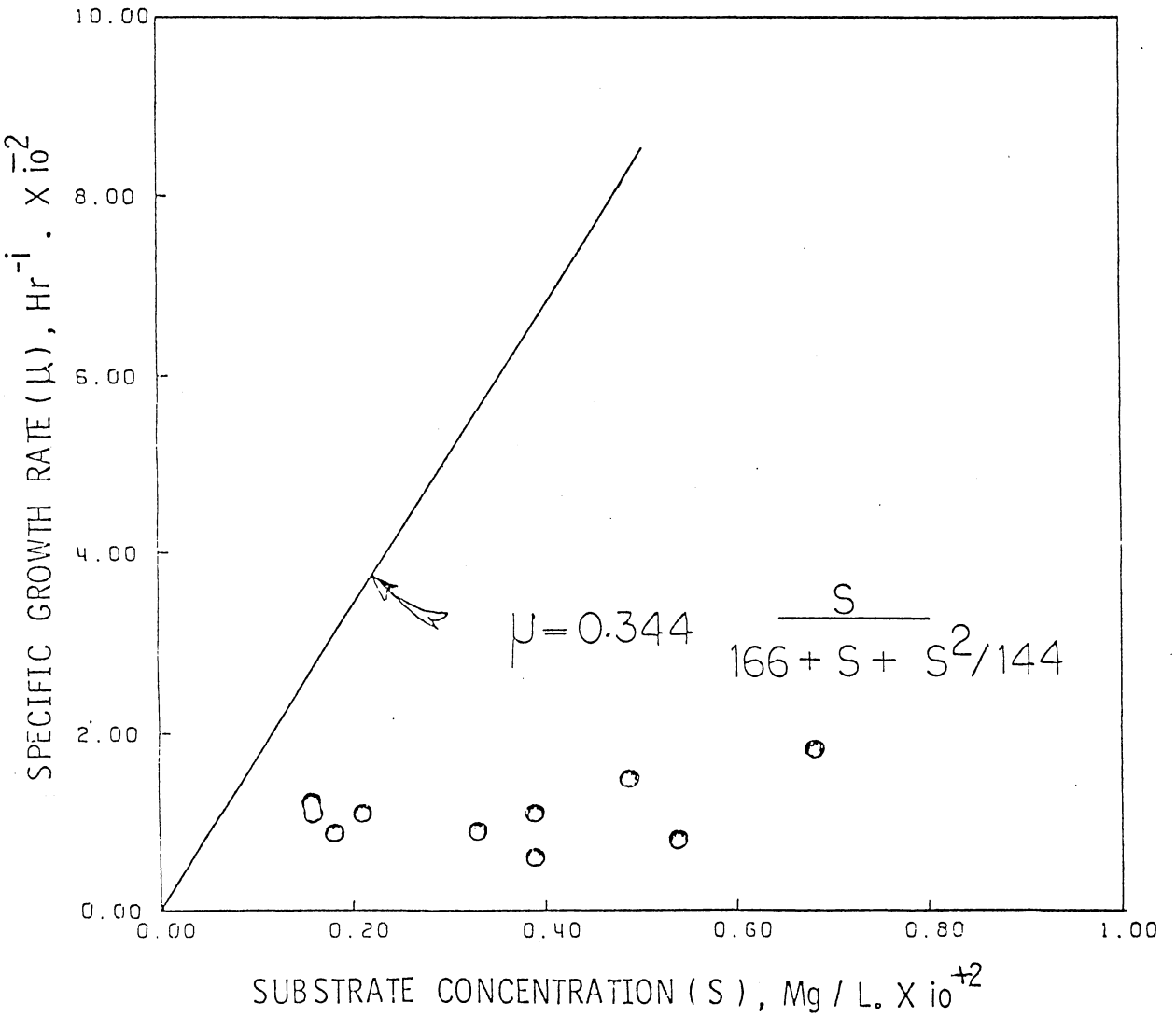

Figure 21. Specific Growth Rate Versus Substrate Concentration at Sludge Age of 4.15 Days for Continuous Flow Unit

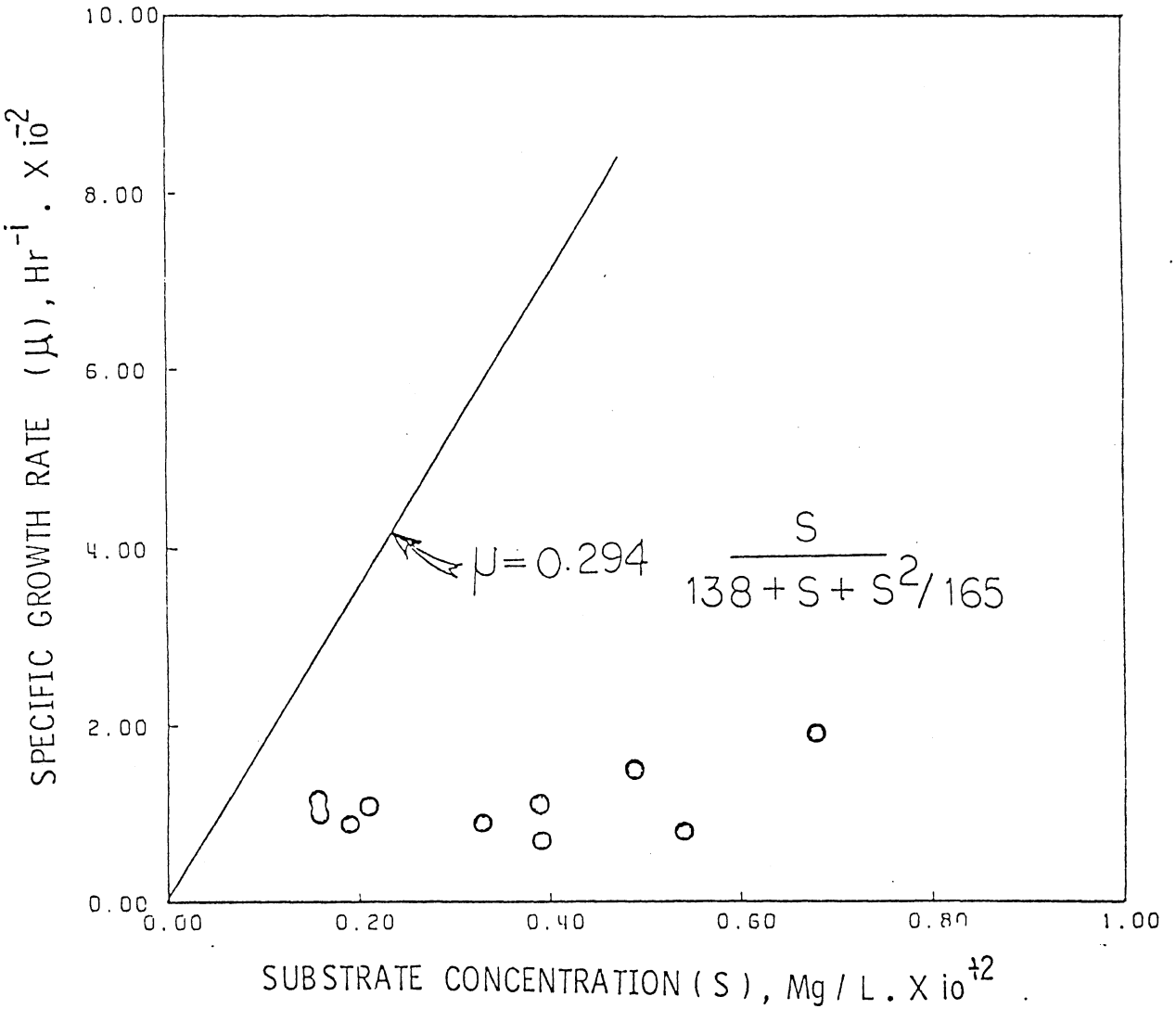

Figure 22. Specific Growth Rate Versus Substrate Concentration at Sludge Age of 3.0 Days for Continuous Flow Unit

$$\mu = \frac{F_w X + (F - F_w) X_e}{VX} + K_d$$

The μ_{obs} values are shown in Table III for different sludge ages (θ_c) of operation. The observed soluble substrate (S_e) in the continuous reactor (in the effluent) for different sludge ages (θ_c) of operation is also shown in Table III. Observed μ_{obs}(μ) versus S_e(s) is shown in Figures 19 through 22, where the predicted μ versus s is plotted. It is seen that the continuous flow growth rates fall well below the ones predicted by the Haldane equation.

CHAPTER V

DISCUSSION

This investigational work was undertaken in order to study the applicability of kinetic relationships to biological treatment of phenolic waste and the inhibition effect of phenol on growth of biomass. The experimental unit was run at different mean cell residence time (θ_c) ranging from 26 days to 3 days.

The C.O.D. removal efficiency of the system ranges from 74 to 94 percent, as shown in Table III and Figure 2. Except for the very low sludge age (θ_c) value of 3.0 days, the C.O.D. removal efficiency did not vary much and stayed relatively high for different sludge age (θ_c) values ranging from 4.15 days to 26.23 days. Similar high efficiency of removal under various mean cell residence times of operation were observed by Lowe (4) as well as other research workers (1) (10) (12).

The observed yield varied appreciably from 0.26 at mean cell residence time (θ_c) of 26.23 days to as high as 0.9 at low sludge age (θ_c) of 3.0 days. Observed yield values as high as 0.9 and above has been observed by Lowe (4). Radhakrishnana and Ray (5) and Yang and Humphrey (8) also got a high observed yield (above 0.8). The possible reason for the high observed yield could be due to the presence of some autotrophic organisms or because of high carbon/oxygen ratio in phenol. Increase of observed yield with decrease in sludge age (θ_c) has been observed in the conventional growth kinetic studies using glucose as a carbon source as

well as phenol. One prime reason seems to be the maintenance energy requirements.

From Figures 5 and 6 the true yield (Y_t) value for the continuous flow unit is found to be 1.19 and the decay coefficient is 0.12 day^{-1}. The author is aware that it is impractical to get a true yield of 1.0 and above, practically and logically. However, mathematically, true yields of 1.0 and above have been reported in the literature. One single continuous yield curve was obtained in the present studies, as shown in Figure 4. Two distinct observed yield (Y_{obs}) curves were obtained by Lowe (4). The same way discontinuous kinetics was observed by Lowe (4) in the plots of P_x versus s, MLSS versus θ_c, F/M versus θ_c, and U versus θ_c. But no such discontinuous kinetics was observed in the present study. If the unit would have been operated at much lower sludge age (θ_c) values other than the one selected for the study, a discontinuous kinetics resulting in two distinct kinetic curves instead of one might have been obtained.

Figures 11 through 14 show the plots of μ versus s as obtained in batch growth study. The predicted values of μ according to the Haldane Equation (F.10) were also plotted as a continuous curve in the same figure in order to see the closeness or diversity between the actual value of μ according to experimental batch growth study and the theoretical value of μ according to the Haldane Equation (F.10). The actual and theoretical values are close to each other. It indicates that the calculated biological constants, μ_{max}, K_s, and K_i are close to actual values observed in the batch growth study experiments. It also shows the presence of inhibitory effect due to phenol and justifies the use of the Haldane Equation (F.10) to describe growth kinetics instead of

Monod's equation. Similar observation has been reported in the literature by Pawlosky and Howell (14).

Figures 19 through 22 show the plot of specific growth rate of μ versus s observed for various sludge age (θ_c) values for continuous unit. In the same figures the theoretical values of μ versus s, according to the Haldane Equation (F.10) were plotted as a continuous curve (st. line). It is evident that in all of the graphs there is wide variation between the actual μ_{obs} and the predicted μ, according to the Haldane Equation (F.10). This indicates that the equations which were developed using the four sets of batch growth study data do not describe the continuous flow study data. One reason can be that at low concentration, phenol may not have an inhibitory effect and the Haldane Equation (F.10) may not be applicable at low concentration. To further describe the continuous flow growth rate, in the Haldane Equation (F.10), μ_{max}, K_s, and K_i values used are the ones obtained from batch growth study experiments. It is possible that the actual growth rate constants in the continuous flow system can be much different from the one obtained from batch growth study. However, it seems over and above this reason, it is quite likely at low concentration the phenol may not have an inhibitory effect and the growth kinetic may now follow Haldane's equation.

CHAPTER VI

CONCLUSIONS

A continuous flow activated sludge unit with internal recycle at different sludge ages (θ_c) using phenol as substrate was operated. Mean cell residence time (θ_c) was used as the operating parameter. This study has led to the following conclusions listed below.

1. Under normal range operation the sludge age (θ_c) does not have any significant effect on the effluent quality. Good treatment efficiency was obtained for the phenolic waste.

2. Relatively high yield was obtained for biomass treating phenolic waste when compared to conventional domestic waste.

3. No discontinuous kinetics was observed in the present study.

4. At higher concentration of phenol as substrate the specific growth rate closely follows the relationship developed by the Haldane equation.

5. Phenol does not have an inhibition effect on specific growth rate at lower concentration.

CHAPTER VII

SUGGESTIONS FOR FUTURE STUDY

The following suggestions are offered for future investigations.

1. Study the microbial populations and types at different sludge ages (θ_c).

2. Study the effect of temperature on phenolic inhibitions.

3. Conduct similar continuous flow studies over a wide range of influent (S_i) concentrations of phenol.

4. Study the effect of hydraulic detention of time and treatment efficiency and phenolic inhibition.

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APPENDIX A

K_i AND μ VALUES OF θ_c

TABLE VI

K_i AND μ VALUES OF θ_c

Serial No.	S, mg/ℓ	μ, hr^{-1}	μ_{max}, hr^{-1}	K_s	K_i	Serial No.	S, mg/ℓ	μ (Data), hr^{-1}	μ (Cal), hr^{-1}
		θ_c = 12.69 Days							
1	50	0.080	0.285	125	800	1	50	0.080	0.076
2	100	0.123	0.285	125	1491	2	100	0.123	0.102
3	200	0.093	0.285	125	139	3	200	0.093	0.100
4	400	0.060	0.285	125	116	4	400	0.060	0.081
5	600	0.070	0.285	125	210	5	600	0.070	0.064
6	800	0.066	0.285	125	253	6	800	0.066	0.052
7	1000	0.046	0.285	125	197	7	1000	0.046	0.043

Average $K_i \rightarrow 183$

TABLE VI (Continued)

Serial No.	S, mg/ℓ	μ, hr^{-1}	μ_{max}, hr^{-1}	K_s	K_i	Serial No.	S, mg/ℓ	μ (Data), hr^{-1}	μ (Cal), hr^{-1}
		θ_c = 7.93 Days							
1	50	0.077	0.357	168	181	1	50	0.077	0.076
2	100	0.119	0.357	168	313	2	100	0.119	0.107
3	200	0.092	0.357	168	98	3	200	0.092	0.114
4	400	0.073	0.357	168	115	4	400	0.073	0.089
5	600	0.088	0.357	168	216	5	600	0.088	0.069
6	800	0.068	0.357	168	198	6	800	0.068	0.056

Average
$K_i \rightarrow 156$

TABLE VI (Continued)

Serial No.	S, mg/ℓ	μ, hr^{-1}	μ_{max}, hr^{-1}	K_s	K_i	Serial No.	S, mg/ℓ	μ (Data), hr^{-1}	μ (Cal), hr^{-1}
		θ_c = 4.15 Days							
1	50	0.078	0.344	166	554	1	50	0.078	0.073
2	100	0.128	0.344	166	3636	2	100	0.128	0.102
3	200	0.091	0.344	166	103	3	200	0.091	0.106
4	400	0.061	0.344	166	95	4	400	0.061	0.082
5	600	0.072	0.344	166	171	5	600	0.072	0.063
6	800	0.065	0.344	166	196	6	800	0.065	0.051
7	1000	0.045	0.344	166	154	7	1000	0.045	0.037

Average $K_i \rightarrow 144$

TABLE VI (Continued)

Serial No.	S, mg/ℓ	μ, hr^{-1}	μmax, hr^{-1}	K_s	K_i	Serial No.	S, mg/ℓ	μ (Data), hr^{-1}	μ (Cal), hr^{-1}
		θ_c = 3.0 Days							
1	50	0.077	0.294	138	859	1	50	0.077	0.072
2	100	0.123	0.294	138	9762	2	100	0.123	0.098
3	200	0.088	0.294	138	121	3	200	0.088	0.101
4	400	0.059	0.294	138	110	4	400	0.059	0.078
5	600	0.069	0.294	138	198	5	600	0.069	0.061
6	800	0.063	0.294	138	229	6	800	0.063	0.049
7	1000	0.043	0.294	138	167	7	1000	0.043	0.041

Average
$K_i \rightarrow 165$

APPENDIX B

CALCULATIONS OF BATCH GROWTH STUDY MODEL AND CONTINUOUS FLOW UNIT MODEL

The batch growth study model calculations of K_i and μ for θ_c = 4.15 days. These calculations are presented below.

Calculation of K_i

S = 1000 mg/ℓ.

$$\mu = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}}$$

$$0.045 = 0.344 \frac{1000}{166.0 + 1000 + \frac{(1000)^2}{K_i}} = 144$$

$\therefore K_i = 144.$

Calculation of μ, hr^{-1}

S = 1000 mg/ℓ.

$$\mu = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}}$$

$$\mu = 0.344 \frac{}{144 + 1000 + \frac{(1000)^2}{144}} = 0.0372$$

$\therefore \mu = 0.0372\ hr^{-1}.$

Calculation of μ

The continuous flow unit's model calculation of μ for θ_c = 4.15 days. The calculation is presented below.

S = 10 mg/μ;

K_s = 166;

$K_i = 144$; and

$\mu_{max} = 0.344\ hr^{-1}$.

$$\mu = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}}$$

$$\mu = (0.344) \frac{(10)}{(166) + 10 + \frac{10^2}{144}} = 0.019$$

$\therefore\ \mu = 0.019\ hr^{-1}$.

VITA[2]

S. N. Vithal Reddy

Candidate for the Degree of

Master of Science

Thesis: APPLICABILITY OF INHIBITORY KINETICS ON BIOLOGICAL SYSTEMS USING PHENOLIC WASTES

Major Field: Bioenvironmental Engineering

Biographical:

Personal Data: Born February 8, 1949, in Zahirabad, Medak (A.P.), India, the son of Saireddy Narsimha Reddy and Saireddy Susilamma.

Education: Graduated from V. V. Ex. High School, Hyderabad, India; received the Pre-University degree from A. U. College, Hyderabad; received the Bachelor of Engineering degree from Osmania University, Hyderabad, in April, 1974; completed requirements for the Master of Science degree at Oklahoma State University, Stillwater, Oklahoma, in December, 1977.

Professional Experience: Served as Junior Engineer in A.P.C.L.M.B. Ltd., Mathani (A.P.), India, from October, 1974, to March, 1975; graduate research assistant, School of Civil Engineering, Oklahoma State University, from September, 1976, to December, 1977.

Membership in Professional Societies: Water Pollution Control Federation, American Water Works Association, American Society of Civil Engineers (Student Member).

Publication: Dr. D. F. Kincannon, D. A. Lowe, and S. N. V. Reddy, "Application of Kinetic Relationships to the Biological Treatment of Phenolic Wastes," presented at A.C.S. Conference, New Orleans, Louisiana, March, 1977.