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A CONTINUOUS FLOW KINETIC MODEL TO PREDICT THE EFFECTS OF TEMPERATURE ON THE TOXICITY OF WASTE TO ALGAE

by

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ABSTRACT

A continuous flow kinetic model to describe and predict the effects of temperature on the toxicity of a specific oil refinery waste to the green alga, *Selenastrum capricornutum,* is developed. The model is based on enzyme inhibition kinetics and is developed using semi-continuous flow and continuous flow algal cultures grown between 20° C (68° F) and 33° C (91° F). Phenol is employed as the controlling inhibitor or toxicant. The model is applied to continuous flow algal cultures exposed to an actual oil refinery waste.

In addition, the maximum specific growth rates, $\hat{\mu}$, the half saturation constants, K_s, and the nutrient utilization constants, K_A and K_B, for two luxury uptake functions are determined for the alga, *Selenastrum capricornutum,* growing in an ammonium-nitrogen limited environment between 20°C (68° F) and 33° C (91° F).

Results indicate that phenol and the oil refinery waste studied exert competitive inhibition of *Selenastrum capricornutum,* and that phenol is more toxic at 24° C (75° F) than at either 20° C (68° F) or 28° C (82° F). In addition, the maximum specific growth rate, $\hat{\mu}$, has a maximum value between 24° C (75°F) and 27°C (81° F). Also, the ammonium-nitrogen half saturation constant, K_s, does not vary with temperature between 20°C (68° F) and 33° C (91° F). The variation of the nutrient utilization constants, K_A and K_B, for the luxury uptake functions is similar to the variation of the maximum specific growth rate, $\hat{\mu}$, for the temperature range studied.

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CHAPTER I

INTRODUCTION

Nature of the Problem

The combination of thermal enrichment and toxic waste discharges is a problem affecting many sections of the environment. Thermal enrichment is the discharge of waste heat to a natural water system. It is often referred to as thermal pollution. Toxic wastes are generated and discharged by all phases of industrial, municipal, and agricultural life. Frequently, toxic wastes are discharged in combination with or adjacent to thermal discharges. In addition, toxic wastes may be discharged at elevated temperatures.

Toxic wastes have been discharged for many decades; however, thermal pollution or discharge of waste heat is of a more recent era (29, 49, 68, 69). Industrial effluents, municipal discharges, electrical power generating facility effluents, and even agricultural runoff, tend to increase the temperature of natural water systems; and all of these wastes may contain toxic substances. Electrical power generating facilities, the major source of thermal pollution (29, 49, 51), are expected to increase their present level of production six times by the year 2000 (87). This unprecedented increase in electrical power production will undoubtedly increase the present level of thermal pollution and will place an additional burden on natural water systems presently receiving toxic wastes.

In addition, many industries such as pulp and paper mills, tanneries, and oil refineries, discharge toxic wastes at extremely high temperatures (30, 58, 63). In essence, they combine toxic wastes and thermal pollution into one waste stream. For instance, a typical oil refinery waste (58) may be discharged at temperatures ranging from 20°C (68°F) to 41°C (106°F). These high temperatures may have a significant effect on the toxicity of a particular waste. The interaction between temperature and toxicity could substantially affect biological treatment efficiency and the assimilative capacity of a receiving stream.

The relationship between temperature and toxicity has not been extensively investigated. Therefore, it is essential that the effects of temperature on the toxicity of various wastes be evaluated, and that mathematical relationships be developed which will describe and predict these effects.

Scope of the Study

In this study a continuous flow kinetic model to describe and predict the effects of temperature on the toxicity of a specific oil refinery waste to a green alga, *Selenastrum capricornutum,* was developed. The model was based on enzyme inhibition kinetics and was developed using semi-continuous and continuous flow algal cultures grown between 20°C (68°F) and 33°C (91° F). Phenol was employed as the controlling inhibitor or toxicant. The model was applied to continuous flow algal cultures exposed to an actual oil refinery waste. In addition, the maximum specific growth rates, $\hat{\mu}$, the half saturation constants, K_s, and the nutrient utilization constants, K_A and K_B, for the luxury uptake functions developed by Toerien et al. (83) were determined for *Selenastrum capricornutum* growing in an ammonium-nitrogen limited environment over the temperature range studied.

Algae were selected as the test organism because: 1) they are the basis for the aquatic food chain and thus are the principal food source for larger aquatic organisms, and 2) they are the primary organism involved in the lagoon treatment of toxic wastes. Thus, interactions between temperature and toxicity which affect algae will affect the total aquatic food chain and also will interfere with certain waste treatment unit operations. In addition, this particular algal species, *Selenastrum capricornutum,* was selected as the test organism, because it has been specified by the Environmental Protection Agency for use in bioassays (26).

Objectives

The general objective of this study was to develop a mathematical model to predict the effects of temperature on the toxicity of oil refinery waste to algae.

To satisfy the above general objective the following specific objectives were undertaken:

a) To determine an acceptable temperature range for conducting the experiment.
b) To determine an acceptable toxicant concentration range for conducting the experiment.
c) To determine the maximum specific growth rate, $\hat{\mu}$, and the half saturation constant, K_s, for *Selenastrum capricornutum* in an

ammonium-nitrogen limited environment over the temperature range studied.

d) To apply the luxury uptake function developed by Toerien et al. (83) to an ammonium-nitrogen limited environment and determine the luxury uptake constants over the temperature range studied.

e) To develop an enzyme inhibition model to describe phenol toxicity to *Selenastrum capricornutum* over the temperature range studied.

f) To apply the enzyme inhibition model developed to an actual oil refinery waste.

Significance

Many lakes, rivers, streams, and estuaries receive both toxic wastes and heated effluents. In addition, as the number of power generating facilities increase, more and more natural water systems will be receiving both heated and toxic wastes. Also, society's demands for more and better products will increase the amount of heated, toxic wastes discharged to the environment. Thus, the effects of temperature on the toxicity of wastes must be defined.

Many studies have been conducted to determine the effects of elevated temperatures on various organisms (10, 13, 21, 25, 68, 90), and several investigators have determined the toxicity of many compounds to various organisms (23, 25, 32, 33, 60). However, very few studies have been made to determine and understand the effects of elevated temperature on the toxicity of compounds to various organisms. This study was designed to provide basic information about the complex relationship between temperature and toxicity of oil refinery wastes to algae. It also provides a basic mathematical model for describing the overall temperature-toxicity relationship.

CHAPTER II

LITERATURE REVIEW

Temperature and Toxicity

The effects of temperature on microorganisms were investigated as early as 1890 (69). Since that time many studies and reviews of the literature related to the effects of temperature on living organisms have been conducted (10, 13, 23, 25, 62, 68, 90). Unfortunately the lack of a standard bioassay procedure, nomenclature for reporting results and the sheer mass of detailed information have made comparison of these various studies virtually impossible (62, 69). In addition, most of these studies were carried out to determine temperature effects alone and do not provide information concerning the relationship between temperature and toxicity.

Recent investigators have indicated that the effect of temperature on the growth of microorganisms may be represented by the traditional Arrhenius equation. Verma and Nepal (86) used the Arrhenius equation to explain the change in growth rate of a bacteria population developed from raw sewage. Their experiments were conducted with batch cultures grown between 20°C (68°F) and 37°C (99°F). Goldman (39) gathered data from the literature on the growth rates of several green algal species and developed an Arrhenius type equation to explain the variation of algal growth rate with temperature. However, his equation does not account for the decline in growth rate of several algal species as the increase in temperature approaches their maximum specific temperature limit. Rye and Mateles (76) have reported that the use of the Arrhenius equation in connection with microorganisms is only valid within a narrow specific temperature range.

As with the effects of temperature on microorganisms, many excellent reviews and papers have been published to describe the toxicity of various compounds to microorganisms (16, 23, 25, 32, 33, 60, 62, 69). The most noteworthy of these is the monumental work of McKee and Wolf (60) which contains over 3800 references. However, the literature is lacking in specific studies that relate to toxicity and temperature.

De Silva (21) has attempted to summarize the available data on the combined effects of temperature and toxic materials to fish. He indicated that, in general, toxicity is increased with increasing temperature. Angelovic, Sigler, and Neuhold (5) have reported that the lethal concentration (LC_{50}) of fluorides decreases with an increase in temperature. A decrease in the LC_{50} indicates an increase in the level of toxicity. Pickering and Henderson (70) have reported that the toxicity of zinc to fathead minnows increases with increases in temperature.

Brown, Jordan, and Tiller (12) reported that the resistance of rainbow trout to phenol poisoning increased with increases in temperature; thus, indicating a decrease in toxicity with increasing temperatures. McClean (61) reported that the toxicity of chlorine to a barnacle larvae and a copepod was unaffected by increases in temperature.

More recently, a review of over 1200 references on temperature and toxicity published by Middlebrooks et al. (62) indicated that the effects of temperature on the toxicity of various compounds varies in accordance with the specific toxic substance and the species tested. They also suggested that future toxicity bioassays be designed to identify the temperature-toxicity relationship and that a standard bioassay procedure be utilized to allow comparison of results.

Oil Refinery Wastes

General characteristics

Oil refinery wastes are heterogenous toxicants and their composition is extremely variable. Therefore, it is impossible to completely and accurately describe or characterize a typical effluent. The characteristics of specific waste discharges for a particular refinery have often appeared in the literature (2, 20, 24, 25, 30, 34, 38, 40, 51, 57, 58, 85). The most common characteristics of oil refinery wastes have been summarized in Table 1. However, the values in Table 1 should not be considered as representing a typical oil refinery waste; rather, they should be viewed as a range of values often encountered with oil refinery discharges. The character of a specific oil refinery waste will depend on the nature of the crude oil being processed, the type of product produced, the type of refinery process employed, and the efficiency of the refinery operation.

Many of the toxic components in an oil refinery waste, even if present in sublethal concentrations, may damage aquatic organisms because of toxicity due to

synergistic effects of several interacting components (16). Similarly, the presence of multiple toxicants, even if present at lethal concentrations, may not exhibit the expected toxic effect because of antagonistic non-toxic effects between interacting compounds. The concentration ranges of several toxicants often found in oil refinery wastes are shown in Table 2. It should be pointed out that Table 2 is not inclusive and that a specific oil refinery waste may contain other toxicants and other concentrations of toxicants; but these are the most typical.

Oil refinery waste toxicity

Investigations of oil refinery waste toxicity are not widely publicized. Case studies which are available deal with a specific oil refinery waste and location; therefore, it is difficult to extrapolate results into generalizations. For instance, Douglas and Irwin (25) have evaluated the relative resistance of 16 species of fish to a specific petroleum refinery waste. Their results do not reveal the relative toxicity of the waste; but rather, they suggest the

Table 1. Summary of oil refinery waste characteristics.

Parameter	Range		Reference No.
	Min	Max	
Temperature ^{o}C	22	41	58
pH	6.2	10.6	1,40,58
BOD_5 mg/l	17.0	280	1,40,85
COD mg/l	140	3,340	1,34,38,40,58,85
Sulfides mg/l	0.0	38	1,40,58,85
Phenol mg/l	0.3	154	1,40,56,58,85
Hardness as $CaCO_3$ mg/l	139	510	58,85
Alkalinity as $CaCO_3$ mg/l	77	356	1,40,58,85
Oil mg/l	23	200	1,85
Phosphorus mg/l	0.0	97	1,58,85
NH_3 mg/l as N	0.0	120	1,38,40,58,85
Chlorides mg/l	19	1,080	1,38,58,85
Sulfates mg/l	0.0	182	85

Table 2. Toxicants commonly found in oil refinery waste.

Toxicant	Ave. Conc. mg/l	Threshold Toxicity (mg/l) Scenedesmus (60)	Ref.
Cadmium	0.04	0.10	65
Chromium	0.28	0.70	65
Copper	0.07	0.15	65
Lead	0.23	2.50	65
Nickel	0.11	1.50	65
Phenol	154.00	40.00	56
Sulfides	24.00	4.0[a]	56
Zinc	0.17	1.0	65

[a]For H_2S.

type of test organism to be utilized in toxicity bioassays. Turnbull et al. (85) reported the 24-hour TL_m (medium tolerance level) of the bluegill sunfish, *Lepomis macrochirus,* to a composite oil refinery waste in terms of a dilution ratio, rather than by the concentration of specific toxicants present in the waste. They reported the 24-hour TL_m to be a 20 percent dilution by volume at 24°C. Graham and Dorris (40) have reported that a 4 to 1 dilution of a particular oil refinery waste in Oklahoma caused chronic toxicity of fathead minnows, *Pimephales promelas.* Again no specific relationship between toxic substances and toxicity was determined. Clemens and Clough (18) attempted to correlate the sulfide concentration and the phenolic concentration of a specific oil refinery waste to the medium lethal dosage (LD_{50}) for fish. No correlation was found between the LD_{50} and the sulfide concentration of the oil refinery waste. However, correlations were found between the LD_{50} and the phenolic concentration in the oil refinery wastes. The phenolic LD_{50}'s reported in terms of dilution volume for *Carassius sp., Notropis sp.,* and *Daphnia sp.* were: 33.1 percent, 18.8 percent, and 15.5 percent respectively.

Specific studies to determine the toxicity of oil refinery wastes to algae could not be found in the literature. However, the threshold toxicity levels for some of the common toxicants in oil refinery were found. The threshold level for *Scenedesmus* for some of these common toxicants is shown in Table 2. It is apparent that for the toxicants listed in Table 2 phenol and sulfides are most often present in toxic concentrations. Thus, phenol and sulfides often control the toxicity of oil refinery wastes.

Sulfides are often present in oil refinery wastes as hydrogen sulfide (H_2S), and are therefore often lost from the waste stream during transport and discharge. Also sulfides are readily removed by various treatment methods, e.g. aeration. Phenol, however, is seldom removed during transport or discharge and is somewhat difficult to treat biologically or chemically. Often it remains in solution and is discharged to a natural water system. Therefore it is likely that phenol is the controlling toxicant in a significant number of oil refinery waste discharges.

Phenol Toxicity

Toxicity to algae

A phenol concentration of 40 mg/l has been reported by McKee and Wolf (60) and Bringmann and Kuhn (11) to cause threshold toxic effects on the alga *Scenedesmus sp.* Kostyaev (48) reported that phenol concentrations from 10 to 40 mg/l stimulated the growth of *Scenedesmus acuminatus,* but concentrations of phenol greater than 50 mg/l retarded the growth rate. He also found that phenol concentrations greater than 500 mg/l prevented growth altogether.

Phenol concentrations less than 40 mg/l have also stimulated the photosynthesis of *Chlorella sp.,* while phenol concentrations greater than 750 mg/l have prevented photosynthesis (50), and concentrations greater than 500 mg/l have only retarded the photosynthetic activity (52). Huang and Gloyna (45) have shown that phenol destroys the chlorophyll in *Chlorella pyenoidosa.* Also, lethal protoplasmic changes in the alga *Ulothrix tenerrima* have been caused by a 5 percent (50 g/l) phenol solution (17).

Biochemical mechanism

The exact mechanism of phenol toxicity is not clearly understood. It has been suggested by Berry and Parkinson (7) that phenol toxicity in bacteria is influenced by variations in temperature. Tibor (82) reported that phenol may not inhibit a single biochemical process, but that it exerts a nonspecific denaturing action on the cell wall. Tomcsik (84) reported that phenol denatures the cytoplasmic membrane in *Bacillus megatherium.* Experiments with phenol and *Escherichia coli* reported by Commager and Judis (19) indicated that the lethal action of phenol is due to effects on cell permeability.

Phenol has been shown to be a competitive inhibitor in experiments with pure enzymes. Kaplan and Laidler (47) and Martinek, Levashov, and Berezin (59) reported that phenol exhibits competitive inhibition with α-chymotrypsin. Stockdale and Selwyn (80) showed that phenol inhibition of lactate dehydrogenase and hexokinase was the competitive type. Wedding, Hansch, and Fukuto (88) reported that phenol was a competitive inhibitor with NAD in the forward direction of the malate dehydrogenase reaction.

In studies with *Staphylococcus bacteria,* phenol has been shown to inhibit the enzymes concerned with oxidation-reduction reactions (37). Phenol has also been shown to inhibit the biosynthesis of catalase in *Staphylococcus aureus* and *Escherichia coli* (37, 44). Enzymatic activity in the bowel of rainbow trout is also reduced by phenol (7, 74).

The effects of phenol on pure enzyme systems have been reported often in the literature; however, the kinetics of phenol toxicity or inhibition of living organisms is quite rare. Very little work has been reported concerning the kinetics of phenol toxicity in algae. However, it is possible that enzyme inhibition kinetics may be applied to algal systems.

Luxury Uptake

Luxury uptake or "excess uptake" of nutrients is a phenomenon which occurs when the rate of nutrient uptake from solution by the organism exceeds the rate at which the nutrient is utilized by the organism for growth or production of cell mass. This phenomenon has been

reported in the literature by several investigators in connection with several algal species (8, 14, 15, 27, 36, 42, 72, 83). In most cases, these algal species were cultured in either a nitrogen or phosphorus limited environment. Caperon and Meyer (14) investigated the steady state growth kinetics of four species of marine phytoplankton in both nitrate and ammonium limited cultures. Luxury uptake occurred in the nitrate limited cultures, but ammonium limited cultures did not exhibit luxury uptake. The luxury uptake of nitrate by marine diatoms has been reported by Epply and Thomas (27).

Porcella et al. (72) reported that the green alga, *Selenastrum capricornutum,* exhibited excess uptake of phosphorus and nitrogen under various initial ratios of nitrate-nitrogen to phosphorus in the culture media. Also, Toerien et al. (83) have reported luxury uptake of phosphorus by *Selenastrum capricornutum.*

Several mathematical models have been developed which incorporate the phenomenon of luxury uptake (8, 36, 42, 83). Grenney, Bella, and Curl (42) have developed a three-compartment model which describes phytoplankton growth in a nitrogen limited environment. Their model is based on the intracellular nitrogen concentration. Bierman, Verhoff, Poulson, and Tenney (8), have developed a multi-nutrient dynamic model which describes the luxury uptake of phosphorus in eutrophic environments. Toerien et al. (83) investigated the luxury uptake of phosphorus by the green alga, *Selenastrum capricornutum,* and they developed a mathematical model, based on Michaelis-Menten (Monod) kinetics, which describes the luxury uptake process. This model will be discussed in greater detail in Chapter III: Theory.

CHAPTER III

THEORY

Inhibition Models

General

The toxic mechanisms which inhibit the growth of microorganisms are not fully understood. Most previous investigators have adopted Michaelis-Menten enzyme inhibition kinetics to describe the inhibition of microorganisms. Although this approach is lacking in theory, it should be pointed out that the Monod relationship (64), which describes the growth of microorganisms in a nutrient limited environment, is similar in form to the Michaelis-Menten equation, which describes the kinetics of enzymatic reactions (22, 89).

Andrews (3, 4) has employed inhibition kinetics to describe the dynamic behavior in anaerobic digestion of sewage sludge. Zines and Rogers (91) described product inhibition of *Klebsiella (Aerobacter) aerogenes* in continuous culture with ethanol being used as the toxicant. Hartman and Laubenberger (43) employed Michaelis-Menten enzyme inhibition kinetics to describe the toxicity of copper and hexavalent chrome to a population of activated sludge bacteria. More recently, Poon and Bhayahi (71) have used Michaelis-Menten enzyme inhibition kinetics to describe the toxicity of silver and nickel to an activated sludge bacteria population and to the bacterium *Geotrichum candidum.*

Because phenol (a toxicant in oil refinery waste) has been shown to inhibit both pure enzymes and enzymes in living organisms, and also, because Michaelis-Menten enzyme inhibition kinetics have been successfully applied by previous investigators to the inhibition of microorganism growth, Michaelis-Menten enzyme inhibition kinetics will be employed in this study to describe the inhibition of growth by algae which have been exposed to oil refinery waste. Phenol does not control the toxicity in all oil refinery wastes; however, it is felt that phenol does control toxicity in a sufficient number of cases to make this study significant.

Chemostat kinetics

The continuous flow system used in this study is defined as a continuous flow stirred tank reactor or chemostat. The functional relationships which define a chemostat have been presented in detail by previous authors (39, 74, 83) and will not be emphasized here. The following basic equations describing chemostat performance will be modified to include the effects of toxicants. The nomenclature of the equations is based on the "Unified Fundamental Symbols for Continuous Cultivation of Microorganisms" developed at the Second Symposium on Continuous Cultivation of Microorganisms held in Prague in 1962 (55). The expressions presented below for the cell concentrations in the chemostat (X_1), the limiting nutrient or substrate concentration (S_1) in the effluent, and the specific growth rate (μ) were developed from material balances for the chemostat.

$$X_1 = \frac{Y}{\mu\theta}(S_o - S_1) \quad \ldots\ldots\ldots\ldots (1)$$

$$S_1 = \frac{K_s\left(\frac{1}{\theta} + k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)} \quad \ldots\ldots\ldots\ldots (2)$$

$$\mu = \frac{1}{\theta} + k_d \quad \ldots\ldots\ldots\ldots (3)$$

in which

Y	=	net cell yield coefficient, or mass of organisms formed per mass of substrate used
μ	=	specific growth rate, time $^{-1}$
θ	=	mean residence time = V/F
V	=	volume of chemostat
F	=	flow rate, volume/time
S_o	=	initial substrate concentration, mass/volume
S_1	=	steady state substrate concentration, mass/volume
K_s	=	half saturation constant, concentration of substrate at which the growth rate is 1/2 of the maximum growth rate, $\hat{\mu}$, mass/volume
$\hat{\mu}$	=	maximum specific growth rate, time^{-1}
k_d	=	specific cellular decay rate, time^{-1}
X_1	=	steady state cell concentration, mass/volume

Inhibition kinetics

The enzyme substrate reaction in microorganisms may be described as follows:

$$E + S \rightleftarrows ES \rightleftarrows E + P \quad \ldots\ldots\ldots\ldots (4)$$

in which

E = free enzyme
S = substrate
ES = bound enzyme or enzyme-substrate complex
P = product

Inhibition of the enzyme (E) in Equation 4 may occur in three ways; namely (1) competitive, (2) uncompetitive, and (3) noncompetitive (89). These three types of inhibition are described in Equations 5, 6, and 7 where E is the free enzyme, I is the inhibitor, EI is the enzyme-inhibitor complex, ES is the enzyme-substrate complex and IES is the inhibitor-enzyme-substrate complex:

COMPETITIVE INHIBITION

$$E + I \underset{\text{addition of substrate}}{\overset{\text{Reversible by}}{\rightleftarrows}} EI \quad \ldots (5)$$

UNCOMPETITIVE INHIBITION

$$ES + I \longrightarrow IES \quad \ldots\ldots\ldots\ldots (6)$$

NONCOMPETITIVE INHIBITION

$$E + I \underset{\text{addition of substrate}}{\overset{\text{Nonreversible by}}{\rightleftarrows}} EI \quad \ldots\ldots (7)$$

Uncompetitive inhibition is distinguished from noncompetitive inhibition, in that noncompetitive inhibition attacks the free enzyme (E), while uncompetitive inhibition attacks the enzyme-substrate complex (ES). Competitive inhibition is defined as the attachment of a pseudosubstrate or substrate analog (I) to the active site of the free enzyme (E); such inhibition may be reversed by increasing the concentration of the substrate in solution (89).

The Michaelis-Menten equations describing these three types of inhibition are shown below using nomenclature based on the "Unified Fundamental Symbols for Continuous Cultivation of Microorganism" developed at the Second Symposium on Continuous Cultivation of Microorganisms held in Prague in 1962 (55).

COMPETITIVE INHIBITION

$$\mu = \frac{\hat{\mu} S_1}{K_s \left(1 + \frac{I}{K_I}\right) + S_1} \quad \ldots\ldots\ldots (8)$$

UNCOMPETITIVE INHIBITION

$$\mu = \frac{\hat{\mu} S_1}{K_s + S_1 \left(1 + \frac{I}{K_I}\right)} \quad \ldots\ldots\ldots (9)$$

NONCOMPETITIVE INHIBITION

$$\mu = \frac{\hat{\mu} S_1}{K_s \left(1 + \frac{I}{K_I}\right) + S_1 \left(1 + \frac{I}{K_I}\right)} \quad \ldots\ldots (10)$$

in which

I = inhibitor concentration
K_I = inhibition constant

By equating Equation 3 with each of the inhibition equations (Equations 8, 9, 10) respectively, it is possible to express growth rate in the chemostat in terms of the inhibitor, I,

COMPETITIVE INHIBITION

$$\mu = \frac{1}{\theta} + k_d = \frac{\hat{\mu} S_1}{K_s \left(1 + \frac{I}{K_I}\right) + S_1} \quad \ldots\ldots (11)$$

UNCOMPETITIVE INHIBITION

$$\mu = \frac{1}{\theta} + k_d = \frac{\hat{\mu} S_1}{K_s + S_1 \left(1 + \frac{I}{K_I}\right)} \quad \ldots\ldots (12)$$

NONCOMPETITIVE INHIBITION

$$\mu = \frac{1}{\theta} + k_d = \frac{\hat{\mu} S_1}{K_s \left(1 + \frac{I}{K_I}\right) + S_1 \left(1 + \frac{I}{K_I}\right)} \quad \ldots\ldots (13)$$

Equations 11, 12, and 13 can each be solved for S_1 and then each value of S_1 substituted into Equation 1 to develop an expression for the cell concentration in the chemostat as a function of inhibitor. (S_o and θ can be measured.)

COMPETITIVE INHIBITION

$$X_1 = \frac{Y}{\mu\theta} \left[S_o - \frac{K_s \left(\frac{1}{\theta} + k_d\right) \left(1 + \frac{I}{K_I}\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)} \right] \quad \ldots\ldots (14)$$

UNCOMPETITIVE INHIBITION

$$X_1 = \frac{Y}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta}+k_d\right)}{\hat{\mu}-\left(\frac{1}{\theta}+k_d\right)\left(1+\frac{I}{K_I}\right)}\right] \quad ..(15)$$

NONCOMPETITIVE INHIBITION

$$X_1 = \frac{Y}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta}+k_d\right)\left(1+\frac{I}{K_I}\right)}{\hat{\mu}-\left(\frac{1}{\theta}+k_d\right)\left(1+\frac{I}{K_I}\right)}\right] \quad ..(16)$$

The cell concentration equation for noncompetitive inhibition (Equation 16) cannot be linearized; however, the cell concentration equations for competitive and uncompetitive inhibition (Equations 14 and 15) may be expressed in linear form as follows:

COMPETITIVE INHIBITION

$$X_1 = \frac{YS_o}{1+\theta k_d} - \frac{\left(\frac{1}{\theta}\right)YK_s}{\hat{\mu}-\left(\frac{1}{\theta}+k_d\right)} - \frac{\left(\frac{K_sY}{K_I\theta}\right)I}{\hat{\mu}-\left(\frac{1}{\theta}+k_d\right)} \quad .(17)$$

UNCOMPETITIVE INHIBITION

$$\frac{1}{X_1} = \frac{1+\theta k_d}{YS_o} - \frac{\theta\hat{\mu}-(1+\theta k_d)}{YK_s} + \frac{(1+\theta k_d)I}{K_IK_sY} \quad (18)$$

The linear form of the competitive inhibition equation (Equation 17) is very useful, however, there are certain inconsistencies associated with the linear form of the uncompetitive inhibition equation (Equation 18). In practice, when K_s is small, the intercept of Equation 18 is a negative value. This intercept represents the inverse of the cell concentration for the case when the inhibitor concentration, I, is zero. When the inhibitor is absent, the cell concentration should be at a maximum and not a negative value. Therefore, application of the linear form of the uncompetitive inhibition equation (Equation 18) is limited.

The uncompetitive cell concentration equation (Equation 15) can be linearized as shown below to eliminate the inconsistency of the previous linear form (Equation 18).

$$X_1 = \frac{YS_o}{1+\theta k_d} - \frac{YK_s}{(\theta\hat{\mu})-(1+\theta k_d)} + \frac{K_IK_sY}{(1+\theta k_d)I} \quad .(19)$$

Equation 19 is the inverse of Equation 18; however, it, too, contains a contradiction. When the inhibitor concentration, I, is equal to zero in Equation 19, the steady state cell concentration, X_1, approaches infinity. A condition of infinite steady state cell concentration cannot exist. Thus, both Equations 18 and 19 are unsuitable for use in data analysis, and the uncompetitive inhibition equation (Equation 15) must be used in a nonlinear form.

Use of inhibition models

It is possible to determine the type of inhibition exerted by a particular toxicant by fitting experimental data to either Equation 15, 16, or 17. It is also possible to determine the value of the inhibition constant, K_I, from the same equations. The inhibition constant, K_I, for competitive inhibition may be determined from the slope of a linear plot of cell concentration, X_1, versus inhibitor concentration, I. This relationship is shown graphically in Figure 1. Alternatively, the competitive inhibition constant, K_I, may be determined from Equation 14 by using nonlinear curve fitting techniques or by solving Equation 14 directly for K_I. The uncompetitive inhibition constant, K_I, and the noncompetitive inhibition constant, K_I, must be determined from Equations 15 and 16, respectively, with nonlinear curve fitting techniques or by solving directly for the respective K_I value.

The effects of bacterial contamination in the inhibited cultures may be accounted for by introducing a term into the inhibition equations (Equations 14, 15, and 16) which represents the bacterial utilization of the inhibitor. The effect of bacteria upon the concentration

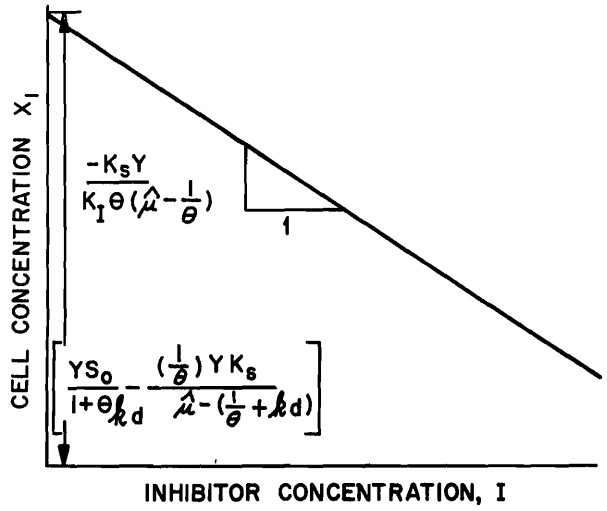


Figure 1. Linear plot of cell concentration, X_1, vs. inhibitor, I, for competitive inhibition.

of inhibitor in the chemostat may be represented by Equation 20.

$$I_e = I_o - K_e B - I_1 \quad \ldots\ldots\ldots\ldots (20)$$

in which

I_e = effective inhibitor concentration, mass/volume

I_o = initial inhibitor concentration, mass/volume

K_e = bacteria utilization constant, dimensionless

I_1 = steady state inhibitor concentration in the chemostat, mass/volume

B = steady state bacteria mass in the chemostat, mass/volume

Substituting Equation 20 into Equations 14, 15, and 16 for I results in the following set of inhibition equations:

COMPETITIVE INHIBITION

$$X_1 = \frac{Y}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta}+k_d\right)\left(1+\frac{I_o - K_e B - I_1}{K_I}\right)}{\hat{\mu} - \left(\frac{1}{\theta}+k_d\right)}\right] \quad (21)$$

UNCOMPETITIVE INHIBITION

$$X_1 = \frac{Y}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta}+k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta}+k_d\right)\left(1+\frac{I_o - K_e B - I_1}{K_I}\right)}\right] \quad (22)$$

NONCOMPETITIVE INHIBITION

$$X_1 = \frac{Y}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta}+k_d\right)\left(1+\frac{I_o - K_e B - I_1}{K_I}\right)}{\hat{\mu} - \left(\frac{1}{\theta}+k_d\right)\left(1+\frac{I_o - K_e B - I_1}{K_I}\right)}\right] \quad (23)$$

If the steady state inhibitor concentration in the chemostat, I_1, is relatively small, it may be neglected, and Equations 21, 22, and 23 may be further simplified. The type of inhibition, the bacteria utilization constant, K_e, and the inhibition constant, K_I, can be determined by fitting experimental data to Equations 21, 22, and 23 using nonlinear curve fitting techniques.

The inhibition constant, K_I, is a measure of the affinity of the inhibitor for the enzyme. In practice then, it is a measure of the toxicity of the particular toxicant to a specific organism—the smaller the inhibitor constant, K_I, the greater the toxicity of the toxicant.

In practice the value of k_d is very small (39, 74, 83) and may be assumed to be zero without introducing serious error. If this is done, the solution and analysis of Equations 14, 15, 16, and also Equations 21, 22, and 23 are greatly simplified (75).

Experiments conducted with a specific organism cell concentration and a series of inhibitor concentrations should reveal the type of inhibition exerted by the toxicant and the values for K_I, which will measure the strength of the toxicant. Experiments conducted at various temperatures should indicate if K_I is a function of temperature and should reveal the effect of temperature on the toxicity of a particular waste to a specific organism.

Luxury Uptake

As discussed in Chapter II: Literature Review, luxury uptake has been observed and reported by several investigators (8, 14, 15, 27, 36, 42, 72, 83). Of these, the relationships developed by Toerien et al. (83) will be used in this study because they were developed using *Selenastrum capricornutum.*

Toerien et al. (83) postulated that the luxury uptake process may be described by the two step mechanism shown in Figure 2. The first step describes the removal of nutrient from the media into the cell. The second step involves the incorporation of the internal nutrient into the cell growth processes and the production of biomass. Luxury uptake occurs when the rate of nutrient removal from the media (nutrient removal velocity) exceeds the rate at which internal nutrient is utilized for synthesis and cellular maintenance (nutrient utilization rate). Consequently, the amount of internal nutrient is increased (and presumably stored) in excess of the amount of nutrient required for synthesis and growth.

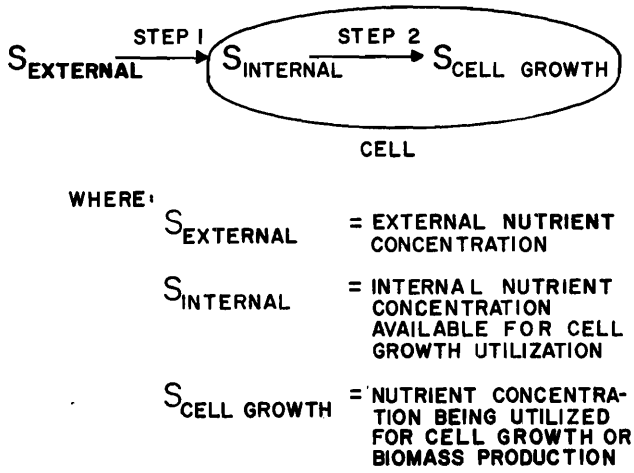
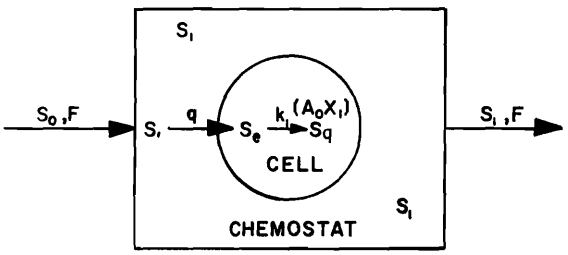


Figure 2. The two step nutrient utilization process postulated by Toerien et al. (83).

Under steady state conditions in a continuous flow system (chemostat) all environmental and physiological properties of the cell are constant. The external nutrient concentration in the chemostat is controlled by the specific growth rate of the organism, μ, according to the Michaelis-Menten-Monod kinetic growth equation. When luxury uptake occurs in the chemostat, the only parameters which are influenced are the steady state cell concentration, X_1, and the cell yield coefficient, Y. In a chemostat without cell recycle and a short hydraulic residence time, θ, (high specific growth rate, μ) the excess nutrient taken up by the organism is not utilized for cell growth before the cells leave the system. Consequently, the cell yield coefficient is relatively low. However, as the hydraulic residence time is increased (specific growth rate decreases), a greater fraction of the excess nutrient is utilized for cell growth before the cell leaves the system, and the cell yield coefficient is relatively high. Also, when the hydraulic residence time approaches infinity ($\theta \rightarrow \infty$), essentially all of the excess nutrient will be utilized for cell growth, and the cell yield coefficient will approach the maximum cell yield coefficient value, Y_{max}.

A flow diagram, developed by Toerien et al. (83), depicting the supply, uptake, and utilization of a specific nutrient is shown in Figure 3. Toerien et al. (83) used this concept to derive several equations which describe the luxury uptake process in *Selenastrum capricornutum*. The derivation of those equations will not be presented here;



A_0 = NUTRIENT CONTENT OF INDIVIDUAL CELL AT INFINITE CELL AGE IN CHEMOSTAT $\frac{S_0 - S_1}{X_{MAX}}$

S_e = NUTRIENT CONCENTRATION AVAILABLE FOR CELL GROWTH INCLUDING EXCESS NUTRIENT UPTAKE AND STORAGE

X_{MAX} = MAXIMUM CELL CONCENTRATION IN CHEMOSTAT AT INFINITE CELL AGE

S_q = NUTRIENT CONCENTRATION UTILIZED FOR GROWTH BY CELL

Figure 3. Definition sketch for a specific nutrient supply, uptake and utilization for growth in a chemostat at steady state (Toerien et al. (83)).

however, the application of these equations will be discussed.

As illustrated in Figure 3, the nutrient passes through the cell wall at some nutrient removal velocity, q, and is then distributed to the cell functional process for biomass synthesis. The nutrient removal velocity in a chemostat may be described in terms of the hydraulic residence time, θ; the specific growth rate, μ; the initial nutrient concentration, S_0; the nutrient concentration at steady state, S_1; the cell yield coefficient, Y, and the steady state cell concentration, X_1, as follows:

$$q = \frac{\mu}{Y} = \frac{S_o - S_1}{X_1 \theta} \quad \ldots\ldots\ldots\ldots (24)$$

The nutrient utilization rate constant, k_1, shown in Figure 3, is a growth parameter which is specific for a particular species, nutrient, and environment. In the absence of any theoretical model to describe this reaction, Toerien et al. (83) assumed the reaction to be first order as follows:

$$\frac{dS_e}{d\theta} = \frac{-dS_q}{d\theta} = -k_1 S_e \quad \ldots\ldots\ldots\ldots (25)$$

in which

S_e = nutrient concentration available for cell growth including "excess" nutrient uptake and storage, mass/volume

S_q = nutrient concentration utilized by cells for growth, $S_q = A_o X_1$, mass/volume

Through a series of rearrangements and substitutions, Equation 25 can be integrated to develop Equation 26. Equation 26 represents the steady state cell concentration, X_1, under luxury uptake conditions in a chemostat.

$$X_1 = X_{max}(1 - e^{-k_1 \theta}) \quad \ldots\ldots\ldots\ldots (26)$$

in which

X_1 = steady state cell concentration, mass/volume

X_{max} = maximum cell concentration at infinite hydraulic residence time, mass/volume

The net cell yield coefficient, Y_n, is the mass of organisms formed per mass of substrate actually used by the organism for cell synthesis, rather than the amount of substrate removed from solution. It is apparent from Equation 26, that the net cell yield coefficient, Y_n, is now a function of the hydraulic residence time, θ, as illustrated in Equation 27.

$$Y_n = \frac{X_1}{S_o - S_1} = \frac{X_{max}(1 - e^{-k_1\theta})}{S_o - S_1}$$

$$= Y_{max}(1 - e^{-\theta/K_A}) \quad \ldots\ldots\ldots (27)$$

in which

Y_{max} = maximum cell yield coefficient

$$Y_{max} = \frac{X_{max}}{S_o - S_1}, \frac{\text{mass cell}}{\text{mass nutrient removal}}$$

K_A = nutrient utilization constant = $1/k_1$, time

In addition to assuming first order kinetics to describe the relationship between nutrient removal and nutrient utilization, Toerien et al. (83) also developed a rectangular hyperbolic function (Michaelis-Menten (Monod)) to describe the variation of net cell yield coefficient, Y_n, with hydraulic residence time, θ, as shown by Equation 28.

$$Y_n = Y_{max}\left(\frac{\theta}{\theta + K_B}\right) \quad \ldots\ldots\ldots (28)$$

in which

K_B = nutrient utilization constant corresponding to the hydraulic residence time at $Y_n = (Y_{max}/2)$, time

From Equations 27 and 28, Toerien et al. (83) developed a set of equations to determine the fraction of excess nutrient uptake, F_e, and the nutrient removal velocity, q. The fraction of excess nutrient uptake is defined as the fraction of the total nutrient uptake which is accumulated and/or stored inside the cell (i.e. not yet utilized for cell growth). The derivation of these equations is reported by Toerien et al. (83) and therefore, will not be presented here. A summary of the equations developed by Toerien et al. (83) is shown in Table 3.

Luxury Uptake, Chemostat Kinetics, and Inhibition Kinetics

The steady state cell concentration, X_1, and the steady state limiting nutrient concentration, S_1, in a chemostat without recycle are described by Equations 1 and 2. These two equations may be combined to express the steady state cell concentration in terms of the steady state limiting nutrient concentration and the cell yield coefficient as follows:

$$X_1 = \frac{Y}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)}\right] \quad \ldots\ldots (33)$$

If the hydraulic residence time, θ, and the cell yield coefficient, Y, are constant (i.e. no luxury uptake), the steady state cell concentration is solely a function of the limiting nutrient concentration, S_o, in the chemostat influent. However, if luxury uptake is occurring in the chemostat, the cell yield coefficient is not a constant, and either Equation 27 or 28 must be substituted into Equation 33 to determine the steady state cell concentration. Substitution of Equation 27 into Equation 33 will result in the "A Form" of the steady state cell concentration equation (Equation 34), and substitution of Equation

Table 3. A summary of luxury uptake equations developed by Toerien et al. (83).

Function	'A' Form Expotential Function	Equation Number	'B' Form Rectangular Hyperbolic Function	Equation Number
Net Cell Yield Coefficient, Y_n	$Y_n = Y_{max}\left(1 - e^{-\theta/K_A}\right)$	27	$Y_n = Y_{max}\left(\frac{\theta}{\theta + K_B}\right)$	28
Fraction of Excess Nutrient Uptake, F_e	$F_e = e^{-\theta/K_A}$	29	$F_e = \left(1 - \frac{\theta}{K_B + \theta}\right)$	30
Nutrient Removal Velocity, q	$q = \frac{\mu}{Y_{max}\left(1 - e^{-\theta/K_A}\right)\left(1 + \theta K_d\right)}$	31	$q = \frac{\mu}{Y_{max}\left(\frac{\theta}{\theta + K_B}\right)\left(1 + \theta K_d\right)}$	32

28 into Equation 33 will result in the "B Form" of the steady state cell concentration equation (Equation 35).

'A FORM'

$$X_1 = \frac{Y_{max}(1 - e^{-\theta/K_A})}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)}\right] \quad (34)$$

'B FORM'

$$X_1 = \frac{Y_{max}\theta}{\mu\theta(\theta + K_B)}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)}\right] \quad . \quad . \quad . (35)$$

Equations 34 and 35 can be used to determine the maximum specific growth rate, μ, and the half saturation constant, K_s, under luxury uptake conditions.

A similar approach may be used with the enzyme inhibition functions (Equations 21, 22, and 23) to develop equations to describe inhibition under luxury uptake conditions. The 'A' and 'B' forms of the resulting equations are listed below:

COMPETITIVE INHIBITION WITH LUXURY UPTAKE

'A FORM'

$$X_1 = \frac{Y_{max}(1 - e^{-\theta/K_A})}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)}\right] \quad (36)$$

'B FORM'

$$X_1 = \frac{Y_{max}\theta}{\mu\theta(\theta + K_B)}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)}\right] \quad (37)$$

UNCOMPETITIVE INHIBITION WITH LUXURY UPTAKE

'A FORM'

$$X_1 = \frac{Y_{max}(1 - e^{-\theta/K_A})}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}\right] \quad (38)$$

'B FORM'

$$X_1 = \frac{Y_{max}\theta}{\mu\theta(\theta + K_B)}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}\right] \quad (39)$$

NONCOMPETITIVE INHIBITION WITH LUXURY UPTAKE

'A FORM'

$$X_1 = \frac{Y_{max}(1 - e^{-\theta/K_A})}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}\right] \quad (40)$$

'B FORM'

$$X_1 = \frac{Y_{max}\theta}{\mu\theta(\theta + K_B)}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}\right] \quad (41)$$

Experimental data can be fit to Equations 36 through 41 using nonlinear curve fitting techniques to determine the type of inhibition, the form of luxury uptake, the bacteria utilization constant, K_e, and the appropriate inhibition constant, K_I.

CHAPTER IV

EXPERIMENTAL PROCEDURES

General

Semi-continuous flow (1, 54) and continuous flow experiments were conducted utilizing phenol as the inhibitor and the alga, *Selenastrum capricornutum*, as the test organism, to develop data to be applied to the basic inhibition equations. Additional continuous flow experiments were conducted with *Selenastrum capricornutum* and an oil refinery waste to apply the inhibition equations developed with the pure phenol toxicant cultures. Semi-continuous flow cultures were also employed as an initial screening study to determine a workable range of phenol concentrations to be used in the later continuous flow experiments. Semi-continuous flow experiments require limited space and have been shown to yield reasonable estimates of continuous flow cultures, chemostats, when the time interval between media addition and withdrawal is relatively small.

The original culture of *Selenastrum capricornutum* was obtained from the Pacific Northwest Environmental Research Laboratory of the Environmental Protection Agency, located at Corvallis, Oregon. Inoculum for both the semi-continuous and continuous flow cultures consisted of 10 ml of a seven day old batch culture. The exact number of cells introduced into individual cultures was not rigidly controlled since these cultures would be measured at steady state conditions and not at a specified time period after inoculation. The cultures were considered to be at steady state when the range in variation in mean algal biomass (measured by cell count) was no more than ± 10 percent during a complete residence time.[1]

Semi-Continuous Flow Cultures

The semi-continuous culture experiments were conducted in duplicate at 20°C, 24°C, and 28° C, and with phenol concentrations of 0, 20, 40, 60, 80, 100, and 120 mg/l. Temperature variation during the experiment was limited to ± 1° C.

The semi-continuous cultures were grown in a constant temperature environmental chamber,[2] in 500 ml Erlenmeyer flasks covered with sterile tissue and 150 ml inverted Griffin beakers. Algal Assay Procedures (26) were employed with the following modifications. Each culture flask contained 250 ml of culture. One-third (83 ml) of that culture was withdrawn and replaced with fresh, sterile nutrient media every 24 hours. Thus, the cultures remained at steady state with a mean residence time of three days.

The nutrient medium employed for the semi-continuous cultures was a modified PAAP solution (74). The medium composition is shown in Tables 4 and 5. The original PAAP medium was modified by replacing $NaNO_3$ with NH_4Cl, to provide a final nitrogen concentration of 2.1 mg/l. Ammonium chloride was used to facilitate analysis of the growth limiting nutrient, nitrogen (79). Sodium bicarbonate was substituted for sodium carbonate to provide buffer capacity and also to provide essential carbon to sustain the algal growth. The final concentration of carbon in the medium was 80 mg/l. The medium was also buffered at pH 7.20 with a 0.03 phosphate buffer solution by varying the ratio of monobasic to dibasic sodium phosphate. This buffer was very satisfactory and pH variation was less than ± 0.10 pH units at steady state.

Cell counts, using a hemacytometer; pH measurements, using a Corning pH meter;[3] and optical density, read at 750 μ m[4] with a 1" cell, were conducted daily on the 83 ml sample withdrawn from each culture. Upon reaching steady state the cultures were also analyzed for ammonia using the indophenol technique (79). Phenol determinations were made with a gas chromatograph[5] equipped with a flame detector and using isothermal operation procedures (31).

During the experiment, attempts were made to limit bacteria; however, bacteria were present in the cultures. No attempt was made to determine the number of bacteria present. Because bacteria were present, the cell

[1] During the toxicant experiments this variation was slightly exceeded for a few cultures (± 15 percent maximum).

[2] Sherer Controlled Environmental Lab, model CEL 37-14, Sherer-Gillet Co., Marshall, Michigan.

[3] Corning Scientific Instruments pH meter, model 7.

[4] Bausch and Lomb, Spectronic 20, Rochester, N.Y.

[5] Hewlett-Packard Research Chromatograph, Model 5750.

Table 4. Macronutrient composition of modified PAAP medium.

Components	Concentration mg/l	Essential Nutrient	Concentration mg/l
NH_4Cl	8.03	N	2.1
K_2HPO_4[a]	3.48	P	0.62
$MgCl_2$	19.00	Mg	9.68
$MgSO_4 \cdot 7H_2O$	49.00	S	6.37
$CaCl_2 \cdot 2H_2O$	14.70	Ca	4.01
$NaHCO_3$	571.43	K	1.56
$FeCl_3$	0.32	Fe	0.11
$Na_2EDTA \cdot 2H_2O$[b]	1.00	C	80.00

[a] 0.03 molar phosphate buffer was also added to control pH.

[b] Na_2EDTA = Disodiumethylenediaminetetraacetic acid.

Table 5. Micronutrient composition of modified PAAP medium.

Component	Concentration µg/l	Essential Nutrient	Concentration µg/l
H_3BO_3	618.40	B	110.00
$MnCl_2$	880.88	Mn	380.00
$ZnCl_2$	109.03	Zn	50.00
$CoCl_2$	2.60	Co	1.18
$CuCl_2$	0.03	Cu	0.01
$Na_2MoO_4 \cdot 2H_2O$	24.20	Mo	9.60
$Na_2EDTA \cdot 2H_2O$	7440.00		

Note: The trace metals and EDTA were combined in a single stock mix at a level of 1000 times the final concentration.

mass values for the semi-continuous flow cultures were calculated from the linear regression equation:

$$SS = 8.25 + 15.8\ (CC) \quad \ldots\ldots\ldots \quad (42)$$

in which

SS = cell mass in mg/l
CC = cell counts x 10^6 per ml

developed by Porcella et al. (72).

Continuous Flow Culture Experiments

The continuous flow experiments were conducted at 20°C, 24°C, 27°C, 28°C, and 32°C without toxicant and at 20°C, 24°C, and 28°C with pure phenol as the toxicant. The experiments involving pure phenol as the toxicant were conducted at hydraulic residence times approximately equal to 1 day, 1.5 days, and 2.0 days. Experiments utilizing oil refinery waste as the toxicant were conducted at 24°C and 28°C with a hydraulic residence time of approximately two days. Temperature variations in the chemostats during both types of experiments were less than ± 1.5°C.

The procedures employed during the continuous flow experiments are outlined by Porcella et al. (72) except that air was not bubbled through the chemostat for mining and to control pH; rather, pH was controlled by the buffer system used in the semi-continuous cultures. Complete mixing was provided by magnetic mixers (39). The medium employed for the continuous experiments was the same as that used in the semi-continuous experiments, except that the ammonium-nitrogen concentration was increased to 4.2 mg/l to increase cell biomass.

Cell counts and pH measurements were conducted daily on a thirty-milliliter sample withdrawn from each chemostat.

In addition, at steady state ammonium-nitrogen concentrations were determined using the indophenol technique (79) on a 50 ml sample withdrawn from each chemostat. Bacteria concentrations were determined by a spread plate technique (6, 73). Phenol measurements on the effluent from the 24°C chemostats were determined with the gas chromatograph (31); however, the phenol concentrations in the effluent from the 20°C and 28°C chemostats were determined by the Direct Photometric Method (6). Suspended solids determinations were conducted by the technique described by Strickland and Parsons (81).

Oil Refinery Waste

The oil refinery waste used in this study was obtained from an oil refinery located in North Salt Lake City, Utah. This refinery consisted of both a crude oil distillation unit and a thermofor catalytic cracking unit. Wastewater from the processes is collected into a single sewer and sent through a sour water stripper. The sour water stripper is designed to remove hydrogen sulfide. The oil refinery waste employed in this study was obtained downstream from the sour water stripper as shown in Figure 4.

Analysis of the oil refinery waste is shown in Table 6. All analyses were performed according to Standard Methods (6), with the exception being the analysis for hydrogen sulfide which was measured with a specific ion electrode (66). The oil refinery waste was stored at 1° C between experiments to prevent microbial breakdown of the constituents.

Data Analysis

Two different computer programs were used to perform linear and nonlinear regression analyses on the data. All computer analyses were performed on a Burroughs Model 6700 computer at the Utah State University Computer Center, Logan, Utah. The computer program used for linear regression analysis is outlined in Appendix

Table 6. Analysis of oil refinery waste used in this study.

Parameter	Concentration mg/l
COD	1010.0
TOC	189.0
NH_3-N	111.21
NO_2-N	0.0028
NO_3-N	1.413
Ortho-PO_4	1.50
Total PO_4	2.685
Phenol	150.00
H_2S	< 1.0
Ag	< 0.05
Cd	< 0.10
Cr (total)	< 0.10
Cu	< 0.10
Fe	0.20
Mn	< 0.10
Pb	< 0.50
Zn	0.20

G. The nonlinear regression analysis was performed by a computer program entitled NLIN developed by Grenney (41). Analyses to determine the mean, variance, and standard deviation of various data groups were performed on a Monroe Model 1665, Electronic Programmable Calculator, with a program furnished by the Monroe Calculator Company.[6] All statistical analyses were performed according to Sokal and Rohlf (78).

Cell mass values (i.e. mg/l) used in the analyses of continuous flow data obtained from cultures with inhibitors at 20°C, 24°C, and 28°C were calculated from a linear regression equation of cell number versus cell mass which is shown in the results section of this report. Semi-continuous cell mass values (i.e. mg/l) were calculated from an equation developed by Porcella et al. (72), as previously explained.

The "goodness of fit" provided by the nonlinear equations developed by regression analysis was measured by determining the correlation coefficient between the observed experimental data and the values predicted by the nonlinear equations. High positive correlation coefficients indicated that the nonlinear regression equation adequately described the experimental data. Negative correlation coefficients indicated that the nonlinear regression equation represented an inverse relationship to the data.

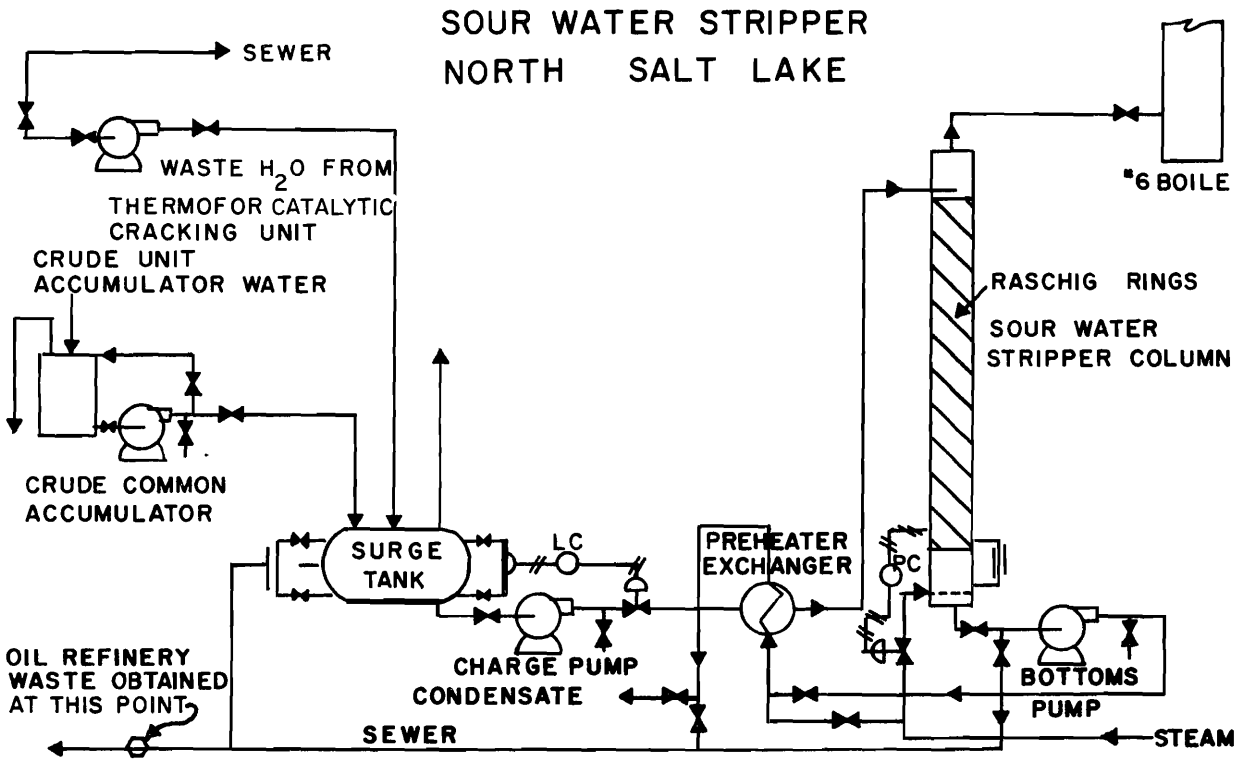


Figure 4. Flow diagram of oil refinery waste system and location of sample point from which oil refinery waste was obtained (56).

[6]Monroe Calculator 6, Division of Litton Industries.

CHAPTER V

RESULTS AND DISCUSSION

Preliminary Studies

Buffer experiments

Initially two buffers were evaluated as a means of controlling pH in semi-continuous and continuous flow cultures of *Selenastrum capricornutum*. A glycylglycine buffer (67) and a phosphate buffer (39) were evaluated in semi-continuous flow cultures of *Selenastrum capricornutum* grown at 25°C with a mean hydraulic residence time of three days. Growth was monitored by measuring the optical density at 750 μm. Both the pH and optical density were measured daily. Results of both the glycylglycine buffer and the phosphate buffer experiments are tabulated in Appendix B, Tables B-1 to B-3. The variation in pH with time for two typical cultures with different glycylglycine buffer concentrations is shown in Figure 5. The higher glycylglycine buffer concentration held the pH relatively constant. However, the initial pH value of the culture was not maintained at either glycylglycine concentration.

Figure 6 shows the variation in cell concentration with time in a typical culture with and without the glycylglycine buffer. It is apparent that the cell concentration increased significantly with increasing glycylglycine concentrations. These cultures were grown under nitrogen limited conditions and should have reached steady state within six days after inoculation. The fact that the cultures with glycylglycine continued to increase in cell mass above those cultures without glycylglycine and did not obtain steady state within six days, indicates that the alga were obtaining some form of nitrogen from the glycylglycine molecule. However, it is unlikely that the *Selenastrum capricornutum* were obtaining the nitrogen directly from the glycylglycine molecule. Rather, it is likely that the nitrogen was split from the glycylglycine molecule by bacteria present in the cultures and then, in turn, taken up by an alga. Attempts were not made to establish the exact cause of the increased growth with increased glycylglycine concentration.

Because the glycylglycine buffer was incapable of maintaining the desired pH and also stimulated algal growth, it was not used in further experiments.

The phosphate buffer system consisted of a one to one ratio of mono to dibasic sodium phosphate. This buffer system was evaluated in the same manner as the glycylglycine buffer. The results are tabulated in Appendix B (Tables B-2 to B-3).

Figure 7 shows the pH variation in a typical phosphate buffered culture. Both the 0.03 and the 0.06 molar phosphate buffers were able to maintain a relatively constant pH under steady state conditions. The pH remained at the desired level of 7.1 to 7.2.

The effects of the phosphate buffer on the steady state cell concentration of two typical cultures are shown

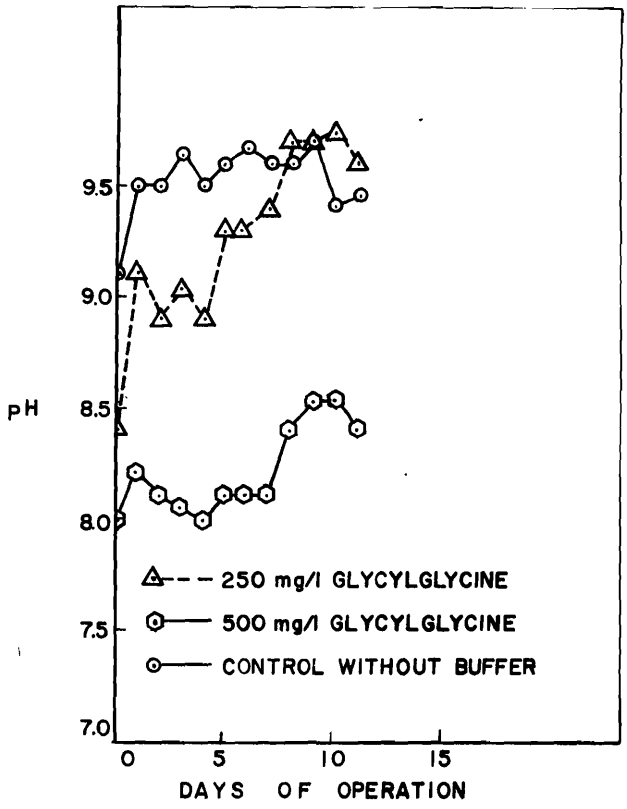


Figure 5. Variation in pH of semi-continuous cultures with various glycylglycine buffer concentrations (N = 1.05 mg/l).

in Figure 8. Apparently the 0.06 molar phosphate buffer exerted an inhibitory effect on *Selenastrum capricornutum*. This phenomenon was also observed to a lesser degree with a 0.05 molar phosphate buffer. However, inhibitory effects were not observed at a 0.03 molar phosphate buffer concentration.

Because a 0.03 molar concentration of phosphate buffer with a one to one ratio of mono to dibasic sodium phosphate provided excellent pH control without inhibitory effects, it was utilized throughout the remainder of this study to control pH in both semi-continuous and continuous flow cultures.

Temperature tolerance experiments

Batch cultures and semi-continuous flow cultures maintained at a three day hydraulic residence time were grown at various temperatures to determine the temperature range acceptable for this study. *Selenastrum capricornutum* cultures were grown at temperatures ranging between 15°C and 40°C. Results of this experiment are summarized in Tables 7 and 8.

The semi-continuous flow cultures of *Selenastrum capricornutum* grew well at 15°C. However, lower temperatures were not employed, because it was felt that lower temperatures would not be needed for this study.

Semi-continuous flow cultures did not (three day residence time) sustain growth above 35°C. Apparently, the maximum specific growth rate of *Selenastrum capricornutum* is less than 0.33 $days^{-1}$ at temperatures above 35°C. However, batch cultures grown at 36°C maintained themselves for 15 days, even though the cell concentration of the cultures continued to decrease. *Selenastrum capricornutum* would not maintain itself in batch cultures above 38°C.

Based on the above results, the temperature-toxicity portion of this study was conducted between 20°C and 33°C.

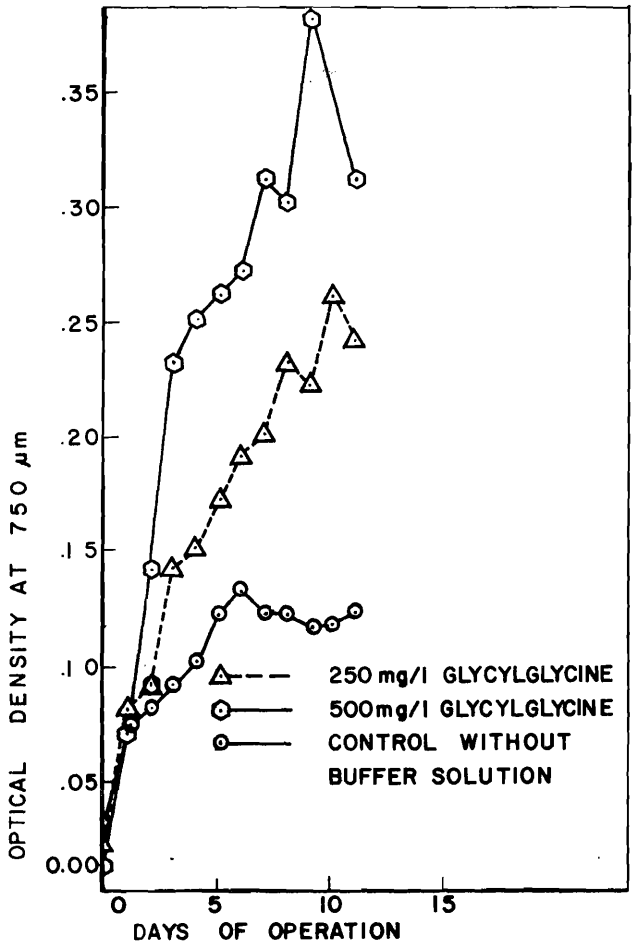


Figure 6. Optical density of semi-continuous cultures with various glycylglycine buffer concentrations (N = 1.05 mg/l).

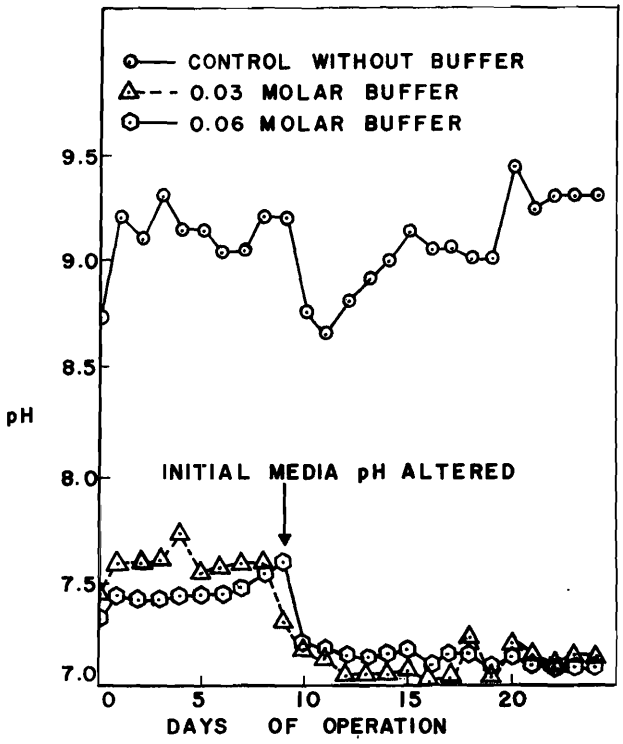


Figure 7. Variation in pH of semi-continuous cultures with various phosphate buffer concentrations (N = 1.05 mg/l).

Phenol tolerance experiments

Semi-continuous flow cultures of *Selenastrum capricornutum* with a three day residence time were grown at various temperatures and phenol concentrations to determine an acceptable phenol concentration range to utilize in future experiments. The results of these experiments are summarized in Appendix B, Table B-4.

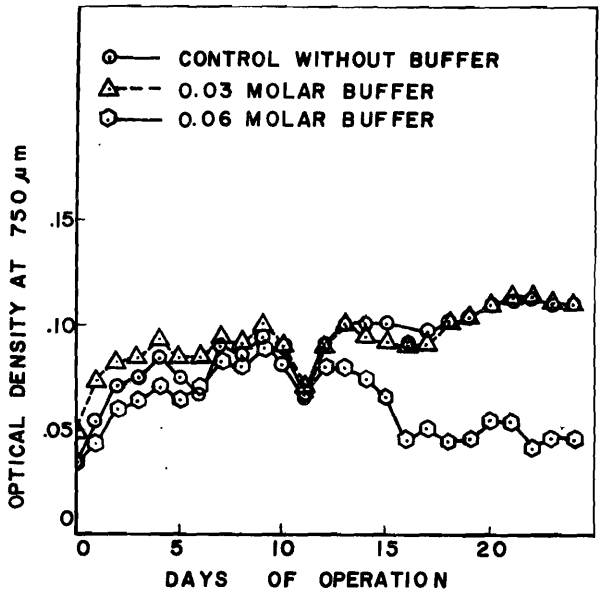


Figure 8. Optical density of semi-continuous cultures with various phosphate buffer concentrations (N = 1.05 mg/l).

The test alga was able to maintain itself in phenol concentrations as great as 240 mg/l at temperatures ranging from 20°C to 33°C. Phenol concentrations greater than 240 mg/l were not tested. However, it is felt that the *Selenastrum capricornutum* could not withstand phenol concentrations significantly greater than 240 mg/l, especially at temperatures above 24°C.

Based on the above results, the temperature-toxicity experiments with semi-continuous flow cultures utilized phenol concentrations ranging from 0 to 120 mg/l, and

Table 7. Batch culture optical density at 36°C, 37°C, 38°C, and 40°C.

Temp. °C	Culture No.	Number of Days After Inoculation		
		0	5	10
36	x	.070	.035	.025
	y	.065	.035	.023
	z	.075	.023	.025
	Ave	.070	.031	.024
37	x	.070	.035	.025
	y	.075	.035	.025
	z	.070	.025	.025
	Ave	.072	.317	.025
38	x	.015	.010	.000
	y	.015	.020	.000
	Ave	.015	.015	.000
40	x	.013	.010	.000
	y	.013	.015	.000
	Ave	.013	.013	.000

Table 8. Steady state optical density and pH of semi-continuous cultures at various temperatures with a 3 day residence time. Values are a 3 day average.

Temp. °C	Culture O.D. at 750 µm			Ave O.D. at 750µm	Culture pH			Ave pH
	A	B	C		A	B	C	
15	.123	.097	.117	.112	9.80	9.95	9.91	9.89
33	.220	.237	.220	.226	9.72	9.75	9.72	9.73
35	.101	.128	.128	.119	9.30	8.95	9.46	9.24
36	.000	.000	.000	.000	7.40	7.40	7.40	7.40

the continuous flow culture experiments utilized phenol concentrations ranging from 0 to 60 mg/l. The range of phenol concentrations was reduced for the continuous flow experiments because it was felt that continuous flow cultures would be more sensitive to the toxicant than the semi-continuous flow cultures.

Semi-Continuous Flow Culture Experiments

General

Semi-continuous flow cultures of *Selenastrum capricornutum* (three day residence time) were grown in phenol concentrations ranging from 0 to 120 mg/l at temperatures of 20°C, 24°C, and 28°C. The results of these experiments are summarized in Table 9. The individual data points presented in Table 9 represent an average of three samples measured during one complete residence time.

Linear regression analysis

The data were fitted to each of the inhibition equations (Equations 15, 16, and 17). A statistically significant correlation was obtained with the linear competitive inhibition equation (Equation 17). No significant correlation could be established for the uncompetitive inhibition equation (Equation 17) because these two equations cannot be applied in a linear form. Results from the linear regression analyses for the competitive inhibition equation at 20°C, 24°C, and 28°C are shown in Figures 9, 10, and 11. The data points appear to be randomly distributed about the regression line and do indicate a significant amount of linearity.

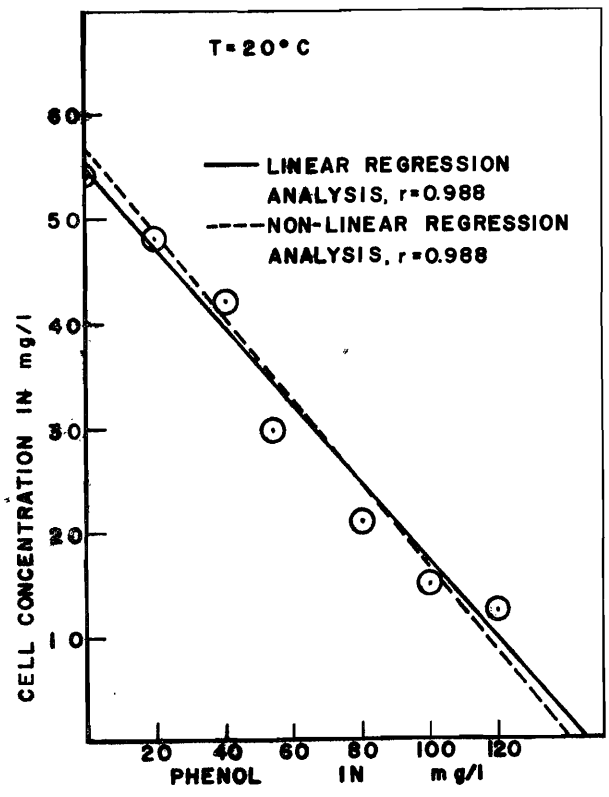


Figure 9. Cell concentration vs. phenol concentration for semi-continuous cultures at 20°C for the competitive inhibition model, using linear regression and nonlinear regression analysis.

Table 9. Cell concentration for semi-continuous experiments.[a]

Phenol Concentration (mg/l)	Cell count x 10^{-6}/ml[a]			Cell mass mg/l[b]		
	20°C	24°C	28°C	20°C	24°C	28°C
0	2.924	4.110	3.334	54.73	73.19	60.92
20	2.566	3.000	2.276	48.79	55.65	44.21
40	2.139	2.658	2.130	42.05	50.25	41.90
60	1.370	2.048	1.913	29.90	40.48	38.48
80	0.798	1.560	1.255	20.85	32.90	28.08
100	0.439	1.113	1.045	15.18	25.84	24.76
120	0.271	0.561	0.236	12.53	17.11	11.98

[a]Mean of the steady state measurements.

[b]Calculated from Equation 42.

The results of the linear regression analysis are summarized in Table 10. Equation 17 does not include a term to account for bacterial effects on the toxicant present. This equation was used for this particular analysis because no measurement of bacteria in the cultures was performed. The maximum specific growth rate, $\hat{\mu}$, and the half saturation constant, K_s, employed for this particular analysis were obtained from the continuous

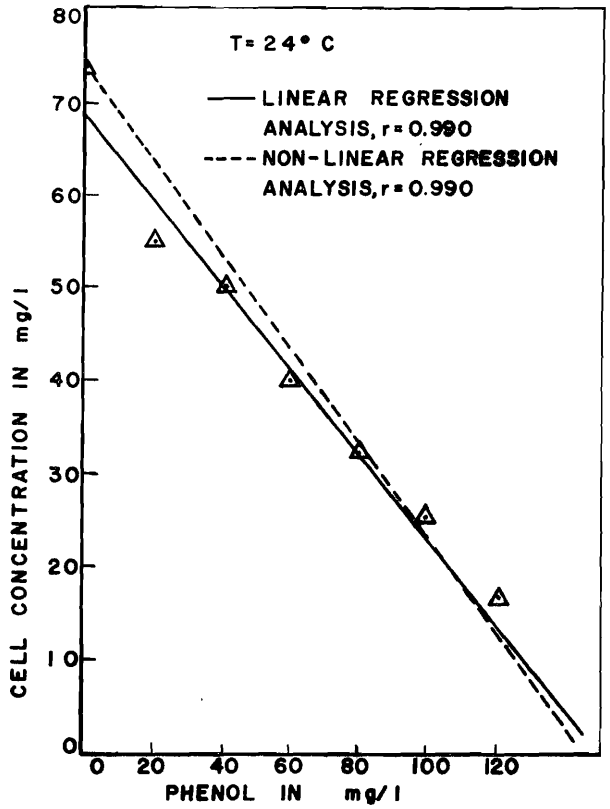


Figure 10. Cell concentration vs. phenol concentration for semi-continuous cultures at 24°C for the competitive inhibition model, using linear regression and nonlinear regression analysis.

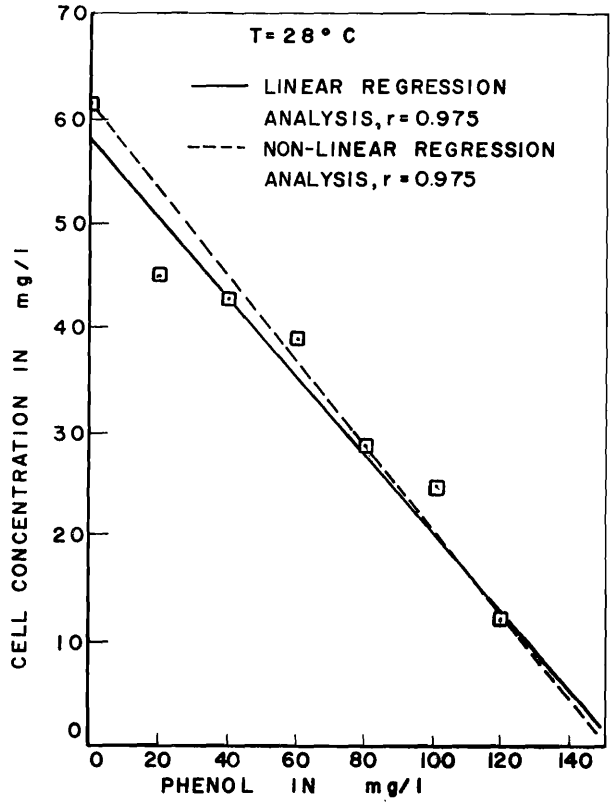


Figure 11. Cell concentration vs. phenol concentration for semi-continuous cultures at 28°C for the competitive inhibition model, using linear regression and nonlinear regression analysis.

Table 10. Semi-continuous culture linear regression analysis for the competitive (X_1 vs. I) model using K_s and $\hat{\mu}$ determined from continuous culture experiment.

Temp. °C	Slope x 10^2	95% Confidence Interval for Slope x 10^2		Inter-cept	95% Confidence Interval for Intercept		Correlation[a] Coefficient
		Max.	Min.		Max.	Min.	
20	-0.384	-0.452	-0.315	55.042	59.117	50.967	-0.988
24	-0.438	-0.511	-0.365	68.475	72.861	64.089	-0.990
28	-0.356	-0.449	-0.264	57.141	62.710	51.572	-0.975

[a] 1 percent significance level correlation coefficient = -0.798.

flow experiments which will be discussed in a later section. A previous analysis of the semi-continuous flow culture data has been reported (75) using values for the maximum specific growth rate, $\hat{\mu}$, and the half saturation constant, K_s, obtained from an equation developed by Goldman (39); however, this present analysis is more accurate because Goldman's equation may not be valid over a wide temperature range.

Goldman (30) developed an Arrenhius type function to describe the variation of $\hat{\mu}$ with temperature. The data employed to derive that function was obtained from several different algal species (both high temperature and low temperature) and indicated that $\hat{\mu}$ for *Selenastrum capricornutum* does not continue to increase with increasing temperature, as will be shown later in this paper.

The correlation coefficients for the linear regression analyses were all greater than 0.970. These values are substantially greater than the 1 percent significance level correlation coefficient of 0.798 and indicate an extremely high correlation of the data with the competitive inhibition equation (Equation 17). The respective inhibition constant, K_I, can be obtained from the slope of the appropriate regression line. The values of the inhibition constant, K_I, obtained in this manner from the linear regression analysis are reported in Table 11. As explained in the Theory Section, the smaller the value of the inhibition coefficient, K_I, the greater the toxicity of the inhibition. Thus, the values of the inhibitor constants, K_I, reported in Table 11, indicate that phenol is more toxic to *Selenastrum capricornutum* at 24°C than it is at 20°C or 28°C. The steady state cell concentrations, X_1, at 24°C are greater than those at 20°C and 28°C because the cultures are closer to the optimum growth temperature for the organism.

Nonlinear regression analysis

The semi-continuous flow culture data were also fitted to the competitive, uncompetitive, and noncompetitive inhibition equations (Equations 14, 15, and 16) using nonlinear regression analysis. Again, the maximum specific growth rates, $\hat{\mu}$, and the half saturation constants, K_s, obtained from the continuous flow culture experiments were employed. Only the competitive inhibition equation (Equation 14) resulted in a significant correlation. The results of the nonlinear regression analysis for the competitive inhibition equation are reported in Table 12. The correlation coefficients obtained are all substantially greater than the 1 percent significance level and indicate a high degree of correlation with the competitive inhibition equation (Equation 14). This was not unexpected since the previous linear regression analysis had indicated a high degree of correlation with the competitive inhibition equation.

Plots of the nonlinear regression analysis are also shown in Figures 9, 10, and 11. Essentially the relationship is linear except for a slight nonlinear portion of the curve at phenol concentrations less than 40 mg/l. The competitive inhibition constants, K_I, developed from the nonlinear regression analysis are reported in Table 11. These values are slightly smaller than those previously developed using linear regression analysis; however, the pattern in toxicity is the same. The phenol is more toxic to the *Selenastrum capricornutum* at 24°C than it is at 20°C or 28°C.

Continuous Flow Culture Experiments

General

The analysis of the continuous flow culture data was divided into four phases. Phase I involved the determination of a function which describes the luxury uptake of ammonium-nitrogen by *Selenastrum capricornutum*. The function utilized for this phase was developed by Toerien et al. (83) as outlined in the Theory

Table 11. Competitive inhibition constants, K_I, obtained from linear regression analyses, and nonlinear regression analysis of the semi-continuous flow data using kinetic constants developed from continuous flow data.

T °C	Linear K_I mg/l	Nonlinear K_I mg/l
20	0.121	.113
24	0.083	.075
28	0.145	.129

Table 12. Results of nonlinear regression analysis of semi-continuous data using the competitive inhibition model and kinetic constants, $\hat{\mu}$, and K_s, obtained from continuous flow data.

T °C	$\hat{\mu}$ days^{-1}	K_s µg/l	K_I mg/l	Correlation[a] Coefficient
20	1.365	5.237	.113	.988
24	1.992	5.237	.075	.990
28	1.391	5.640	.129	.975

[a] 1 percent significance level correlation coefficient = 0.798.

Section of this report. During Phase II analysis, the maximum specific growth rates, $\hat{\mu}$, and the half saturation constants, K_s, for *Selenastrum capricornutum* were developed for 20°C, 24°C, 27°C, 28°C, and 33°C. These constants were developed using the Michaelis-Menten (Monod) equation with linear and nonlinear regression analysis. Phase III analysis involved the use of the function developed in Phase I and the constants developed in Phase II to determine the continuous flow inhibition function for phenol. Finally, in Phase IV, the continuous flow inhibition function developed in Phase III was applied to an actual oil refinery waste.

Figures F-1 - F-17, Appendix F, show the variation in steady state cell concentration, X_1, with time during the entire continuous flow experiment. These figures also indicate the effect of adding toxicant to the cultures after they reach steady state.

Phase I: Luxury uptake

General. The luxury uptake function was developed in four parts. Part I involved the determination of the maximum yield coefficient, Y_{max}. In Part II, the variation of the yield coefficient with the hydraulic residence time, θ, and the nutrient utilization constants, K_A and K_B, for *Selenastrum capricornutum* grown at 20°C, 24°C, 27°C, 28°C, and 33°C were determined. Part III verified the function and constants developed in Part II by predicting the nutrient removal velocity, q. In Part IV, the function and constants developed in Part II were verified by predicting the fraction of excess nutrient uptake, F_e. All analyses were performed using nonlinear regression analysis.

Part I: Maximum yield coefficient, Y_{max}. The maximum yield coefficient, Y_{max}, was defined in the Theory Section as the yield coefficient measured in a continuous flow culture with an infinite hydraulic residence time, θ (83). The maximum cell yield coefficient is measured when the steady state cell concentration, X_1, is at a maximum (i.e. $Y_{max} = X_{max}/S_o - S_1$). In practice, this means achieving a steady state cell concentration in a chemostat at a given hydraulic residence time and then discontinuing feeding the culture. The culture will then reach a maximum cell concentration, X_{max}, at the infinite residence time, θ_∞ (83). This type of an experiment was conducted with the *Selenastrum capricornutum* at 20°C, 24°C, 27°C, 28°C, and 33°C. During this experiment, the limiting ammonium-nitrogen concentration in the chemostats was 4.2 mg/l.

The results of the experiment to determine the maximum yield coefficient are tabulated in Appendix C, Tables C-1 to C-7. A summary of the maximum yield coefficients obtained for the various temperatures is presented in Table 13. The values obtained for the maximum yield coefficient indicate that this parameter does vary significantly with temperature and that it has a maximum value at 24°C. The parameter decreases with temperatures above and below 24°C. This pattern is very similar to that exhibited by the maximum specific growth rate, $\hat{\mu}$.

Part II: Luxury uptake function (Y vs θ). Under luxury uptake conditions, the cell yield coefficient, Y, varies with the hydraulic residence time, θ (Equations 27 and 28). Data from the continuous flow culture experiments at 20°C, 24°C, 27°C, 28°C, and 33°C, were fitted to Equations 27 and 28 using nonlinear regression analysis, and the nutrient utilization constants, K_A and K_B, were determined. The data employed during this analysis are tabulated in Appendix C, Tables C-8 to C-12. A summary of the nonlinear regression analysis and the correlation coefficients obtained are presented in Table 13. Figures 12 through 16 represent the relationships between the data and functions developed from Equations 27 and 28.

Both Equations 27 ('A Form') and 28 ('B Form') provide a significant correlation for the data at the 5 percent significance level, except for the 27°C and 33°C cases. The lack of correlation at 27°C and 33°C is probably due to insufficient data collection. However, since Equations 27 and 28 appear to adequately describe the data at the other temperatures, the constants developed by these two equations will be used in further analysis of the 27°C and 33°C data even though a significant correlation was not obtained. This concession is made, realizing that although some error may be introduced in future analysis; such an assumption is superior to excluding from further analysis.

In general, high correlation is obtained from both Equation 27 ('A Form') and Equation 28 ('B Form') and it is difficult to determine which equation provides a better fit to the data. However, Equation 27 ('A Form') has a higher correlation coefficient for three out of the five cases analyzed and on that basis appears to better represent the data. This is similar to what Toerien et al. (83) reported on the luxury uptake of phosphorus by *Selenastrum capricornutum.*

Part III: Nutrient removal velocity, q. Luxury uptake occurs when the nutrient removal velocity, q, exceeds the nutrient utilization rate, as pointed out in Chapter III Theory. Without luxury uptake, the nutrient removal velocity is a linear function of growth rate. However, under luxury uptake conditions the nutrient removal velocity is a nonlinear function of growth rate. Equation 31 ('A Form') and Equation 32 ('B Form') were developed from Equations 27 and 28 to describe the variation of the nutrient removal velocity as a function of growth rate. A summary of the nonlinear regression analysis of Equations 31 and 32 is presented in Table 14. The correlation coefficients are all significant at the 5 percent level. However, the correlation coefficients for three of the five cases are higher for Equation 32 ('B Form') than for Equation 31 ('A Form'). This is opposite

to what was found in the previous section. The figures representing these relationships are presented in Appendix C, Figures C-1 to C-5, and a typical plot of the relationship is presented in Figure 17. This figure is for the 24°C case and exhibits a somewhat better fit than do the other cases. This is particularly true for the fit of Equation 31 ('A Form'). The better fit obtained in the 24°C case is probably due to the availability of a greater number of data points for analysis as compared to the other cases.

Part IV: Fraction of excess nutrient, F_e. Under luxury uptake conditions, the cell yield coefficient is not constant, and the nitrogen concentration within an

Table 13. Variation is maximum yield coefficient with temperature and a comparison of nonlinear regression analyses of Equation 27, $Y_n = Y_{max}(1-e^{-\theta/K_A})$, with Equation 28 $Y_n = Y_{max}(\theta / \theta+K_B)$ for the continuous flow Data.

Temp. °C	Y_{max} mg Cell / mg NH_4-N	Equation 27		Equation 28		5% Significant Level for Corr. Coeff.
		K_A (days)	Correlation Coefficient	K_B (days)	Correlation Coefficient	
20	22.01	1.05	.934	0.558	.931	.632
24	61.20	3.16	.908	2.460	.899	.404
27	33.44	1.30	.470[a]	0.825	.442[a]	.754
28	29.94	1.36	.800	0.750	.804	.413
33	25.57	0.84	-.459[b]	0.427	-.472[b]	.707

[a]Nonsignificant at 5% level.

[b]Significant, but in an inverse relationship.

Table 14. Comparison of nonlinear fit of Equations 31 and 32 to nutrient removal velocity, q, and fraction of excess uptake, F_e, with continuous flow data.

Temp. °C	Correlation Coeff. for q		Correlation Coeff. for F_e		5% Significant Level for Corr. Coeff.
	Eq. 31	Eq. 32	Eq. A	Eq. B	
20	.923	.919	.940	.938	.632
24	.920	.925	.907	.899	.404
27	.897	.905	.462[a]	.434[a]	.754
28	.986	.983	.798	.804	.413
33	.773	.789	.824	.833	.707

[a]Nonsignificant at 5% level.

individual cell varies with the hydraulic residence time, θ. The amount of excess or stored nitrogen in the cell may be expressed as the fraction of excess nutrient uptake, F_e, which has been previously discussed in the Theory Section. Equations 29 and 30 were developed from Equations 27 and 28 to describe the variation of the fraction of excess nutrient uptake as a function of hydraulic residence time.

Using the nutrient utilization constants, K_A and K_B, previously developed, the data were fitted to Equations 29 and 30. The results of the regression analyses are presented in Table 14, and plots of the resulting equations are shown in Appendix C, Figures C-6 and C-10.

The correlation coefficients resulting from the regression analysis are all significant at the 5 percent level except for the 27°C cultures. Again, the lack of correlation for the 27°C case is attributable to a lack of data and the clustering of the data. In each case, Equation 30 ('B Form') appears to provide a better fit for the data. A typical plot of the resulting equations is presented in Figure 18. This particular figure does not indicate the better fit of Equation 30 as compared to Equation 29; however, because the amount of data analyzed in the 24°C cases enables a good fit for both equations.

These results support the validity of the nutrient utilization constants, K_A and K_B, developed for the luxury uptake equations (Equations 27 and 28) and further support their use in future analysis to determine kinetic growth constants and inhibition constants.

Phase II: Kinetic growth constants

General. The maximum specific growth rate, $\hat{\mu}$, and the half saturation constant, K_s, for *Selenastrum capricornutum* were determined from the continuous flow experimental data obtained at 20°C, 24°C, 27°C, 28°C, and 33°C. These analyses were performed using both linear and nonlinear regression techniques. Also, an attempt was made to determine these same parameters from equations which included the luxury uptake function.

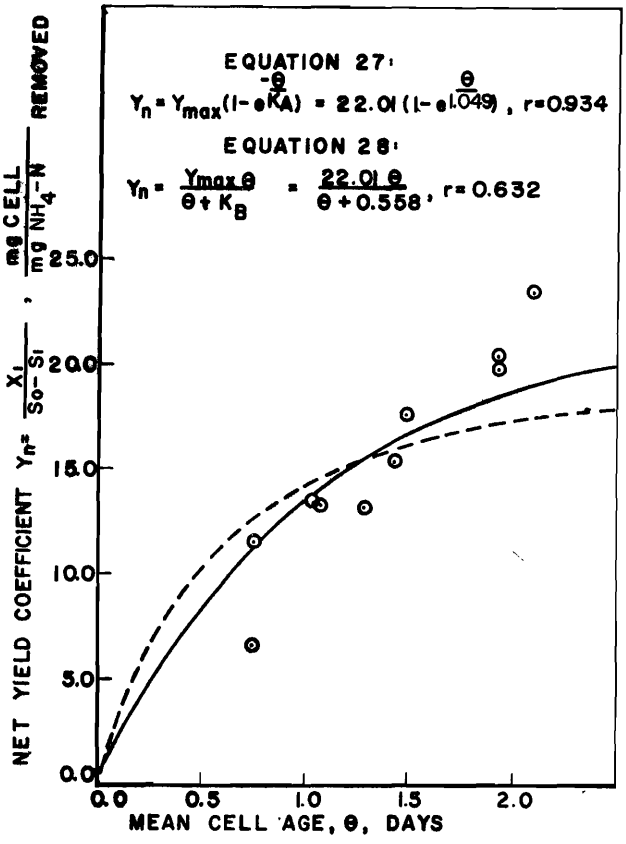


Figure 12. Net cell yield coefficient as a function of mean cell age, θ, at 20°C.

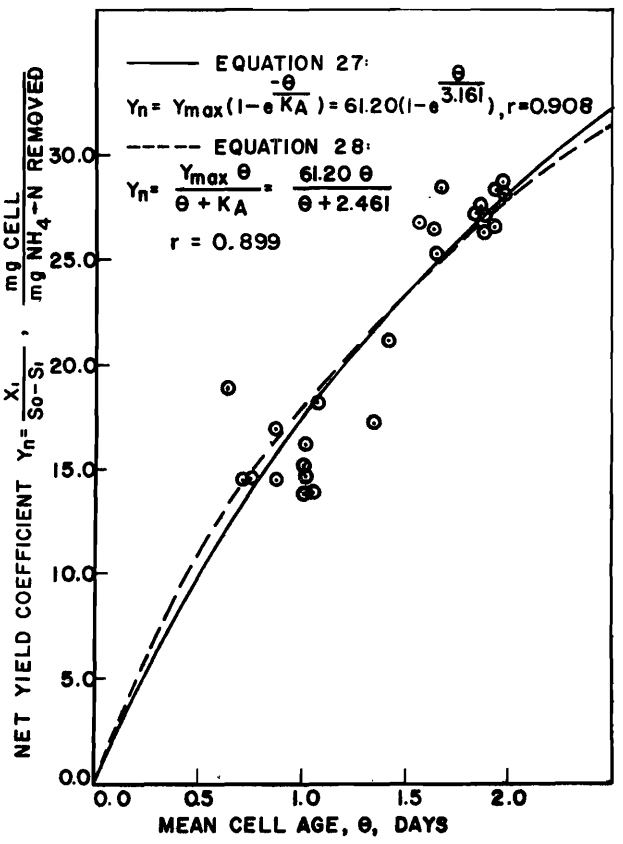


Figure 13. Net cell yield coefficient as a function of mean cell age, θ, at 24°C.

The continuous flow experimental data utilized in these analyses are tabulated in Appendix D, Tables D-1 to D-22. In addition, a summary of the measured and calculated data utilized in these analyses are recorded in Appendix D, Tables D-23 to D-27.

Nonlinear regression analysis. The data were fitted, using nonlinear regression techniques, to the Michaelis-Menten (Monod) equation (Equation 43) which describes microbial growth in a nutrient limited environment

$$\mu = \frac{\hat{\mu} S_1}{K_s + S_1} \quad \ldots\ldots\ldots\ldots\ldots\ldots (43)$$

The analyses were first performed including all the data points and then repeated excluding those data points which lay near the upper extreme of the function. The results of both analyses are reported in Table 15.

Analysis of the data excluding the data points near the upper extreme of the function are justified because only two observations of questionable reliability were made in that region, and because these data points are far removed from the main body of data, which allows these two points to exert an undue effect on the character of the function and on the parameters developed. Also, the values obtained for the maximum specific growth rate, $\hat{\mu}$, of 1.544 days^{-1} at 24°C, using all of the data do not agree with reported values for *Selenastrum capricornutum* (39, 72, 83). However, the value obtained by excluding the data points (1.992 days^{-1}) is in good agreement with previous reports (39, 72, 83). Also, the correlation coefficients, except for the 28°C case, are higher when the data points are excluded.

The lack of correlation for the 28°C case where selected data have been excluded from the analysis is attributed to the exclusion of the two data points which lie above the computed curve. The exclusion of these data points from the correlation calculation was necessary because the computed function is mathematically undefined for measured data points which lie above the computed curve. Therefore, a calculated data point to correlate with the measured data point cannot be deter-

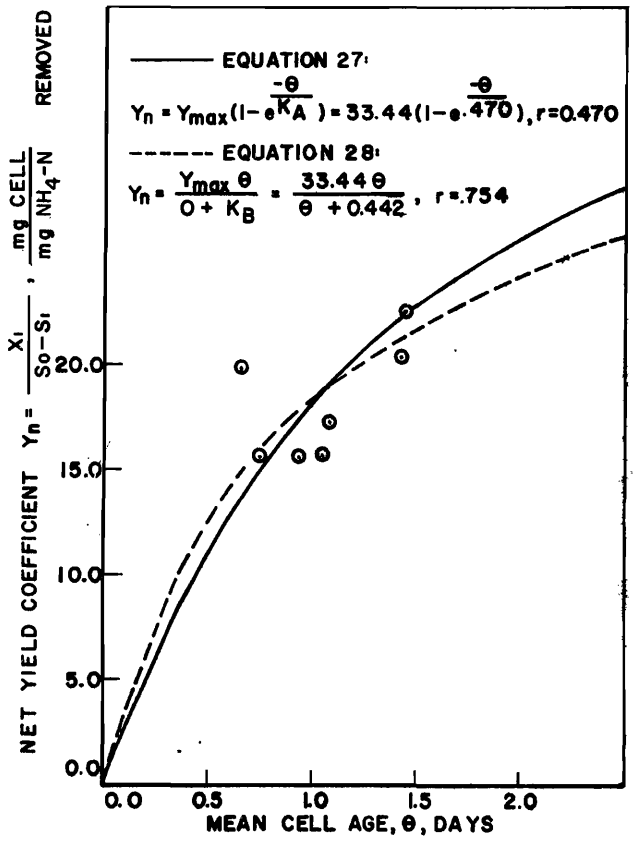


Figure 14. Net cell yield coefficient as a function of mean cell age, θ, at 27°C.

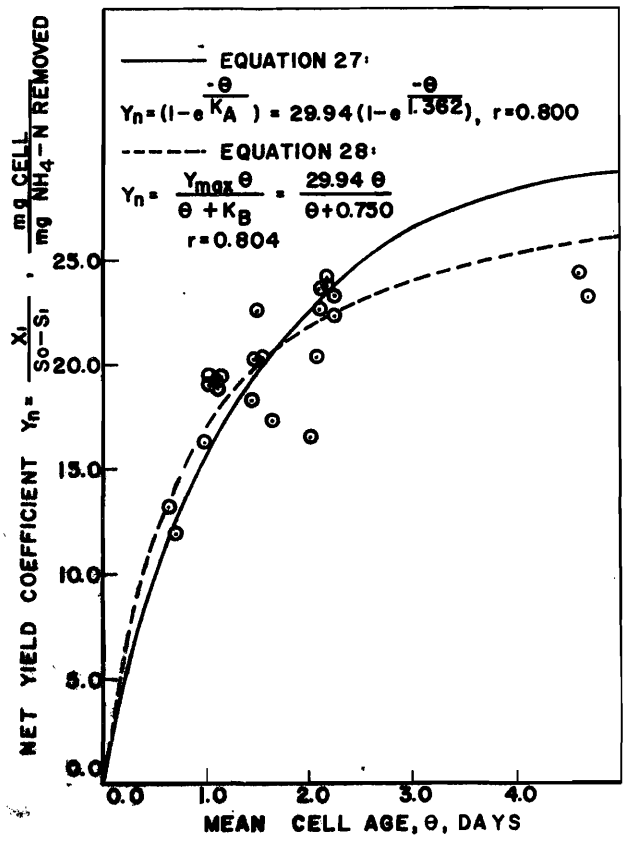


Figure 15. Net cell yield coefficient as a function of mean cell age, θ, at 28°C.

Table 15. Values for maximum specific growth rate, $\hat{\mu}$, and half saturation constant, K_s, developed from linear and nonlinear analysis of Michaelis-Menten (Monod) equation for ammonium-nitrogen limitation of continuous cultures.

Temp. °C	Linear Analysis $\left(S_1 \text{ vs } \frac{S_1}{\mu}\right)$			Nonlinear Analysis Including all Data Points			Nonlinear Analysis Excluding Various Data Points				5% Significance Level for Correlation Coefficient
	$\hat{\mu}$ $days^{-1}$	K_s µg/l	Correlation Coefficient	$\hat{\mu}$ $days^{-1}$	K_s µg/l	Correlation Coefficient	$\hat{\mu}$ $days^{-1}$	K_s µg/l	Correlation Coefficient	No. of Data Pts. Excluded	
20	1.363 ±.0105[a]	5.550 ± 3.136[a]	.999	1.398	5.237	.975	1.365	5.237	.999	1	.602
24	1.541 ±.0132	6.232 ± 0.402	.999	1.544	5.251	.996	1.992	5.237	.995	1	.396
27	1.526 ±.0333	7.848 ± 3.427	.999	1.540	5.246	.989	1.412	5.237	.995	1	.707
28	1.516 ±.0527	19.532 ± 3.289	.996	1.596	5.248	.799	1.390	5.564	.100[b]	2	.374
33	1.446 ±.1416	73.192 ±46.924	.992	1.274	5.505	.949	1.274	5.503	.949	1	.666

[a] 90 percent confidence interval.

[b] Correlation coefficient nonsignificant because the computed equation is invalid near $\hat{\mu}$ at 28°C.

mined. Without a corresponding calculated data point, a correlation calculation between the measured data point and the calculated data point is impossible. This situation did not occur for any of the other temperatures analyzed. Even though the calculated correlation coefficient for the 28°C case is not significant, inspection of Figure D-4, Appendix D, indicates an excellent fit of the data. Therefore, the maximum specific growth rates, μ, and the ammonium-nitrogen half saturation constants, K_s, obtained from the analysis which excluded some of the data will be used in future analyses.

Figure 19 is a comparison of the computed curves developed for the various temperatures. (See Appendix D, Figures D-2 to D-5, for a comparison of measured data with computed curves.) This figure illustrates, as do the values in Table 15, that the ammonium-nitrogen half saturation constant, K_s, for *Selenastrum capricornutum* is quite small and does not vary significantly with temperature. The ammonium-nitrogen half saturation constant, K_s, ranges from 5.237 μg/l ammonium-nitrogen at 20°C to 5.503 μg/l ammonium-nitrogen at 33°C. This indicates that *Selenastrum capricornutum* should be able to compete favorably with other organisms in an ammonium-nitrogen limited environment over a fairly wide temperature range.

The maximum specific growth rates, $\hat{\mu}$, as illustrated in Figure 19 and tabulated in Table 15, of *Selenastrum capricornutum* is greatly affected by temperature. Apparently, the maximum specific growth rate has an optimum or maximum value between 24°C and 27°C. At temperatures on either side of this optimum temperature, the maximum specific growth rate decreases rapidly. This indicates that the ability of *Selenastrum capricornutum* to compete with other algal species is greatly influenced by the temperature of the surrounding environment.

Linear regression analysis. The Michaelis-Menten (Monod) equation (Equation 43) can be transformed into three linear expressions as follows:

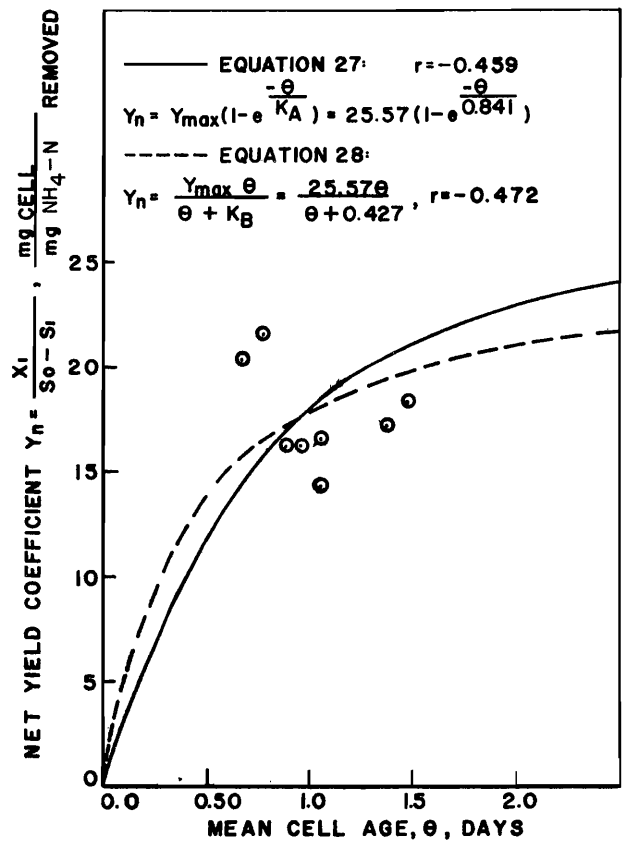


Figure 16. Net cell yield coefficient as a function of mean cell age, θ, at 33°C.

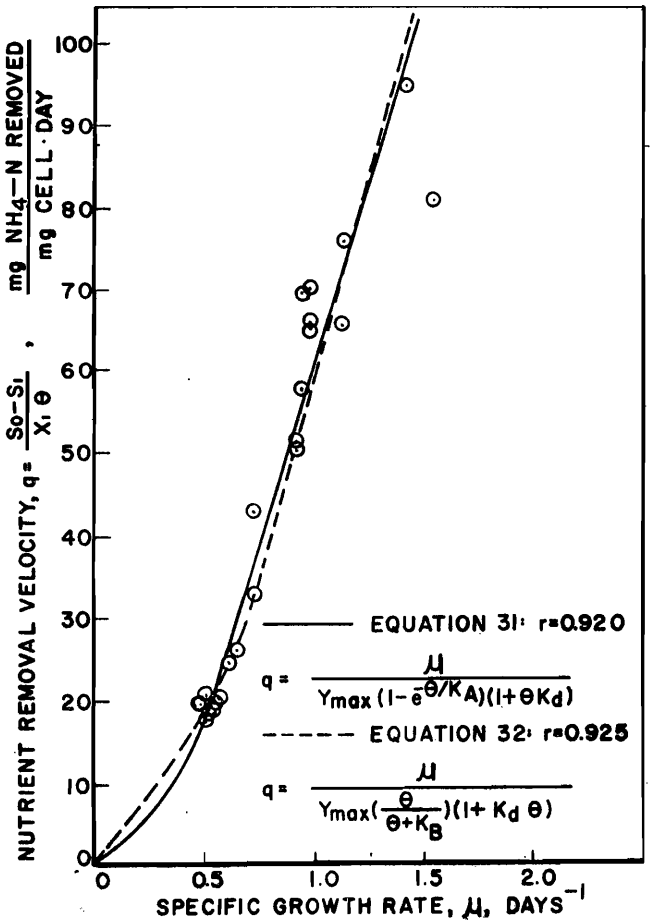


Figure 17. Comparison of nutrient removal velocity, q, and specific growth rate, μ, at 24°C.

$$\frac{1}{\mu} = \left(\frac{K_s}{\hat{\mu}}\right)\left(\frac{1}{S_1}\right) + \frac{1}{\hat{\mu}} \quad \ldots\ldots\ldots (44)$$

$$S_1 = \left(\frac{S_1}{\mu}\right)\hat{\mu} + K_s \quad \ldots\ldots\ldots (45)$$

$$\mu = \hat{\mu} - K_s\left(\frac{\mu}{S_1}\right) \quad \ldots\ldots\ldots (46)$$

The data were fitted to each of these expressions using the linear regression computer program in Appendix E. The results of this linear regression analysis are shown in Table 16. The data only fit one of these three expressions (Equation 45) satisfactorily.

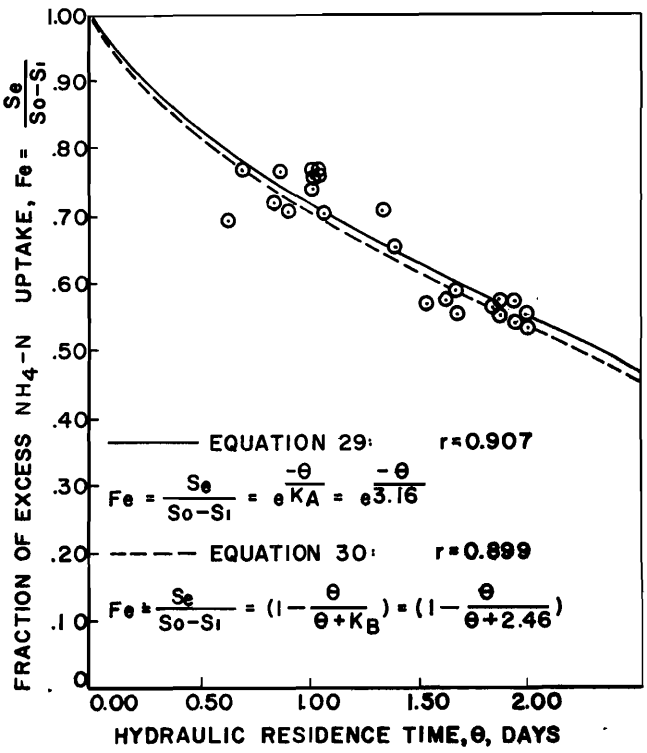


Figure 18. Fraction of "excess" ammonium-nitrogen uptake as a function of hydraulic residence time, θ, at 24°C.

As shown in Table 16, only the correlation coefficients for Equation 45 are significant at the 5 percent level. Figures D-6 to D-10, Appendix D, indicate the type of fit obtained for Equation 45. These figures show that the correlation coefficients indicate a better fit than may exist. The highly significant correlations obtained are due to a grouping of the data at the two extremes of the curve rather than to a uniform distribution of the data along the curve. Therefore, the reliability of the kinetic constants obtained from the linear regression analysis is questionable.

Figure 20 presents the linear regression equations developed from Equation 45. (See Appendix D, Figures D-6 to D-10, for the relationship of the measured data to the linear regression equations.) This figure and the values reported in Table 16 indicate that the ammonium-nitrogen half saturation constant, K_s, for *Selenastrum capricornutum* at 20°C, 24°C, and 27°C are very similar and only slightly increase with increasing temperature. This agrees with the nonlinear regression analysis of the previous section. However, the ammonium-nitrogen half saturation constants for *Selenastrum capricornutum* at 28°C and 33°C increase dramatically with increasing temperature. Since in the linear regression analysis of Equation 45, the half saturation constant is obtained from the intercept of the function, the half saturation constant values are highly susceptible to error. This is especially true when analyses are performed on data which are grouped at the two extremes of the curve.

The values for the maximum specific growth rate, $\hat{\mu}$, obtained from the expressions in Figure 20 are reported in Table 16 and are compared with the values obtained from the nonlinear regression analysis in Table 15. The linear values agree fairly well with the nonlinear values except in the 24°C case. The value of 1.541 days^{-1} obtained from linear regression of the 24°C case is inconsistent with previous reports (39, 72, 83). This inconsistency is probably due to the data grouping and to errors in data collection.

The values of the maximum specific growth rates obtained from the linear regression do indicate a pattern similar to those values obtained from the nonlinear regression analyses. (See Table 15.) In both instances the maximum specific growth rates approach a maximum value between 24°C and 27°C and then decline as the temperature continues to increase.

The linear transformation of the Michaelis-Menten (Monod) equations (Equations 44, 45, and 46) can introduce serious error into the data analysis. At least two of the linear forms must be used for each data analysis to obtain reliable values for the kinetic constants, $\hat{\mu}$ and K_s. Each of the linear transformations contains the value of either $\hat{\mu}$ or K_s in the intercept constant. Thus, small deviations in the slope of the linear transform will have a tremendous effect on the particular intercept value and

hence, the value of $\hat{\mu}$ or K_s. Therefore, great care must be exercised in fitting data to the linear transforms. The data must be evenly distributed alone and randomly scattered about the regression line to obtain reliable values for $\hat{\mu}$ and K_s. As illustrated by the preceding data analysis, the linear transforms (Equations 44, 45, and 46) should only be applied when facilities for nonlinear regression analysis are not available.

Since the reliability of the linear regression analysis is questionable due to the data grouping at the two extremes of the curve and because some of the values for the half saturation constant and the maximum specific growth rate do not agree with previous reports (39, 72, 83) or with the values obtained from the nonlinear regression analysis, the kinetic constants ($\hat{\mu}$ and K_s) obtained from the linear regression analysis will not be used in future analysis of the data. Instead, the values obtained from the nonlinear regression analyses (excluding extreme data points) will be used to develop the luxury uptake functions for kinetic constants and the inhibition functions.

Kinetic constants and luxury uptake. The maximum specific growth rate, $\hat{\mu}$, and the half saturation constant, K_s, may be obtained from Equations 34 and 35 under luxury uptake conditions. An attempt was made to obtain values for the kinetic constants from these two equations using nonlinear regression analysis of the continuous flow culture data. However, the computer program NLIN (41) was unable to develop the kinetic constants to fit the data because the program did not have the latitude to select the appropriate values for the function. Therefore, the kinetic constants ($\hat{\mu}$ and K_s) obtained from the previous nonlinear regression analysis (excluding luxury uptake) were substituted into the function, and a measure of the correlation between the measured data from the experiment and the calculated data from the function were determined.

Figures 21 to 25 present the relationship between the luxury uptake functions using the nonlinear regression kinetic constants and the measured experimental data. The results of the correlation analysis are summarized in Table 17. These figures and Table 17 indicate that

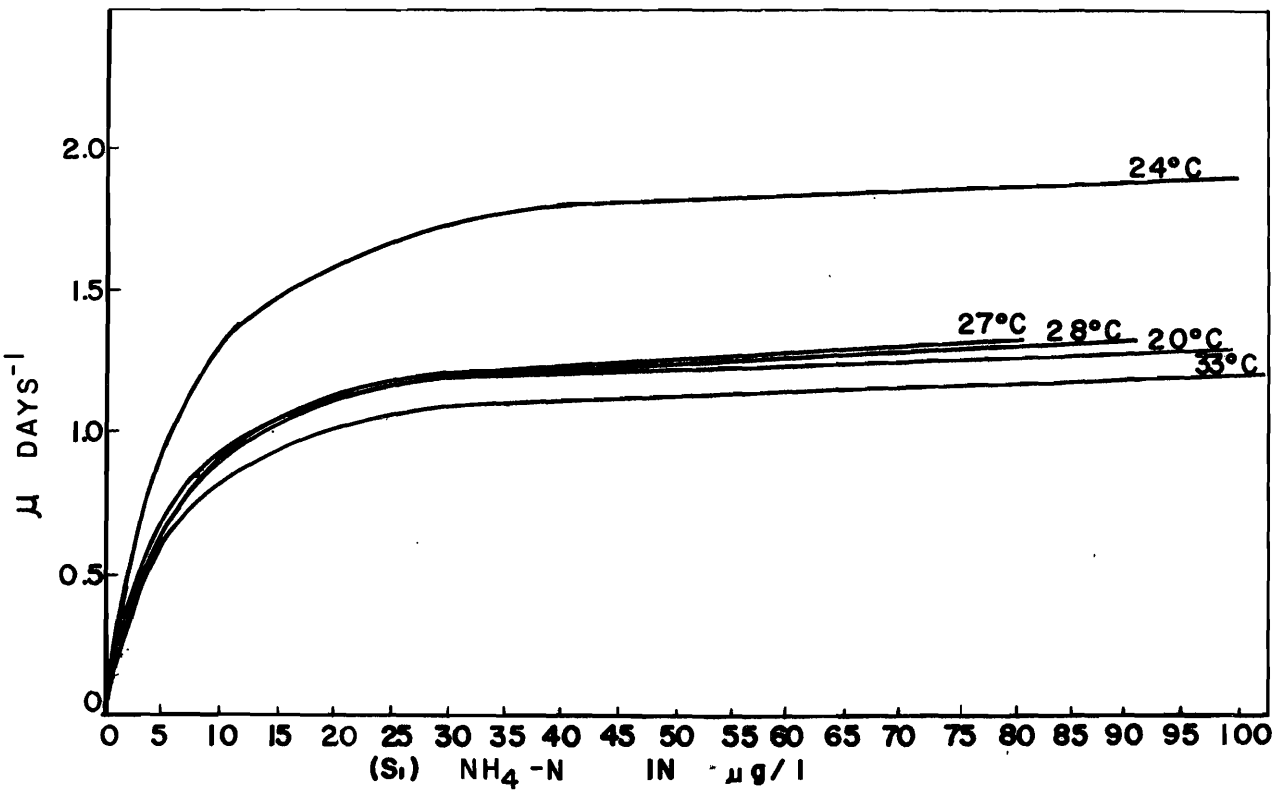


Figure 19. Michaelis-Menten (Monod) kinetic model variation with temperature.

Table 16. Comparison of kinetic constants obtained from various linear transformation of the Michaelis-Menten (Monod) equation.

Temp. °C	$\frac{1}{\mu} = \left(\frac{K_s}{\hat{\mu}}\right)\left(\frac{1}{S_1}\right) + \frac{1}{\hat{\mu}}$			$S_1 = \left(\frac{S_1}{\mu}\right)\hat{\mu} + K_s$			$\mu = \hat{\mu} - K_s\left(\frac{\mu}{S_1}\right)$			5% Significance Level for Correlation Coefficient
	$\hat{\mu}$ $days^{-1}$	K_s µg/l	Correlation Coefficient	$\hat{\mu}$ $days^{-1}$	K_s µg/l	Correlation Coefficient	$\hat{\mu}$ $days^{-1}$	K_s µg/l	Correlation Coefficient	
20	1.245	4.151	.679	1.363	5.550	.999	1.142	-2.581	-.583	.632
24	0.750	2.545	.123	1.541	6.232	.999	0.752	2.781	.142	.404
27	1.147	1.398	.578	1.526	7.848	.999	1.161	-1.044	-.442	.754
28	0.675	2.328	.140	1.516	19.532	.995	0.624	1.315	.165	.413
33	1.076	7.506	.650	1.446	73.192	.992	1.121	-0.084	-.555	.707

substituting the kinetic constants ($\hat{\mu}$ and K_s) into Equations 34 and 35 results in an expression which describes the data very well. The correlation coefficients are all significant at the 5 percent level. This suggests that the kinetic constants ($\hat{\mu}$ and K_s) may not be seriously affected by luxury uptake.

Table 17. **Correlation coefficients developed from luxury uptake kinetic growth equation with maximum specific growth rate, $\hat{\mu}$, and half saturation constant, K_s, developed from nonluxury uptake form of Michaelis-Menten (Monod) equation.**

Temp. °C	Correlation Coefficient		5% Significance Level for Correlation Coefficient
	Equation 34	Equation 35	
20	.859	.627	.576
24	.937	.931	.388
27	.812	.809	.666
28	.785	.786	.396
33	.679	.776	.632

Phase III: Continuous flow phenol inhibition

General. Three different concentrations of phenol were added to the continuous flow cultures at 20°C, 24°C, and 28°C. The steady state measurements for these cultures are recorded in Appendix E, Tables E-1 to E-12. A summary of this data is tabulated in Tables 18, 19, and 20. Figures F-1 to F-17, Appendix F, represent the transition from the noninhibited steady state to the phenol inhibited steady state.

Direct measurement of algal cell mass (mg/l) was not possible in the inhibited cultures due to the presence of bacterial mass. Therefore, the concentration of algae present was determined by a direct cell count and was then converted to algal cell mass by using a set of linear regression equations relating cell number to cell mass. These equations were developed from the cell number and cell mass measurements obtained from the cultures before toxicant was added. These linear regression equations are presented in Table 21. The correlation coefficients for these equations are highly significant and indicate a high

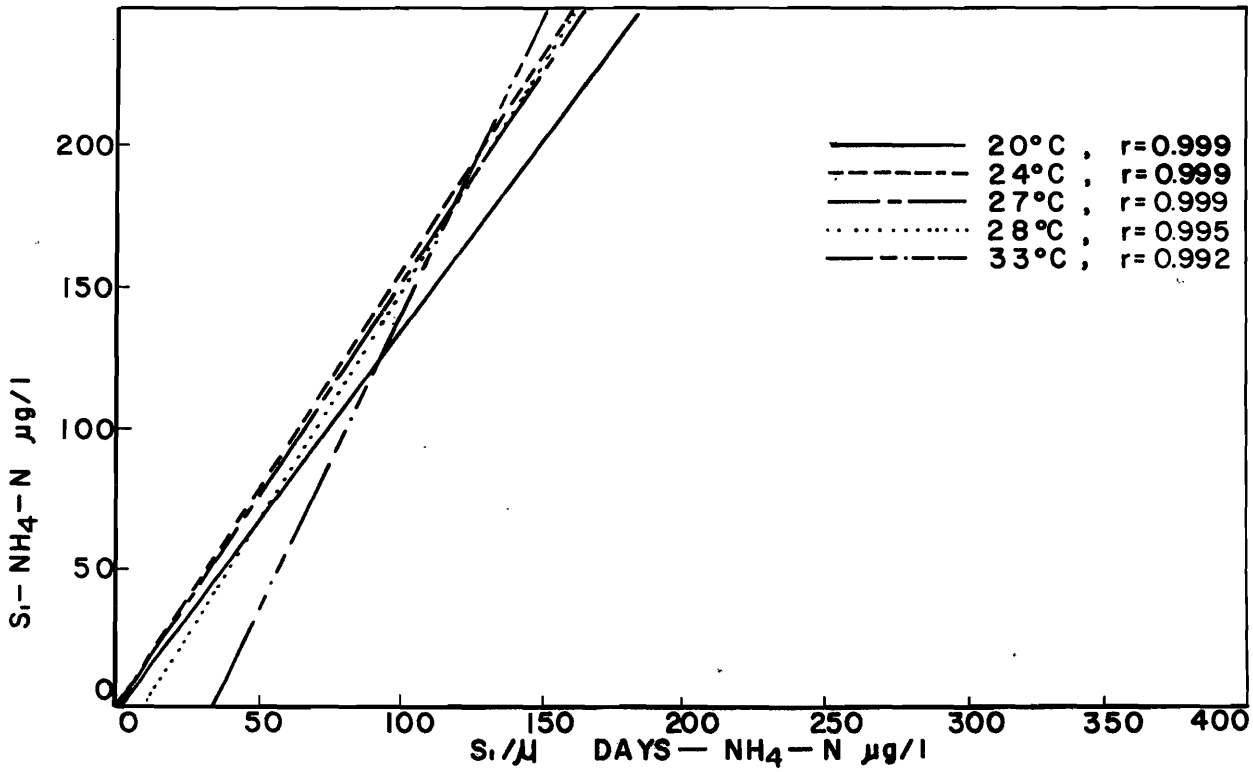


Figure 20. **Comparison of linear transformation of Michaelis-Menten (Monod) equation, $S_1 = (S_1/\mu)\hat{\mu} - K_s$.**

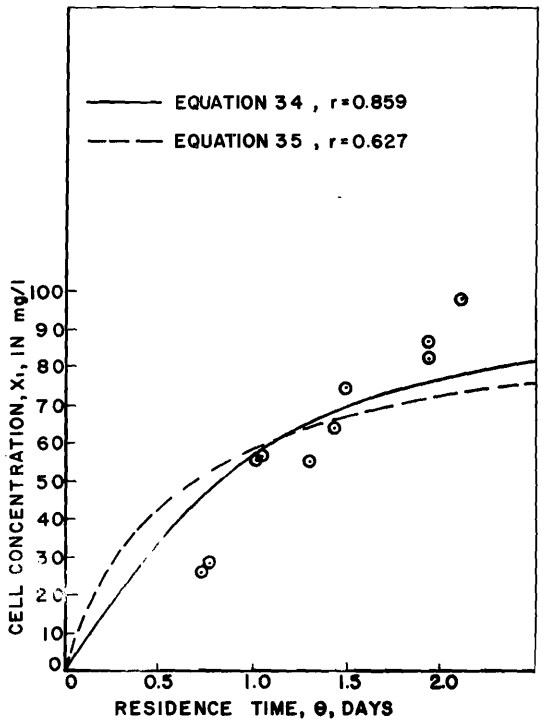


Figure 21. Steady state cell concentration, X_1, as a function of residence time, θ, with a varying yield coefficient at 20°C.

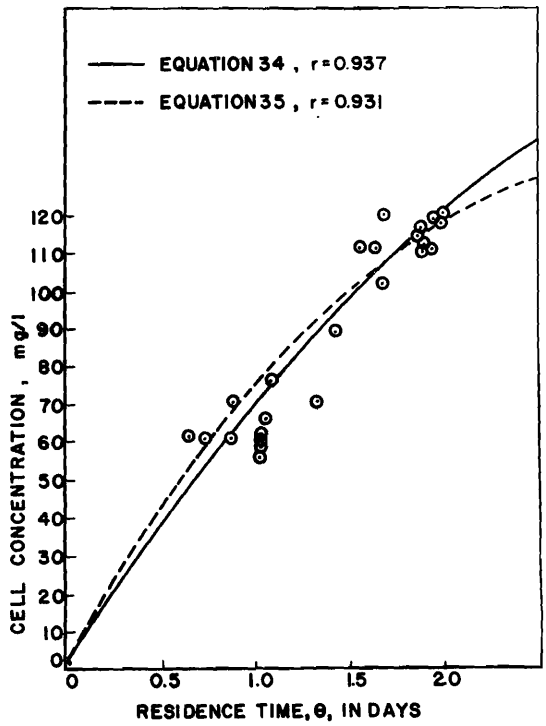


Figure 22. Steady state cell concentration, X_1, as a function of residence time, θ, with a varying yield coefficient at 24°C.

Table 18. Continuous flow steady state data with toxicant at 20°C.

Phenol Conc. mg/l	Residence Time								
	1.07 Days			1.40 Days			1.92 Days		
	X_1 Cells/$.01 mm^3$	X_1^a mg/l	$\frac{1}{X_1}$ 1/mg	X_1 Cells/$.01 mm^3$	X_1 mg/l	$\frac{1}{X_1}$ 1/mg	X_1 Cells/$.01 mm^3$	X_1 mg/l	$\frac{1}{X_1}$ 1/mg
0.0	112.33	44.70	.0224	253.33	67.41	.0148	447.11	98.63	.0101
20.0	95.67	42.01	.0238	96.00	42.06	.0238	187.00	56.73	.0176
30.0	44.67	33.80	.0296	64.33	36.96	.0271	53.33	35.19	.0284
40.0	24.67	30.57	.0327	23.33	30.36	.0329	27.00	30.95	.0323

$^aY = 0.1611 X_1 + 26.60$

Y = mg/l

X_1 = Cell/.01 mm^3

residence time have a greater effect on the phenol toxicity equation for *Selenastrum capricornutum* than do changes in temperature.

Phase IV: Oil refinery waste toxicity

General. Continuous flow culture experiments with the oil refinery waste were conducted at 24°C and 28°C. These experiments were designed to apply Equations 36 and 37 (competitive inhibition) and their associated constants, K_I and K_e, developed in the previous sections to an actual oil refinery waste. The experimental results are presented in Appendix E, Tables E-8, E-11, and E-12, and a summary of the regression analyses are presented in Table 26.

The amount of oil refinery waste added to the cultures was based on the phenol concentration of the waste. However, the oil refinery waste also had a high ammonium-nitrogen concentration. Thus, while the cultures were inhibited by the phenol in the waste, they were also "spiked" by the ammonium-nitrogen present in the waste.

Predicted cell mass. The predicted values for the steady state cell concentration, X_1 (mg/l), were calculated for the luxury uptake competitive inhibition equations (Equations 36 and 37) using the continuous flow kinetic constants ($\hat{\mu}$ and K_s) presented in Table 15 and the inhibition constants (K_I and K_e) presented in Table 23. These predicted steady state cell concentrations were then compared to the measured steady state cell concentrations. The measured steady state cell concentration appears to be substantially smaller than the predicted steady state cell concentration. The ratio of the measured steady state cell concentrations to the predicted steady state cell concentrations appears to be relatively constant and suggests that the constants employed to determine the predicted values need to be adjusted. The analysis indicated that the competitive inhibition constants, K_I, at 24°C for both Equations 36 and 37 needed to be multiplied by a factor of 0.086; that at 28°C, the

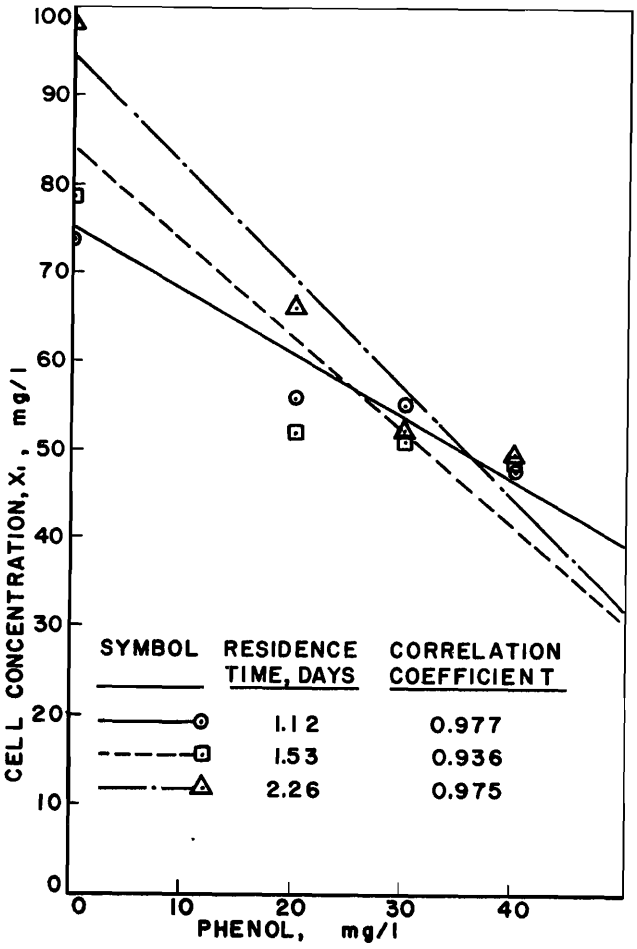


Figure 31. Nonlinear 'B Form' of competitive inhibition equation (X_1 vs. I) for continuous flow data at 28°C (Equation 37).

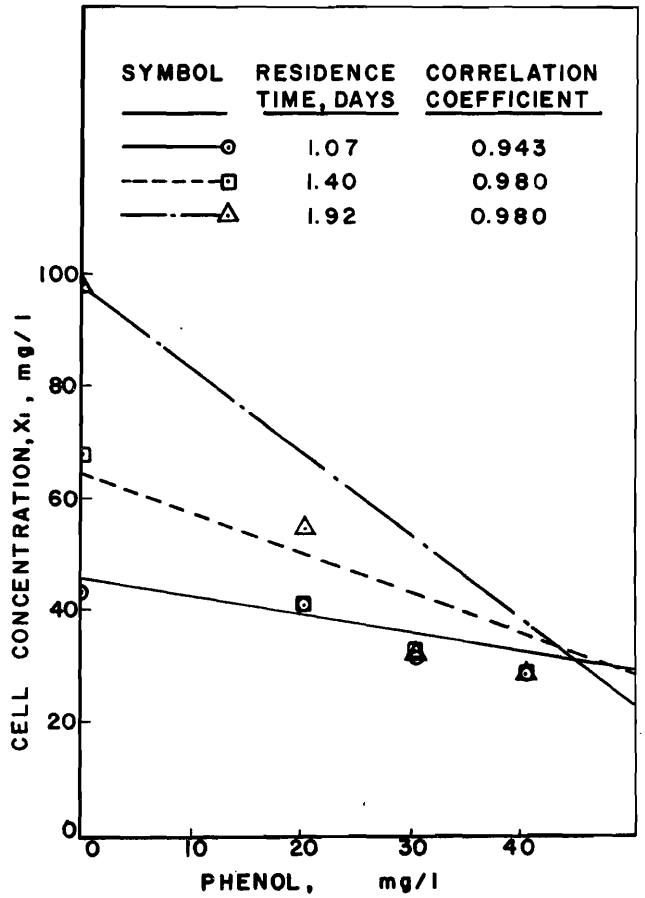


Figure 32. Linear form of competitive inhibition equation (X_1 vs. I) for continuous flow data at 20°C (Equation 17).

Table 24. Linear regression analysis of continuous flow data using the linear competitive inhibition equation (Equation 17).

Temp. °C	Residence Time (Days)	Slope with 95% Confidence Limits	Intercept with 95% Confidence Limits	Correlation[a] Coefficient
20	1.07	-0.3683 ±0.3969	46.058 ± 8.9296	.943
	1.40	-0.9296 ±0.5734	65.113 ± 12.9185	.980
	1.92	-1.7778 ±1.0874	95.372 ± 24.4699	.980
24	1.01	-0.3257 ±0.6097	63.292 ± 18.2903	.852
	1.68	-1.0587 ±1.2025	99.112 ± 36.0742	.937
	2.00	-1.2531 ±1.6851	103.270 ± 50.5516	.915
28	1.12	-0.6142 ±0.4096	72.189 ± 9.2157	.977
	1.53	-0.7635 ±0.8733	74.820 ± 19.6496	.936
	2.26	-1.2608 ±0.8618	94.785 ± 19.3897	.976

[a] 10 percent significant value of the correlation coefficient is .910.

Table 25. Comparison of continuous flow culture competitive inhibition constants, K_I, from linear regression (Equation 17) and nonlinear regression analysis (Equations 38 and 39).

Temp. °C	Equation 17 Linear Regression			Equation 38 Nonlinear: 'A Form'			Equation 39 Nonlinear: 'B Form'		
	θ, Days			θ, Days			θ, Days		
	1.0	1.5	2.0	1.0	1.5	2.0	1.0	1.5	2.0
20	.417	.096	.039	.207	.091	.049	.200	.094	.053
24	.243	.057	.038	.182	.047	.031	.161	.047	.032
28	.306	.127	.046	.279	.094	.043	.256	.094	.047

competitive inhibition constant for Equation 36 needed to be multiplied by a factor of 0.126; and that the competitive inhibition constant for Equation 37 needed to be multiplied by 0.106.

The predicted values for the steady state cell concentration were then recalculated using the adjusted inhibition constants. The results are tabulated in Table 26. Statistical comparison of the adjusted predicted steady state cell concentrations and the measured steady state cell concentrations resulted in a correlation coefficient of 0.72 at 24°C for both Equations 36 and 37, and in correlation coefficients of 0.97 and 0.98 for Equations 36 and 37, respectively, at 28°C. The 24°C correlation coefficients, though relatively high, is statistically non-significant at the 10 percent level, while both correlation coefficients at 28°C are statistically significant above the 10 percent level.

The lack of correlation at 24°C appears to be caused by the steady state cell concentration associated with the 40 mg/l phenol concentration. While the predicted and measured steady state cell concentration associated with the 20 mg/l phenol concentration agree very well with each other, the measured steady state cell concentration associated with the 40 mg/l phenol concentration is much higher than the predicted steady state cell concentration. The measured steady state cell concentrations at 40 mg/l phenol, is also inconsistent with the steady state cell concentration associated with the 60 mg/l phenol concentration. Thus, it is possible that the particular steady state cell concentration associated with the 40 mg/l phenol concentration is subject to excessive experimental error. The lack of significant statistical correlation at 24°C could also be due to the inherent difficulties associated with obtaining statistical significance with only three data points. In addition, the correlation could be biologically significant without being statistically significant.

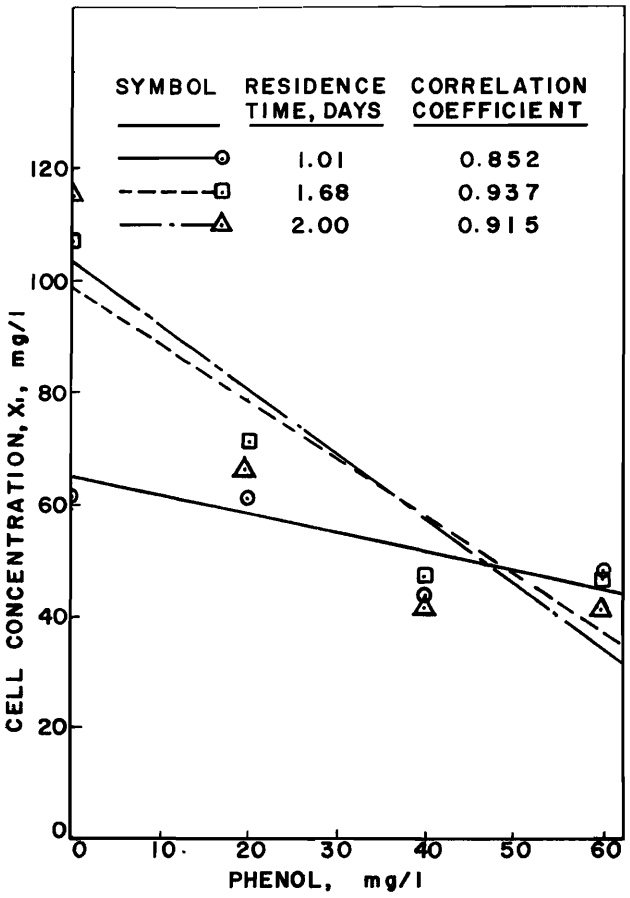


Figure 33. Linear form of competitive inhibition equation (X_1 vs. I) for continuous flow data at 24°C (Equation 17).

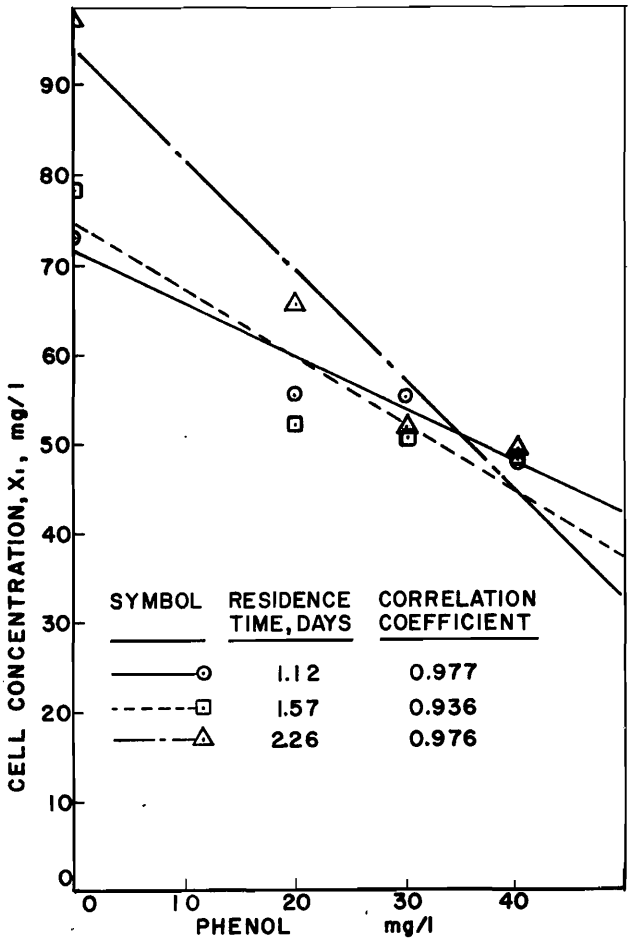


Figure 34. Linear form of competitive inhibition equation (X_1 vs. I) of continuous flow data at 28°C (Equation 17).

Table 26. Results of continuous flow experiment with oil-refinery waste.

Temp. °C	Oil Refinery Waste o mg/l	μ $days^{-1}$	S_o NH_4-N mg/l	S_1 NH_4-N $\mu g/l$	Measured X_1 Cells/0.1mm^3	Measured X_1 mg/l	Predicted X_1 mg/l		Measured X_1 / Predicted X_1		Adjusted K_I mg/l		Adjusted X_1 mg/l		Correlation[a] Coefficient for Adjusted Values	
							Eq. 36 'A Form'	Eq. 37 'B Form'	Eq. 36 'A Form'	Eq. 37 'B Form'	Eq. 36 'A Form'	Eq. 37 'B Form'	Eq. 36 'A Form'	Eq. 37 'B Form'	Eq. 36 'A Form'	Eq. 37 'B Form'
24	20	.501	14.83	23.84	298	69.11	403.81	383.48	.17	.18	.0027	.0028	68.58	77.52	.72	.72
	40	.466	29.66	28.76	1112	154.25	807.46	766.96	.19	.20			137.16	155.04		
	60	.488	44.48	1052.00	904	132.49	1211.19	1150.44	.11	.12			205.74	232.56		
28	20	.486	14.83	183.85	362	85.02	243.45	304.63	.35	.28	.0054	.0050	83.83	85.83	.97	.98
	30	.444	22.25	10.53	807	133.52	365.17	457.02	.36	.29			125.74	128.74		
	40	.443	44.48	17.34	1007	155.32	486.90	608.78	.32	.26			167.46	171.66		

[a] 10 percent significance level correlation coefficient = 0.95.

Effects of nitrogen "spiking." The oil refinery waste contained a high concentration of ammonium-nitrogen (see Table 6). Because the cultures were grown under ammonium-nitrogen limiting conditions, the addition of the oil refinery waste actually increased the steady state cell concentration, X_1, of the cultures. However, the increase was far below that predicted by the amount of ammonium-nitrogen added.

Because the cultures were "spiked" with ammonium-nitrogen, it is possible that they could have been limited by another nutrient instead of ammonium-nitrogen. Comparison of the nutrient concentrations in the influent substrate (see Tables 4 and 5) with the amount of cell mass produced in the chemostats indicates that oil refinery waste additions required to produce a phenol concentration greater than 20 mg/l added enough ammonium-nitrogen to cause the cultures to be carbon limited. However, if the amount of carbon present in the oil refinery waste were taken into account the cultures were still ammonium-nitrogen limited.

Oil refinery waste toxicity. In order for Equations 36 and 37 to adequately describe the oil refinery waste toxicity, it was necessary to divide the competitive inhibition constants, K_I, derived from the pure phenol experiments by an approximate factor of ten. This indicates that the oil refinery waste was approximately ten times more toxic to the *Selenastrum capricornutum* than was the pure phenol. The increased toxicity of the oil refinery waste could have been due to toxic synergistic effects generated between the phenol and other substances present in the waste. The analysis of the waste indicated that most of the toxic substances present in the waste were below their respective threshold toxicity values for *Scenedesmus sp.* However, *Selenastrum capricornutum* could be more sensitive to these substances than *Scenedesmus sp.*, or slight synergistic effects between various substances could be highly toxic to the *Selenastrum capricornutum.*

CHAPTER VI

EVALUATION

Phenol and Temperature Tolerance of Selenastrum Capricornutum

Temperature tolerance

In general, *Selenastrum capricornutum* is well suited for temperature related bioassays. The organism will grow in batch cultures at temperatures up to 38°C and can maintain itself quite well in continuous flow cultures at temperatures up to 35°C. The lowest temperature at which *Selenastrum capricornutum* will grow and reproduce was not determined in this study; however, the results of the study did indicate that the organism will grow at temperatures as low as 15°C. Thus, it appears as though *Selenastrum capricornutum* will grow and reproduce over a wide range of temperatures.

Phenol tolerance

The phenol tolerance of *Selenastrum capricornutum* appears to be less than the phenol tolerance reported for other algal species. Previous investigators (48, 50) have reported that phenol concentrations less than 40 mg/l have stimulated the growth of *Scenedesmus sp.* and *Chlorella sp.;* however, they have also indicated that phenol concentrations as low as 20 mg/l have inhibited the growth of *Selenastrum capricornutum.* The threshold toxicity value of phenol for *Scenedesmus sp.* has been reported to be 40 mg/l (60); however, 40 mg/l phenol in a continuous flow culture of *Selenastrum capricornutum* reduced the steady state cell concentration over 50 percent. Although threshold toxicity values are determined in batch cultures rather than in continuous flow cultures, the type of culture method employed should not produce such a dramatic difference in the results. Thus, *Selenastrum capricornutum* is apparently more sensitive to phenol toxicity than are many other algal species.

Luxury Uptake

Luxury uptake of ammonium-nitrogen by *Selenastrum capricornutum* has not been previously reported in the literature. However, the organism has been reported to exhibit luxury uptake of phosphorus and nitrate (72, 83).

The equations developed by Toerien et al. (83) to describe the luxury uptake of phosphorus by *Selenastrum capricornutum* were also used in the present study to describe the luxury uptake of ammonium-nitrogen by *Selenastrum capricornutum.* In the phosphorus luxury uptake study, Toerien et al. (83), concluded that the 'A Form' of the luxury uptake equations (Equations 27, 29, and 31) provided a better correlation for the experimental data than did the 'B Form' of the luxury uptake equations (Equations 28, 30, and 32). However, they indicated that the 'B Form' of the luxury uptake equations did provide a high degree of correlation for the experimental data. The same phenomenon is exhibited in this study. In general, the 'A Form' of the luxury uptake equations (Equations 27, 29, and 31) provides a better correlation for the experimental data at all temperatures studied.

The nutrient utilization constants, K_A and K_B, represent the inverse of the nutrient utilization rate constant (i.e. $K_A = 1/k_1$ and $K_B = 1/k_1$). As the value of the nutrient utilization constants increase, the value of the nutrient utilization rate constant decreases and a greater amount of 'removed' nutrient is accumulated or stored within the cell. When no luxury uptake occurs the nutrient utilization constants, K_A and K_B, are equal to zero and the net cell yield coefficient, Y_n, is equal to the maximum cell yield coefficient, Y_{max}.

The nutrient utilization constants, K_A and K_B, for the temperatures studied during this investigation are presented in Table 13. The values for the nutrient utilization constants, K_A and K_B, for phosphorus luxury uptake at 24°C as reported by Toerien et al. (83) were 4.78 days and 2.98 days respectively. These values agree very well with the nutrient utilization constants, K_A and K_B, at 24°C for luxury uptake of ammonium-nitrogen determined in the present study (K_A = 3.16 days, and K_B = 2.46 days).

Toerien et al. (83) did not conduct experiments to determine the variation of the phosphorus nutrient utilization constants, K_A and K_B, with temperature. However, the results of this study indicate that the ammonium-nitrogen nutrient utilization constants, K_A and K_B, vary with temperature in a manner similar to that of the maximum specific growth rate, $\hat{\mu}$. That is, the ammonium-nitrogen nutrient utilization constants, K_A and K_B, reach a maximum value between 24°C and 27°C and then decline in value as the temperature is either

increased or decreased beyond that maximum temperature value. This indicates that luxury uptake of ammonia-nitrogen by *Selenastrum capricornutum* reaches a maximum between 24°C and 27°C and then declines as the temperature is either increased or decreased beyond that point.

Kinetic Constants, μ and K_s

Half saturation constant, K_s

The half saturation constants, K_s, determined by the linear regression of the continuous flow culture data are shown in Table 15. These results indicate that the half saturation constant, K_s, increases directly with an increase in temperature. However, the linear regression analysis of the continuous flow culture data was influenced tremendously by one or two outlying points and is not considered reliable.

The half saturation constants, K_s, developed by the nonlinear regression analysis of the continuous flow culture data are also reported in Table 15. These results indicate that the half saturation constant for ammonium-nitrogen does not vary significantly with temperature. The half saturation constants range from 5.237 NH_4-N μg/l at 20°C to 5.356 NH_4-N μg/l at 33°C. The average half saturation constant, K_s, for all the temperatures studied was 5.356 NH_4-N μ g/l.

Shelef et al. (77) reported that the nitrate-nitrogen half saturation constant for *Chlorella pyrenoidosa* increased slightly between 19°C and 28.5°C; however the value of the nitrate-nitrogen half saturation constant, K_s, more than doubled at temperatures between 28.5°C and 39.2°C.

In the present study, the ammonium-nitrogen half saturation constant remained constant from 20°C to 27°C and then only increased 0.119 NH_4-N μg/l between 27°C and 33°C. This slight increase in the value of the ammonium-nitrogen half saturation constant may be an artifact of the regression analysis employed to determine the numerical values, rather than to a biochemical rate change in the alga.

The average nonlinear ammonium-nitrogen half saturation constant value of 5.356 NH_4-N μg/l for *Selenastrum capricornutum* compares favorably with ammonia-nitrogen half saturation constants reported for other species. Eppley and Thomas (27) have reported the ammonium-nitrogen half saturation constants for various marine diatoms grown at 26°C to vary from 1.40 NH_4-N μ g/l to 21.0 NH_4-N μg/l. Also, the green alga *Dunaliella tertiolecta* has been reported to have an ammonium-nitrogen half saturation constant of 1.40 NH_4-N μg/l (28). However, Caperon and Meyer (15) reported the ammonia-nitrogen half saturation constant, K_s, for *Dunaliella tertiolecta* to be 2.38 NH_3-N μg/l. In addition, the ammonia-nitrogen half saturation constant for a natural population of marine phytoplankton has been reported to range from 1.40 NH_3-N μg/l to 18.2 NH_3-N μ g/l (53).

Maximum specific growth rate, $\hat{\mu}$

The values of the maximum specific growth rates, $\hat{\mu}$, determined by linear regression analysis of the continuous flow culture data do show a great deal of variation with changes in temperature and, as discussed previously, are not considered reliable.

The maximum specific growth rates, $\hat{\mu}$, determined by nonlinear regression analysis of the continuous flow culture data, as reported in Table 15, indicate a definite variation with temperature. Essentially, the maximum specific growth rate increased from 1.365 days^{-1} at 20°C to a maximum value of 1.992 days^{-1} at 24°C and then decreased to 1.274 days^{-1} at 33°C. This variation was somewhat different than that reported by Shelef et al. (77) for *Chlorella pyrenoidosa* (high temperature strain). They reported a continual increase in the maximum specific growth rate at temperatures between 19°C and 39.2°C. The difference in the response of maximum specific growth rate in the present study and that reported by Shelef et al. (77) could be due to the nature of the different algal species studied. Goldman (39) developed a continually increasing function to express the variation of the maximum specific growth rate with temperature for *Selenastrum capricornutum.* However, his equation is only valid for a limited temperature range, which he does not define.

The maximum specific growth rate developed in the present study at 24°C is 1.992 days^{-1}. This value compares favorably to the value of 1.85 days^{-1} developed by Toerien et al. (83) at 24°C. However, the maximum specific growth rate of 1.412 days^{-1} at 27°C developed in the present study is substantially less than the value of 2.45 days^{-1} at 27°C reported by Goldman (39). The discrepancy in the 27°C values could be due to experimental error in both cases. For instance, the value in the present study was developed using nonlinear regression techniques and is therefore greatly influenced by the scatter of the data. Goldman's value (39) was determined experimentally and is therefore greatly affected by the ability of the culture to respond quickly and consistently to environmental changes when the culture is near $\hat{\mu}$. Observations made during this present study indicate that the maximum value of $\hat{\mu}$ occurs near 27°C and that *Selenastrum capricornutum* is very sensitive to environmental changes at that temperature. On two separate occasions, steady state cultures growing at 27°C were severely upset by temperature variations of less than 0.5°C.

Inhibition

Phenol inhibition

A comparison of the inhibition constants, K_I, developed from the semi-continuous and continuous flow cultures is presented in Table 27. Both the semi-continuous and continuous flow culture data indicate that phenol is more toxic to *Selenastrum capricornutum* at 24°C than it is at any of the other temperatures studied. Apparently, the toxic effect of phenol is closely associated with the maximum specific growth rate of the organism. Thus, the maximum toxicity of phenol appears to occur at the temperature corresponding to the optimum temperature for growth of the organism.

In addition, the inhibition constants, K_I, for the semi-continuous flow cultures are significantly greater than the corresponding inhibition constant determined from the continuous flow culture data. This indicates that *Selenastrum capricornutum* may be able to develop a certain amount of tolerance to phenol if the phenol concentration does not cause immediate acute toxicity of the organism. Thus, cultures of *Selenastrum capricornutum* exposed to large "shock" doses of phenol may recover over a period of time.

Competitive inhibition

The analysis of the experimental data indicated that both phenol and the oil refinery waste exhibit competitive inhibition of *Selenastrum capricornutum.* Competitive inhibition occurs when the inhibitor behaves as a pseudo-substrate or substrate analog and attaches itself to the active site of the enzyme. This type of inhibition has been previously reported for phenol inhibition of pure enzyme systems (47, 59, 80, 88).

The fact that phenol acts through a competitive inhibition mechanism in *Selenastrum capricornutum* suggests that phenol inhibition of the organism is specific for a particular enzyme or that phenol inhibits several enzymes by the same biochemical mechanism. If phenol is inhibiting several enzymes by a similar mechanism, the competitive inhibition constants developed in this study represent the slowest or the rate controlling enzymatic step of the biochemical synthetic process in *Selenastrum capricornutum.* However, the results of this study do not indicate which enzymes are involved in the phenol inhibition process. Additional research would be required to determine the exact biochemical pathways associated with the phenol inhibition process.

The results of this study do clearly indicate that phenol inhibition of *Selenastrum capricornutum* can be described by the luxury uptake competitive inhibition equations (Equations 36 and 37) and that these same equations can be used to describe the inhibition of *Selenastrum capricornutum* by an actual oil refinery waste. This study also indicates that both phenol toxicity and oil refinery waste toxicity are greatly influenced by changes in temperature.

Table 27. Summary of competitive inhibition constants, K_I, from semi-continuous and continuous cultures.

Temp. °C	Residence Time, θ (Days)	Continuous Culture[a] K_I in mg/l (Phenol)		Semi-Continuous Culture K_I in mg/l (Phenol)	Oil Refinery Waste K_I in mg/l Phenol	
		Eq. 36 'A Form'	Eq. 37 'B Form'		Eq. 36 'A Form'	Eq. 37 'B Form'
20	1.0	.207	.200			
	1.5	.091	.094			
	2.0	.049	.053			
	3.0			.113		
24	1.0	.182	.161			
	1.5	.047	.047			
	2.0	.031	.032		.0027	.0028
	3.0			.075		
28	1.0	.279	.256			
	1.5	.094	.094			
	2.0	.043	.047		.0054	.0058
	3.0			.129		

[a]Determined by nonlinear regression.

CHAPTER VII

SUMMARY

A continuous flow kinetic model to describe and predict the effects of temperature on the toxicity of a specific oil refinery waste to the alga *Selenastrum capricornutum* was developed. The model was based on enzyme inhibition kinetics and was developed using semi-continuous and continuous flow algal cultures grown at temperatures between 20°C (68°F) and 33°C (91°F). Phenol was employed as the controlling toxicant. Continuous flow algal cultures exposed to an actual oil refinery waste were used to apply the model to actual conditions.

The luxury uptake competitive inhibition equation, Equation 36, described the toxicity of phenol and oil refinery waste to the alga *Selenastrum capricornutum.* However, Equation 37 also described the phenomenon very well. Competitive inhibition constants, K_I, and the bacteria utilization constants, K_e, associated with Equation 36 are summarized in Table 28.

The toxicity of the phenol and the oil refinery waste appears to be greater at 24°C than at any other temperature studied. Apparently, the effect of temperature on the toxicity of phenol and oil refinery waste to *Selenastrum capricornutum* is similar to the effect of temperature on the maximum specific growth rate, $\hat{\mu}$, also toxicity increased as the time of exposure to the toxicant was increased.

The maximum specific growth rates and the ammonium-nitrogen half saturation constants, K_s, for temperatures between 20°C (68°F) and 33°C (91°F) were developed for the alga *Selenastrum capricornutum.* The most probable values for these kinetic constants are presented in Table 29. The luxury uptake function developed for *Selenastrum capricornutum* by Toerien et al. (83) was verified for the luxury uptake of ammonium-nitrogen. In addition the most probable nutrient utilization constants, K_A, was developed and these values are presented in Table 29.

Table 28. Summary of the competitive inhibition constants, K_I, and bacteria utilization constant, K_e, associated with phenol and oil refinery waste toxicity of *Selenastrum capricornutum* in Equation 36.

Temp. °C	Residence Time, θ, in days	Phenol Toxicity		Oil Refinery Waste Toxicity	
		K_I mg/l	K_e	K_I mg/l	K_e
20	1.0	0.207	.001		
	1.5	0.091	.001		
	2.0	0.049	.001	0.027	0.001
24	1.0	0.182	.001		
	1.5	0.047	.001		
	2.0	0.031	.001		
28	1.0	0.279	.017		
	1.5	0.094	.001		
	2.0	0.043	.001	0.054	0.001

Table 29. Most probable maximum specific growth rate, μ, ammonium-nitrogen half saturation constants, K_s, and the nutrient utilization constants, K_A, for *Selenastrum capricornutum.*

Temp. °C	$\hat{\mu}$ days^{-1}	K_s µg/l	K_A[a] days
20	1.365	5.237	1.05
24	1.992	5.237	3.16
27	1.412	5.237	1.30
28	1.390	5.564	1.36
33	1.274	5.503	0.84

[a]Values for 'A Form,' Equation 27.

CHAPTER VIII

CONCLUSIONS

From the results of this study the following conclusions can be made:

1. Glycylglycine does not maintain a constant pH (pH 7.1 to 7.2) in a semi-continuous flow culture (three day residence time) of *Selenastrum capricornutum* and is not suitable for use in bioassays.
2. *Selenastrum capricornutum* in bacterized cultures were able to obtain nitrogen either directly or indirectly from glycylglycine.
3. A 0.03 molar phosphate buffer system provides excellent pH control (pH 7.1 to 7.2) in continuous flow bioassays using *Selenastrum capricornutum.*
4. Phosphate concentrations greater than 0.05 molar inhibit the growth of *Selenastrum capricornutum.*
5. *Selenastrum capricornutum* will grow in batch and semi-continuous flow cultures at 15°C and probably at temperatures below 15°C.
6. Batch cultures of *Selenastrum capricornutum* will grow at 37°C, but will not grow at 38°C.
7. *Selenastrum capricornutum* grown between 20°C and 33°C exhibited luxury uptake of ammonium-nitrogen.
8. The equations developed by Toerien et al. (83) to describe the luxury uptake of phosphorus by *Selenastrum capricornutum* also describe the luxury uptake of ammonium-nitrogen by *Selenastrum capricornutum.*
9. Although both Equations 27 and 28, developed by Toerien et al. (83), describe the variation in cell yield of *Selenastrum capricornutum* under luxury uptake conditions, Equation 27, shown below, provides the best "fit" for the data.

$$Y_n = Y_{max}\,(1 - e^{-\theta/K_A}) \quad \ldots\ldots (27)$$

10. The maximum cell yield coefficient, Y_{max}, for *Selenastrum capricornutum* has a maximum value of 61.20 mg cell/mg NH_4-N at 24°C and decreases in value as the temperature decreases to 20°C or increases to 33°C.
11. The nutrient utilization constants, K_A and K_B, for Equations 27 and 28, have a maximum value of 3.16 days and 2.46 days, respectively, at 24°C and decrease in value as the temperature increases to 33°C or decreases to 20°C. This indicates that luxury uptake of ammonium-nitrogen reaches a maximum between 24°C and 27°C and then declines as the temperature is increased or decreased.
12. The nonlinear relationship between the nutrient removal velocity, q, and the specific growth rate, μ, for *Selenastrum capricornutum* grown between 20°C and 33°C is best described by Equation 32.

$$q = \frac{\mu}{Y_{max}\left(\frac{\theta}{\theta + K_B}\right)(1 + k_d\theta)} \quad \ldots (32)$$

13. The nonlinear relationship between the fraction of excess nutrient, F_e, and the hydraulic residence time, θ, in continuous flow cultures of *Selenastrum capricornutum* grown between 20°C and 33°C is best described by Equation 30.

$$F_e = \left(1 - \frac{\theta}{\theta + K_B}\right) \quad \ldots\ldots\ldots (30)$$

14. The ammonium-nitrogen half saturation constant, K_s, for *Selenastrum capricornutum* does not vary with temperature between 20°C and 33°C.
15. The average ammonium-nitrogen half saturation constant, K_s, for *Selenastrum capricornutum* between 20°C and 33°C is 5.356 μg/l NH_4-N.
16. The maximum specific growth rate, $\hat{\mu}$, of *Selenastrum capricornutum* has a maximum value between 24°C and 27°C.
17. The maximum specific growth rates, $\hat{\mu}$, of *Selenastrum capricornutum* grown at 20°C, 24°C, 27°C, 28°C, and 33°C are respectively 1.365 $days^{-1}$, 1.992 $days^{-1}$, 1.412 $days^{-1}$, 1.390 $days^{-1}$, and 1.274 $days^{-1}$.

18. Bacterized semi-continuous flow cultures (three day residence time) of *Selenastrum capricornutum* grown between 20°C and 28°C can withstand phenol concentrations greater than 240 mg/l, however a practical phenol concentration limit is 120 mg/l.
19. In continuous flow cultures (one day residence time) of *Selenastrum capricornutum* grown between 20°C and 28°C, a phenol concentration of 40 mg/l reduced the steady state cell concentration, X_1, by over 50 percent.
20. Phenol exerts competitive inhibition of *Selenastrum capricornutum* in both semi-continuous and continuous flow cultures grown betteen 20°C and 28°C.
21. The competitive inhibition of *Selenastrum capricornutum* by phenol can be best described by the linear form of the competitive inhibition equation, Equation 17.

$$X_1 = \frac{YS_o}{1+\theta k_d} - \frac{\left(\frac{1}{\theta}\right) YK_s}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)} - \frac{\left(\frac{K_s Y}{K_I \theta}\right) I}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)} \quad \ldots (17)$$

22. In continuous flow luxury uptake cultures of *Selenastrum capricornutum* grown between 20°C and 28°C, the competitive inhibition exerted by phenol is described equally well by either of the nonlinear luxury uptake competitive inhibition equations, Equations 36 or 37.

$$X_1 = \frac{Y_{max}(1 - e^{-\theta/K_A})}{\mu\theta}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)}\right] \quad \ldots (36)$$

$$X_1 = \frac{Y_{max}\theta}{\mu\theta(\theta + K_B)}\left[S_o - \frac{K_s\left(\frac{1}{\theta} + k_d\right)\left(1 + \frac{I_o - K_e B - I_1}{K_I}\right)}{\hat{\mu} - \left(\frac{1}{\theta} + k_d\right)}\right] \quad \ldots (37)$$

23. The bacteria utilization constants, K_e, developed from Equations 36 and 37 are very small (less than 0.017) and indicate that bacteria present during the experiment had an insignificant effect upon the algal cultures.
24. The competitive inhibition constants, K_I, for both semi-continuous flow and continuous flow cultures of *Selenastrum capricornutum* are less at 24°C than at either 20°C or 28°C.
25. Phenol is more toxic to *Selenastrum capricornutum* at 24°C than at either 20°C or 28°C.
26. The longer *Selenastrum capricornutum* is exposed to phenol, the more toxic the substance is to the organism.
27. In continuous flow cultures of *Selenastrum capricornutum*, changes in hydraulic residence time, θ, have a greater effect on the toxicity of phenol to the organism than do changes in temperature.
28. The toxicity of oil refinery waste to *Selenastrum capricornutum* can be described by Equations 17, 36, and 37.
29. Oil refinery waste exerts competitive inhibition of *Selenastrum capricornutum.*
30. Oil refinery waste is approximately ten times more toxic than pure phenol to *Selenastrum capricornutum.*
31. Oil refinery waste is more toxic to *Selenastrum capricornutum* grown at 24°C than at 28°C.

CHAPTER IX

RECOMMENDATIONS

To make the results of this study more useful it is recommended that the following be completed:

1. Further studies be conducted to determine the minimum and maximum temperatures for *Selenastrum capricornutum* at which growth will occur.
2. Studies be conducted to determine the physiological condition of *Selenastrum capricornutum* under various temperatures and limiting nutrient conditions.
3. The maximum specific growth rates, $\hat{\mu}$, and the half saturation constants, K_s, be determined for *Selenastrum capricornutum* for various limiting nutrients at 2°C intervals over the growth temperature range for the organism.
4. The inhibition constants, K_I, for the toxicity of several different oil refinery wastes be determined and compared.
5. The inhibition constants, K_I, for a wide range of wastes and temperatures be determined and compared to aid in defining the effects of temperature on toxicity.
6. Studies similar to this present experiment be conducted with a standard test fish.

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APPENDIXES

Appendix A

Symbols and Notation

Symbols–Notation

The following symbols are used in this text.

A	=	nutrient content of individual cell at hydraulic residence time, θ , S_o-S_1/X_1, mass nutrient/ mass cell
A_o	=	nutrient content of individual cell at infinite hydraulic residence time, S_o-S_1/X_1, mass nutrient/mass cell
B	=	steady state bacteria mass in the chemostat, mass/volume
CC	=	cell counts x 10^6 per ml
^oC	=	degrees Centigrade
E	=	free enzyme mass/volume
EI	=	enzyme-inhibitor complex, mass/volume
ES	=	bound enzyme or enzyme-substrate complex
E*	=	energy of activation, kcal
F	=	flow rate, volume/time
F_e	=	fraction of excess nutrient uptake, dimensionless
^oF	=	degrees Fahrenheit
I	=	inhibitor concentration, mass/volume
I_e	=	effective inhibitor concentration, mass/ volume
I_o	=	influent inhibitor concentration, mass/volume
I_1	=	steady state inhibitor concentration, mass/ volume
IES	=	inhibitor-enzyme-substrate complex
k_d	=	specific cellular decay rate, $time^{-1}$
k_1	=	nutrient utilization rate constant, $time^{-1}$
K_A	=	nutrient utilization constant for the 'A Form' of the luxury uptake function, days
K_B	=	nutrient utilization constant for the 'B Form' of the luxury uptake function, days
K_e	=	bacteria utilization constant, dimensionless
K_I	=	inhibitor constant, mass/volume
K_S	=	half saturation constant, mass/volume
P	=	product, mass/volume
q	=	nutrient removal velocity, $time^{-1}$
S	=	substrate, mass/volume
S_e	=	nutrient concentration available for cell growth including excess nutrient uptake and storage, mass/volume
S_o	=	initial substrate or influent substrate concentration
S_q	=	nutrient concentration utilization for growth by cell, mass/volume
S_1	=	steady state substrate concentration, mass/ volume
SS	=	cell mass, mass/volume
$S_{external}$	=	external nutrient concentration, mass/volume
$S_{internal}$	=	internal nutrient concentration available for cell growth utilization, mass/volume
$S_{cell\ growth}$	=	nutrient concentration being utilized for cell growth or biomass production, mass/volume
T	=	temperature
V	=	volume
X_1	=	steady state cell concentration, mass/volume
X_{max}	=	maximum cell concentration at infinite hydraulic residence time, mass/volume
Y	=	cell yield, mass of organisms formed per mass of substrate removed, mass cell/mass nutrient
Y_n	=	net cell yield coefficient, mass cell/mass nutrient Y
Y_{max}	=	maximum cell yield coefficient = X_{max}/S_o-S_1, mass cell/mass nutrient removed
μ	=	specific growth rate, $time^{-1}$
$\hat{\mu}$	=	maximum specific growth rate, $time^{-1}$
θ	=	mean residence time, V/F, days
%	=	percent

Appendix B

Preliminary Studies

Table B-1. Summary of results for semi-continuous flow cultures with glycylglycine buffer and a limiting nitrogen concentration of 1.05 mg/l.

Number of Days	Glycylglycine Concentration in mg/l																		Temp. °C
	250						500						1000						
	O.D.[a] 750 μm			pH			O.D. 750 μm			pH			O.D. 750 μm			pH			
	Cont.[b]	A[c]	B	Cont.	A	B	Cont.	A	B	Cont.	A	B	Cont.	A	B	Cont.	A	B	
1	.0	.015	.020	8.1	8.4	8.4	.0	.01	.015	8.1	8.2	8.2	.0	.01	.018	7.9	8.0	8.0	28
2	.0	.070	.080	8.1	8.9	9.1	.0	.069	.080	8.2	8.6	8.7	.0	.069	.072	8.0	8.2	8.2	27
3	.0	.09	.09	8.2	8.8	8.9	.0	.09	.10	8.1	8.5	8.5	.0	.09	.10	8.0	8.1	8.1	27
4	.0	.12	.14	8.2	8.9	9.05	.0	.12	.14	8.0	8.5	8.6	.0	.09	.14	7.8	8.05	8.01	25
5	.0	.15	.14	7.9	8.9	8.8	.0	.2	.19	7.9	8.4	8.5	.0	.23	.19	7.8	8.0	8.0	25
6	.011	.16	.17	8.2	9.2	9.3	.005	.21	.20	7.9	8.8	8.8	.0	.25	.22	7.8	8.1	8.2	25
7	.01	.16	.19	8.0	9.15	9.3	.005	.21	.23	7.8	8.7	8.8	.0	.26	.24	7.65	8.1	8.1	24
8	.03	.16	.20	8.05	9.25	9.4	.02	.189	.23	7.75	8.7	9.95	.0	.27	.26	7.5	8.1	8.1	24
9	.07	.18	.23	8.3	9.45	9.7	.04	.25	.23	7.7	8.8	9.15	.1	.31	.29	7.7	8.4	8.4	22
10	.09	.190	.22	8.45	9.45	9.7	.08	.20	.22	8.15	8.9	9.15	.1	.30	.26	7.95	8.55	8.50	22
11	.10	.21	.26	8.55	9.6	9.85	.16	.20	.22	8.30	8.95	9.25	.08	.38	.30	7.95	8.55	8.49	24
12	.10	.22	.24	8.15	9.5	9.60	.17	.19	.21	8.20	8.75	8.95	.13	.31	.29	7.80	8.4	8.35	24

[a] Optical density.

[b] Control: No algal inoculum.

[c] Culture identification.

Table B-2. Summary of results for semi-continuous flow cultures with phosphate buffer with a limiting nitrogen concentration of 1.05 mg/l.

Number of Days	Phosphate Conc. = 0.02 molar						Phosphate Conc. = 0.03 molar						Temp. °C
	O.D.[a] 750 μm			pH			O.D. 750 μm			pH			
	Cont.[b]	A[c]	B	Cont.	A	B	Cont.	A	B	Cont.	A	B	
1	.00	.04	.04	7.4	7.6	7.6	.00	.05	.05	7.4	7.45	7.45	24
2	.00	.070	.075	7.80	7.85	7.95	.00	.055	.073	7.5	7.60	7.60	26
3	.00	.070	.075	7.80	8.30	8.32	.00	.083	.09	7.5	7.60	7.60	26
4	.00	.080	.080	7.90	8.30	8.32	.00	.085	.09	7.5	7.62	7.60	26
5	.00	.080	.085	8.00	8.30	8.30	.00	.095	.095	7.65	7.75	7.75	26
6	.00	.081	.079	7.75	7.95	7.95	.00	.085	.080	7.48	7.55	7.55	25
7	.00	.08	.08	7.60	7.90	7.93	.00	.085	.09	7.50	7.58	7.55	26
8	.00	.09	.09	7.60	7.80	7.80	.00	.095	.10	7.50	7.60	7.63	27
9	.00	.089	.084	7.60	7.81	7.83	.00	.093	.10	7.50	7.60	7.60	27
10	.00	.090	.090	7.39	7.30	7.30	.00	.100	.100	7.28	7.30	7.32	26
11	.00	.085	.090	7.26	7.33	7.36	.01	.09	.098	7.13	7.19	7.20	26
12	.00	.070	.090	7.35	7.27	7.36	.02	.07	.082	7.13	7.13	7.13	25
13	.00	.090	.090	7.17	7.23	7.24	.055	.09	.10	7.05	7.05	7.05	26
14	.00	.15	.13	7.18	7.23	7.25	.08	.10	.10	7.05	7.06	7.05	26.5
15	.00	.10	.10	7.20	7.30	7.32	.08	.095	.09	7.05	7.05	7.05	27
16	.02	.95	.95	7.26	7.30	7.31	.09	.093	.093	7.07	7.07	7.07	26
17	.06	.10	.10	7.25	7.25	7.20	.085	.090	.080	7.00	7.00	7.00	26
18	.09	.09	.10	7.35	7.35	7.35	.09	.090	.090	7.05	7.05	7.15	27
19	.095	.11	.11	7.50	7.45	7.45	.095	.100	.100	7.15	7.25	7.25	27
20	.1	.11	.11	7.40	7.35	7.40	.10	.105	.110	7.05	7.05	7.05	25.5
21	.105	.065	.12	7.55	7.55	7.60	.11	.11	.114	7.20	7.20	7.20	25.5
22	.105	.135	.12	7.45	7.45	7.50	.11	.115	.114	7.15	7.15	7.15	25.5
23	.10	.135	.12	7.40	7.40	7.45	.11	.115	.120	7.10	7.10	7.10	26.0
24	.105	.13	.12	7.45	7.45	7.47	.112	.110	.115	7.15	7.10	7.15	26
25	.105	.13	.12	7.45	7.45	7.50	.113	.115	.115	7.115	7.10	7.15	26

[a] Optical density.

[b] Control: No algal inoculum.

[c] Culture identification.

Table B-3. Summary of results for semi-continuous flow cultures with phosphate buffer with a limiting nitrogen concentration of 1.05 mg/l.

Number of Days	Phosphate Conc. = 0.06 molar						Control Without Buffer						Temp. °C
	O. D.[a] 750 μm			pH			O. D. 750 μm			pH			
	Cont.[b]	A[c]	B	Cont.	A	B	Cont.	A	B	Cont.	A	B	
1	.00	.035	.035	7.3	7.32	7.33	.00	.035	.04	8.9	8.75	8.85	24
2	.00	.045	.051	7.35	7.45	7.45	.00	.055	.055	8.8	9.2	9.25	26
3	.00	.060	.060	7.38	7.41	7.41	.00	.07	.070	8.6	9.1	9.20	26
4	.00	.065	.060	7.40	7.43	7.45	.00	.075	.076	8.9	9.3	9.30	26
5	.00	.07	.07	7.40	7.45	7.45	.00	.085	.085	8.7	9.15	9.20	26
6	.00	.065	.070	7.43	7.45	7.45	.00	.075	.073	8.7	9.15	9.25	25
7	.00	.07	.07	7.40	7.45	7.45	.00	.068	.073	8.5	9.05	9.15	26
8	.00	.083	.085	7.45	7.49	7.49	.005	.09	.09	8.5	9.05	9.17	27
9	.00	.080	.085	7.55	7.55	7.60	.002	.085	.089	8.0	9.20	9.15	27
10	.00	.090	.095	7.55	7.50	7.65	.045	.092	.092	9.05	9.20	9.20	26
11	.00	.090	.092	7.19	7.2	7.21	.060	.080	.085	8.8	8.75	8.88	26
12	.005	.069	.079	7.17	7.18	7.18	.045	.065	.07	8.55	8.65	8.75	25
13	.005	.080	.080	7.10	7.15	7.15	.075	.090	.090	8.64	8.80	8.85	26
14	.00	.080	.080	7.10	7.13	7.13	.090	.10	.10	8.52	8.90	9.05	26
15	.005	.074	.075	7.15	7.15	7.15	.080	.10	.10	8.80	9.00	9.15	26.5
16	.000	.065	.070	7.18	7.18	7.18	.085	.10	.09	8.60	9.15	9.30	27
17	.000	.045	.055	7.10	7.10	7.10	.085	.09	.098	8.70	9.05	9.15	27
18	.00	.056	.050	7.15	7.15	7.15	.09	.095	.10	8.75	9.05	9.15	26
19	.01	.045	.050	7.20	7.15	7.20	.10	.10	.11	8.75	9.00	9.15	26
20	.00	.045	.055	7.10	7.05	7.05	.09	.105	.11	8.65	9.00	9.10	27
21	.005	.055	.055	7.10	7.10	7.05	.095	.11	.115	9.2	9.45	9.40	27
22	.00	.055	.050	7.10	7.10	7.10	.095	.11	.115	8.9	9.25	9.30	25.5
23	.00	.040	.045	7.10	7.10	7.10	.10	.113	.12	9.00	9.30	9.35	25.5
24	.00	.045	.045	7.15	7.10	7.10	.098	.11	.12	8.95	9.30	9.40	25.5
25	.00	.045	.040	7.15	7.10	7.18	.10	.11	.12	8.05	9.30	9.40	26

[a] Optical density.

[b] Control: No algal inoculum.

[c] Culture identification.

Table B-4. Results of preliminary experiment to determine the range of phenol concentration for study.

Phenol Concentration in mg/l	Cell Concentration in Cells/0.1mm^3[a]			
	20°C	24°C	28°C	33°C
0	277	340	272	112
0	272	360	300	missing
100	33	43	48	6
100	36	50	65	6
120	17	36	33	4
120	17	29	50	7
140	10	19	25	5
140	10	27	15	7
160	9	14	7	5
160	8	7	8	4
180	6	9	6	2
180	5	contam.	7	contam.
200	4	5	5	contam.
200	5	3	5	contam.
220	5	6	contam.	contam.
220	6	9	4	4
240	5	4	5	2
240	6	7	2	3

[a]Average of 3 samples.

Appendix C

Luxury Uptake Data

Table C-1. Maximum growth yield coefficient data for chemostats numbers 19 and 20 at 20°C with 4.2 mg/l nitrogen.

Chemostat No.	Days of Operation	Temp °C	pH	OD 750 μm	X_1 cell/$_3$ .1mm^3	X_1 mg/l
	8	19.0	7.15	.175	454	90.0
	9	19.5	7.18	.175	457	89.0
19	10	19.5	7.20	.177	457	89.0
	11	19.5	7.18	.182	474	87.5
Ave		19.375	7.177	.177	460.500	88.875
SD		.216	.017	.002	7.889	.892
Var		.046	0.000	.000	62.250	.796
	8	18.5	7.15	.180	406	100.00
	9	18.0	7.19	.180	406	90.00
20	10	18.5	7.21	.173	416	94.00
	11	18.5	7.28	.182	409	100.00
Ave		18.375	7.207	.178	409.250	96.000
SD		.216	.047	.003	4.085	4.242
Var		.046	.002	.000	16.687	18.000
Overall						
Ave		18.875				92.437
SD		.544				4.699
Var		.296				22.089

Table C-2. Maximum growth yield coefficient data for chemostats numbers 8, 12, and 16 at 24°C with 4.2 mg/l nitrogen.

Chemostat No.	Days of Operation	Temp °C	pH	OD 750 μm	X_1 cell/ .1 mm^3	X_1 mg/l
	4	25.0	7.22	.260	1,368	253.33
8	5	24.5	7.28	.256	968	280.00
	6	24.5	7.25	.256	1,220	260.00
Ave		24.666	7.250	.257	1,185.333	264.443
SD		.235	.024	.001	165.128	11.332
Var		.055	.000	.000	27,267.555	128.419
	4	25.0	7.22	.355	824	260.00
12	5	25.0	7.28	.355	864	260.00
	6	25.0	7.25	.355	848	250.00
Ave		25.000	7.250	.355	845.333	256.666
SD		.000	.024	.000	16.438	4.714
Var		.000	.000	.000	270.222	22.222
	4	25.5	7.21	.250	1,004	250.00
16	5	25.0	7.28	.255	892	240.00
	6	25.0	7.25	.256	912	260.00
Ave		25.166	7.246	.253	936.000	250.000
SD		.235	.028	.002	48.771	8.164
Var		.055	.000	.000	2,378.666	66.666
Overall						
Ave		24.50				257.036
SD		.026				10.357
Var		.073				107.273

Table C-3. Maximum growth yield coefficient data for chemostats numbers 1, 2, 3, and 4, at 27°C with 4.2 mg/l nitrogen.

Chemostat No.	Days of Operation	Temp °C	pH	OD 750 µm	X_1 cell/ .1 mm^3	X_1[a] mg/l
1	18	27.0	7.18	.191	331	103.00
	19	27.0	7.20	.198	304	113.34
Ave						
SD						
Var						
2	18	27.0	7.25	.251	526	151.67
	19	27.0	7.26	.253	654	150.00
Ave						
SD						
Var						
3	18	27.0	7.27	.268	787	146.67
	19	27.0	7.27	.278	725	145.00
Ave						
SD						
Var						
4	18	27.0	7.28	.324	783	159.97
	19	27.0	7.28	.337	723	167.33
Ave						
SD						
Var						
Overall						
Ave		27.00				142.212
SD		0.000				20.902
Var		0.000				436.890

[a]Average of 2 samples.

Table C-4. Maximum growth yield coefficient data for chemostats numbers 17, 18, 19, and 20, at 27°C with 4.2 mg/l nitrogen.

Chemostat No.	Days of Operation	Temp °C	pH	OD 750 µm	X_1 cells/ .1mm^3	X_1[a] mg/l
17	18	28.0	7.29	.243	701	150.00
	19	26.5	7.28	.264	645	138.34
Ave						
SD						
Var						
18	18	28.0	7.29	.212	542	131.67
	19	26.5	7.28	.224	593	131.67
Ave						
SD						
Var						
19	18	28.5	7.29	.250	586	135.00
	19	27.0	7.29	.258	741	148.34
Ave						
SD						
Var						
20	18	28.5	7.29	.262	704	138.33
	19	26.5	7.27	.239	682	138.33
Ave						
SD						
Var						
Overall						
Ave		27.437				138.960
SD		-				6.452
Var		-				41.627

[a]Average of 2 samples.

Table C-5. Maximum growth yield coefficient data for chemostats numbers 19 and 20 at 28°C with 4.2 mg/l nitrogen.

Chemostat No.	Days of Operation	Temp °C	pH	OD 750 μm	X_1 cells/.1mm^3	X_1[a] mg/l
	19	28.0	7.29	.242	720	130.00
19	20	28.0	7.29	.239	740	119.67
	21	28.0	7.29	.241	720	120.00
Ave		28.000	7.290	.240	726.666	123.223
SD		.000	.000	.001	9.428	4.793
Var		.000	.000	.000	88.888	22.979
	19	28.0	7.29	.220	715	138.33
20	20	28.0	7.29	.220	706	126.67
	21	28.0	7.29	.220	715	120.00
Ave		28.000	7.290	.220	712.000	128.333
SD		.000	.000	.000	4.242	7.574
Var		.000	.000	.000	18.000	57.381
Overall						
Ave		28.000				125.777
SD		.000				6.834
Var		.000				46.709

[a] Average of two replicates.

Table C-6. Maximum growth yield coefficient data for chemostats numbers 1, 2, 3, and 4, at 32°C with 4.2 mg/l nitrogen.

Chemostat No.	Days of Operation	Temp °C	pH	OD 750 μm	X_1 cells/.1mm^3	X_1[a] mg/l
	33	32.0	7.23	.170	270	101.50
1	34	32.5	7.23	.172	260	115.00
Ave						
SD						
Var						
	33	32.0	7.25	.181	271	106.25
2	34	32.5	7.25	.181	278	103.75
Ave						
SD						
Var						
	33	32.0	7.23	.150	256	110.00
3	34	33.0	7.22	.152	235	110.00
Ave						
SD						
Var						
	33	32.0	7.23	.165	256	103.75
4	34	33.0	7.22	.163	249	93.75
Ave						
SD						
Var						
Overall						
Ave		32.375				105.500
SD		-				6.036
Var		-				36.438

[a] Average of two samples.

Table C-7. Maximum growth yield coefficient data for chemostats numbers 17, 18, 19, and 20 at 32°C with 4.2 mg/l nitrogen.

Chemostat No.	Days of Operation	Temp °C	pH	OD 750 μm	X_1 cells/$.1mm^3$	X_1[a] mg/l
17	33	32.0	7.27	.210	453	105.00
	34	32.0	7.26	.207	408	107.50
Ave						
SD						
Var						
18	33	32.0	7.28	.209	554	108.75
	34	32.0	7.27	.205	520	108.75
Ave						
SD						
Var						
19	33	32.0	7.26	.222	685	115.00
	34	32.0	7.24	.218	625	113.33
Ave						
SD						
Var						
20	33	32.0	7.25	.210	606	103.34
	34	32.0	7.23	.203	543	110.00
Ave						
SD						
Var						
Overall						
Ave						108.959
SD						3.643
Var						13.268

[a] Average of two samples.

Table C-8. Continuous flow luxury uptake data at 20°C.

θ days	μ $days^{-1}$	S_1 $\mu g/l$	X_1 mg/l	$S_o - S_1$ $\mu g/l$	$\frac{X_1}{S_o - S_1}$ $\frac{mg\ cell}{mg\ NH_4\text{-}N}$	$\frac{S_o - S_1}{X_1 \theta}$ $\frac{\mu g\text{-}NH_4\text{-}N}{mg \cdot cell \cdot day}$	S_e[a] $\mu g/l$	$\frac{S_e}{S_o - S_1}$
.73	1.370	2268.11	26.40	3973.19	6.645	206.164	2785.19	0.701
.75	1.340	1690.54	29.60	2509.46	11.795	113.039	1177.46	0.469
1.01	0.990	7.47	56.41	4192.53	13.455	73.587	1654.08	0.395
1.04	0.963	4.33	57.04	4195.67	13.550	70.728	1628.87	0.388
1.29	0.727	4.66	55.77	4195.34	13.293	58.332	1685.69	0.402
1.44	0.696	3.39	64.10	4196.61	15.274	45.465	1312.11	0.313
1.49	0.675	4.72	74.99	4195.28	17.874	37.547	820.73	0.196
1.91	0.527	5.37	87.18	4194.63	20.784	25.191	271.53	0.065
1.91	0.525	3.83	83.98	4196.17	19.830	26.160	417.07	0.099
2.10	0.476	4.03	98.72	4195.97	23.527	20.240	246.43	0.059

[a] $S_e = S_o - S_1 - A_o X_1$

$A_o = 0.045$ mg NH_4-N/mg cell

Table C-9. Continuous flow luxury uptake data at 24°C.

θ days	μ $days^{-1}$	S_1 µg/l	X_1 mg/l	$S_o - S_1$ µg/l	$\frac{X_1}{S_o - S_1}$ $\frac{mg\ cell}{mg\ NH_3\text{-}N}$	$\frac{S_o - S_1}{X_1 \theta}$ $\frac{\mu g\text{-}NH_3\text{-}N}{mg \cdot cell \cdot day}$	S_e [a] µg/l	$\frac{S_e}{S_o - S_1}$
0.654	1.530	842.45	63.46	3357.55	18.901	80.899	2323.15	0.692
0.709	1.410	6.88	61.54	4193.12	14.676	96.102	3190.02	0.761
0.878	1.140	14.53	71.15	4185.47	16.999	67.147	3025.73	0.723
0.881	1.140	5.32	61.54	4194.68	14.671	77.369	3191.58	0.760
1.003	0.996	1.88	63.02	4198.12	15.011	66.416	3170.89	0.755
1.013	0.987	3.24	61.87	4196.68	14.743	66.960	3188.20	0.760
1.016	0.983	4.71	57.51	4195.29	13.708	71.800	3257.88	0.777
1.033	0.966	1.40	58.05	4198.60	13.826	70.017	3252.39	0.775
1.058	0.945	5.60	67.79	4194.40	16.162	58.482	3089.42	0.737
1.073	0.932	14.99	76.45	4185.01	18.268	51.018	2938.88	0.702
1.358	0.737	14.81	71.63	4185.19	17.115	43.025	3017.62	0.721
1.407	0.711	9.14	88.94	4190.86	21.222	33.490	2741.14	0.654
1.546	0.646	3.14	113.10	4196.86	26.949	24.002	2353.33	0.561
1.636	0.611	3.97	111.67	4196.03	26.613	22.968	2375.81	0.566
1.670	0.600	4.91	105.11	4195.09	25.055	23.899	2481.80	0.592
1.680	0.595	1.83	120.00	4198.17	28.584	20.824	2242.17	0.534
1.850	0.540	3.16	114.12	4196.84	27.192	19.879	2336.68	0.557
1.860	0.538	4.27	116.66	4195.73	27.804	19.336	2294.17	0.547
1.886	0.530	2.86	110.00	4197.14	26.208	20.231	2404.14	0.573
1.893	0.528	5.15	113.33	4194.85	27.016	19.049	2347.57	0.560
1.930	0.518	5.09	111.11	4194.91	26.487	19.562	2383.82	0.568
1.946	0.513	5.30	118.89	4194.70	28.343	18.131	2256.79	0.538
1.966	0.508	4.36	117.79	4195.64	28.074	18.118	2275.66	0.542
1.980	0.505	4.98	120.90	4195.02	28.820	17.524	2224.35	0.530

[a] $S_e = S_o - S_1 - A_o X_1$ $\quad A_o = 0.0163$ mg NH_3-N/mg cell

Table C-10. Continuous flow lucury uptake data at 27°C.

θ days	μ $days^{-1}$	S_1 $\mu g/l$	X_1 mg/l	$S_o - S_1$ $\mu g/l$	$\frac{X_1}{S_o - S_1}$ $\frac{mg\ cell}{mg\ NH_4\text{-}N}$	$\frac{S_o - S_1}{X_1 \theta}$ $\frac{\mu g\text{-}NH_4\text{-}N}{mg \cdot cell \cdot day}$	S_e [a] $\mu g/l$	$\frac{S_e}{S_o - S_1}$
0.659	1.519	808.40	67.79	3391.60	19.98	75.92	1364.68	.40
0.732	1.367	196.60	61.97	4003.40	15.74	88.25	2150.50	.53
0.926	1.080	2.91	72.12	4197.09	17.18	62.85	2040.70	.49
1.085	0.922	8.93	65.39	4191.07	15.60	59.07	2235.91	.53
1.094	0.914	15.48	72.12	4184.52	17.23	53.04	2028.13	.48
1.434	0.698	6.20	84.62	4193.80	20.17	34.56	1663.66	.40
1.441	0.694	2.87	96.15	4197.13	22.90	30.29	1322.25	.32

[a] $S_e = S_o - S_1 - A_o X_1$

$A_o = 0.0299$ mg NH_4-N/mg cell

Table C-11. Continuous flow luxury uptake data at 28°C.

θ days	μ $days^{-1}$	S_1 $\mu g/l$	X_1 mg/l	$S_o - S_1$ $\mu g/l$	$\frac{X_1}{S_o - S_1}$ $\frac{mg\ cell}{mg\ NH_4\text{-}N}$	$\frac{S_o - S_1}{X_1 \theta}$ $\frac{\mu g\ NH_4\text{-}N}{mg \cdot cell \cdot day}$	S_e [a] $\mu g/l$	$\frac{S_e}{S_o - S_1}$
0.640	1.565	770.70	45.19	3429.30	13.18	118.57	1938.03	0.57
0.705	1.415	738.92	41.30	3461.08	11.93	118.87	2098.18	0.61
0.990	1.009	9.98	68.59	4190.02	16.37	61.70	1926.55	0.46
1.013	0.986	5.59	80.77	4194.41	19.26	51.26	1529.00	0.36
1.026	0.975	6.27	80.77	4193.73	19.26	50.61	1528.32	0.36
1.036	0.966	17.20	80.77	4182.80	19.30	49.99	1517.39	0.36
1.103	0.908	10.92	75.00	4189.08	17.90	50.64	1714.08	0.41
1.124	0.895	11.85	81.41	4188.15	19.43	45.77	1501.62	0.36
1.453	0.690	9.43	76.28	4190.57	18.20	37.80	1673.33	0.40
1.460	0.685	7.03	95.51	4192.97	22.77	30.07	1041.14	0.25
1.480	0.676	6.38	82.69	4193.62	20.33	34.28	1464.85	0.35
1.526	0.645	11.15	85.26	4188.85	20.35	32.19	1374.61	0.33
1.610	0.623	11.45	72.43	4188.55	17.30	35.92	1798.36	0.43
2.033	0.491	12.87	69.23	4187.13	16.53	29.75	1902.54	0.45
2.066	0.485	12.78	99.36	4187.22	23.73	20.40	908.34	0.22
2.066	0.485	10.83	94.23	4189.17	22.49	21.52	1072.58	0.26
2.080	0.481	11.58	84.61	4188.42	20.20	23.80	1392.29	0.33
2.176	0.461	9.88	100.00	4190.12	23.86	19.26	890.12	0.21
2.196	0.458	9.94	101.28	4190.06	24.17	18.83	847.82	0.20
2.246	0.446	12.13	93.59	4187.87	22.34	19.92	1099.40	0.26
2.246	0.445	8.29	97.43	4191.71	23.24	19.15	976.52	0.23
4.634	0.216	8.21	101.92	4191.79	24.31	8.88	828.43	0.20
4.756	0.210	11.85	96.63	4188.15	23.07	9.11	999.36	0.24

[a] $S_e = S_o - S_1 - A_o X_1$

$A_o = 0.033$ mg NH_4-N/mg cell

Table C-12. Continuous flow luxury uptake data at 32°C.

θ days	μ $days^{-1}$	S_1 $\mu g/l$	X_1 mg/l	$S_o - S_1$ $\mu g/l$	$\frac{X_1}{S_o - S_1}$ $\frac{mg\ cell}{mg\ NH_4\text{-}N}$	$\frac{S_o - S_1}{X_1 \theta}$ $\frac{\mu g\ NH_4\text{-}N}{mg \cdot cell \cdot day}$	S_e[a] $\mu g/l$	$\frac{S_e}{S_o - S_1}$
0.697	1.435	1665.98	51.92	2534.02	20.49	70.02	2030.07	.80
0.777	1.265	802.95	74.04	3397.05	21.79	59.59	2894.96	.85
0.895	1.117	69.69	66.35	4130.31	16.06	69.53	1536.03	.37
0.945	1.058	122.56	66.35	4077.44	16.27	65.03	1483.16	.36
1.082	.925	247.33	66.35	3952.67	16.78	55.06	1358.39	.34
1.085	.921	72.22	59.14	4127.78	14.32	64.33	1815.41	.44
1.380	.725	211.73	68.75	3988.27	17.24	42.04	1300.15	.33
1.440	.693	13.36	77.89	4186.64	18.60	37.33	1141.14	.27

[a] $S_e = S_o - S_1 - A_o X_1$

$A_o = 0.0391$ mg NH_4-N/mg cell

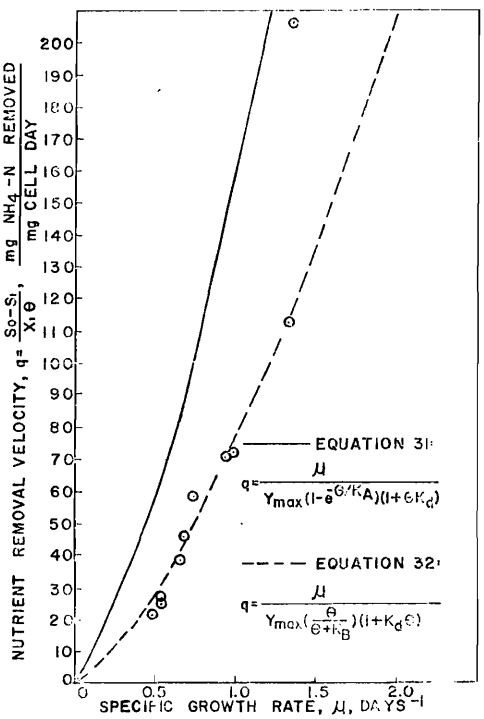

Figure C-1. Comparison of nutrient removal velocity, q, and specific growth rate, μ, at 20°C.

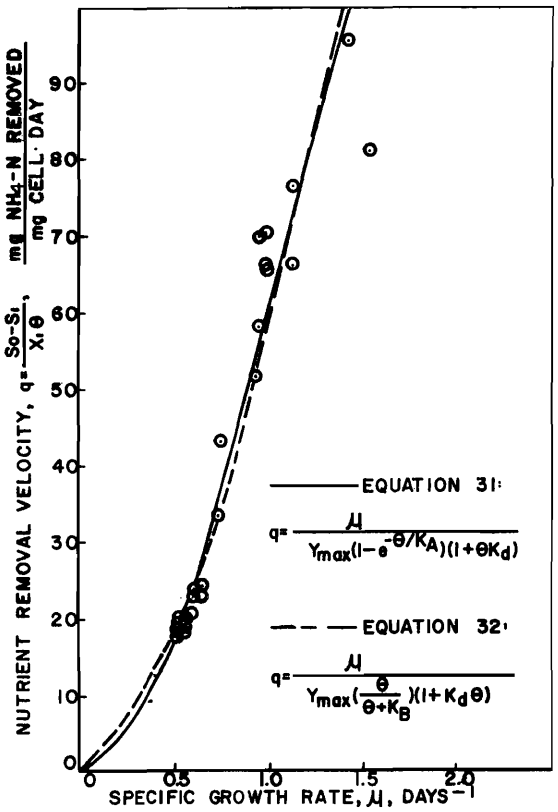

Figure C-2. Comparison of nutrient removal velocity, q, and specific growth rate, μ, at 24°C.

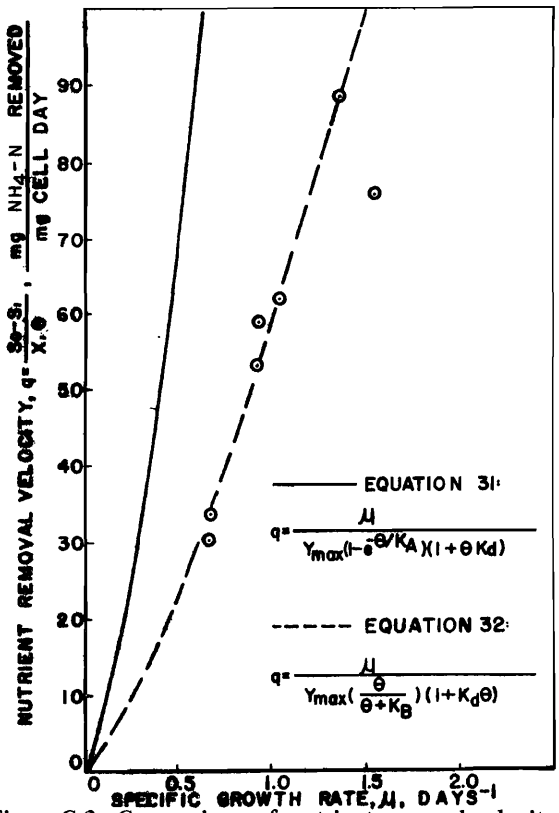

Figure C-3. Comparison of nutrient removal velocity, q, and specific growth rate, μ, at 27°C.

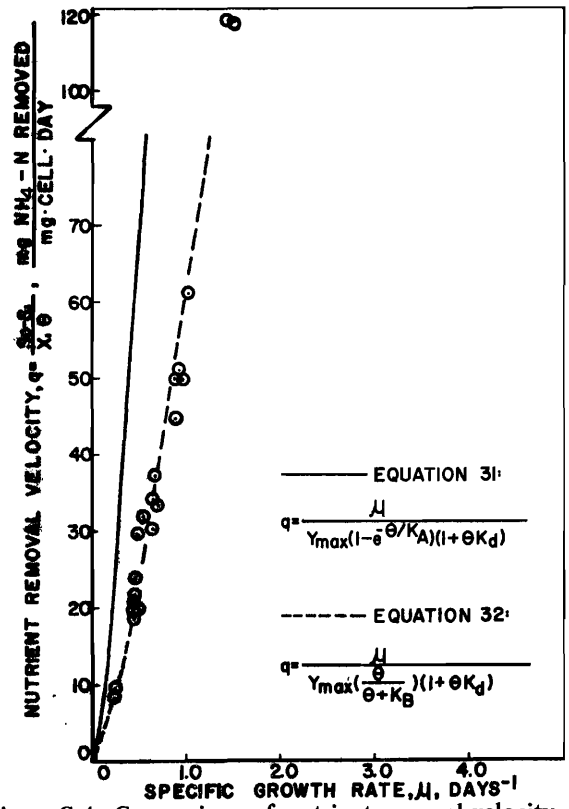

Figure C-4. Comparison of nutrient removal velocity, q, and specific growth rate, μ, at 28°C.

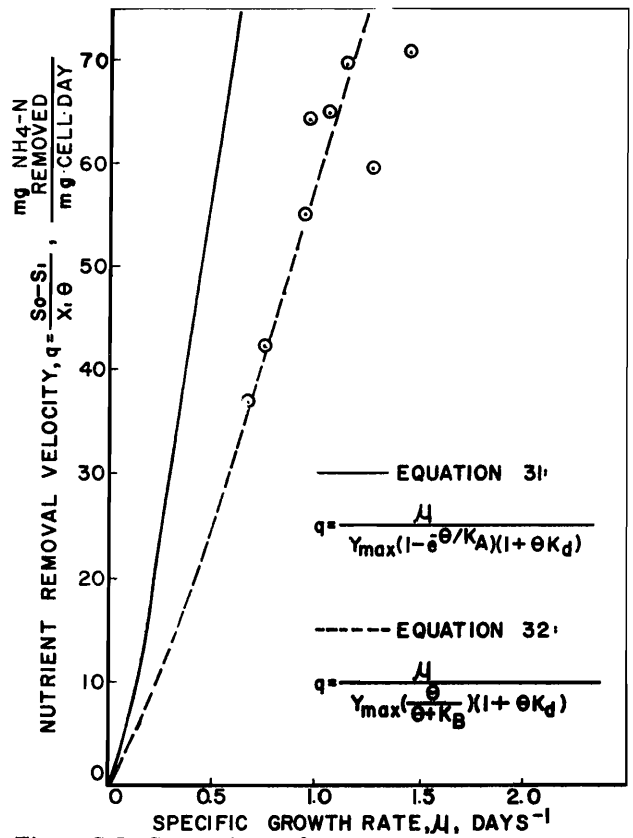

Figure C-5. Comparison of nutrient removal velocity, q, and specific growth rate, μ, at 33°C.

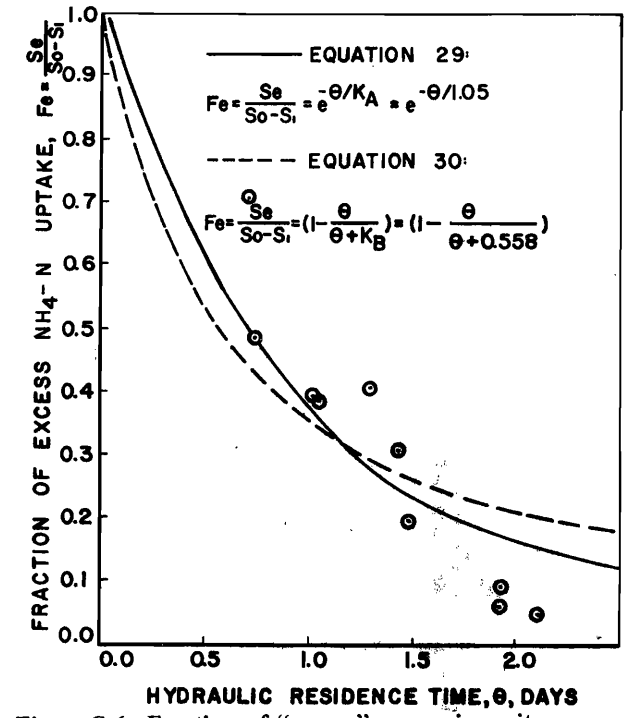

Figure C-6. Fraction of "excess" ammonium-nitrogen uptake as a function of hydraulic residence time, θ, at 20°C.

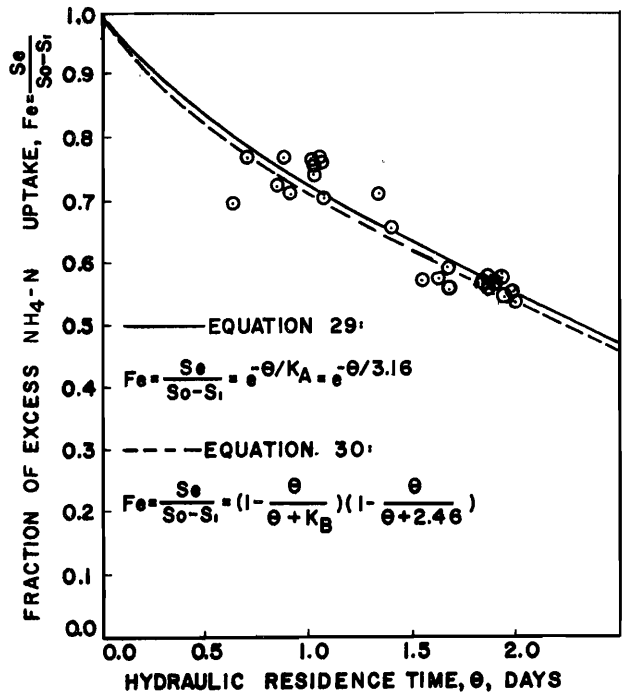

Figure C-7. Fraction of "excess" ammonium-nitrogen uptake as a function of hydraulic residence time, θ, at 24°C.

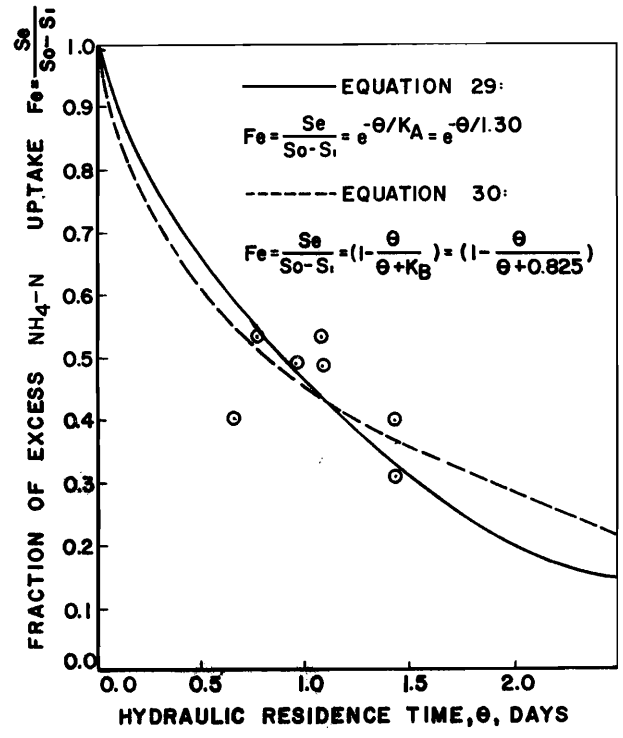

Figure C-8. Fraction of "excess" ammonium-nitrogen uptake as a function of hydraulic residence time, θ, at 27°C.

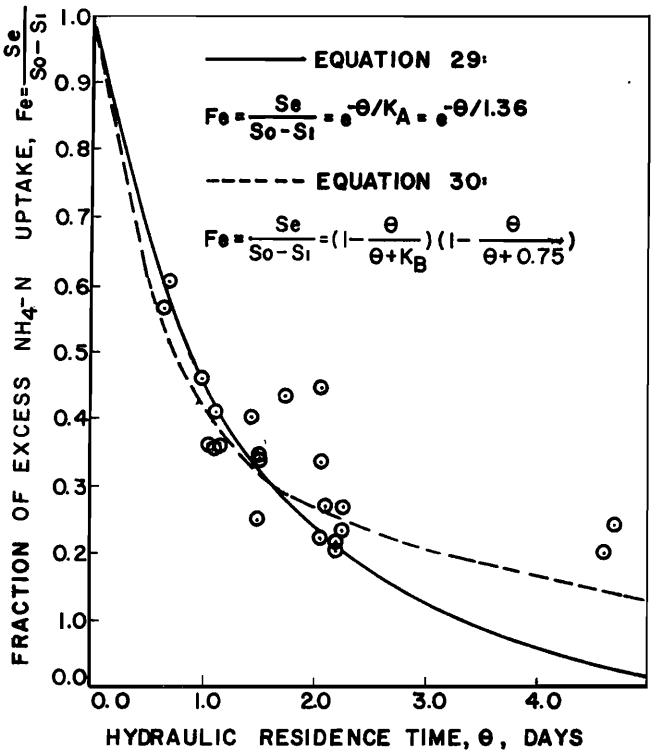

Figure C-9. Fraction of "excess" ammonium-nitrogen uptake as a function of hydraulic residence time, θ, at 28°C.

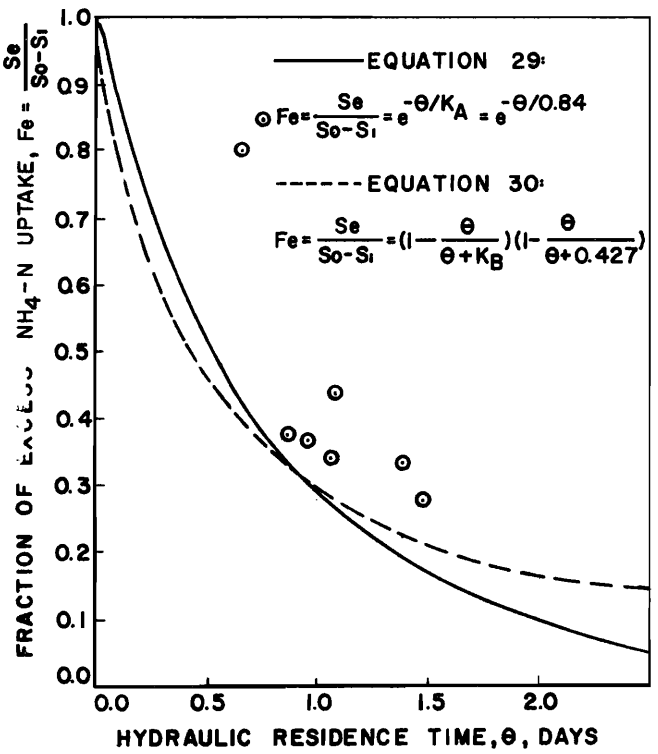

Figure C-10. Fraction of "excess" ammonium-nitrogen uptake as a function of hydraulic residence time, θ, at 32°C.

Appendix D

Continuous Flow Kinetic Data Without Toxicant

Table D-1. Continuous flow steady state data for chemostats numbers 1, 2, 3, and 4, at 20°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	13			Excessive Bacteria Growth						
1	14			"						
	15			"						
Ave										
SD[a]										
Var[b]										
	13	0.98	1.024	18.0	7.03	.070	115	57.69	c	c
2	14	1.03	0.970	18.5	7.04	.062	115	42.31	7.61	188
	15	1.03	.968	18.0	7.03	.058	110	69.23	7.33	299
Ave		1.01	.99	18.17	7.03	.0633	113.33	56.41	7.47	243.50
SD		0.024	.026	.236	.005	.005	2.357	11.028	0.140	55.50
Var		.0005	.0006	.0555	0.0000	0.0000	5.556	121.60	0.020	3080
	13			Excessive Bacteria Growth						
3	14			"						
	15			"						
Ave										
SD										
Var										
	13	0.99	1.009	18.5	7.06	.067	109	57.69	c	150
4	14	1.08	.930	19.0	7.07	.060	115	65.38	4.99	185
	15	1.05	.951	18.5	7.04	.071	110	48.07	3.67	182
Ave		1.040	.963	18.67	7.06	.066	111.33	57.04	4.33	172.33
SD		0.037	.033	.236	.0124	.0045	2.625	7.08	.660	15.83
Var		.001	.0011	.0556	.0002	0.0000	6.889	50.15	.4356	250.89

[a] Standard deviation.

[b] Variance.

Table D-2. Continuous flow steady state data for chemostats numbers 5, 6, 7, and 8, at 20°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	13	1.30	.771	19.0	7.09	.088	207	44.23	8.29	210
5	14	1.43	.700	19.0	7.10	.080	209	59.61	2.36	214
	15	1.41	.709	18.5	7.10	.085	200	63.47	3.33	181
Ave		1.29	.727	18.83	7.10	.084	205.33	55.770	4.66	201.67
SD		.184	.032	.236	.005	.003	3.858	8.310	2.597	14.70
Var		.034	.001	.056	.000	.000	14.88	69.069	6.745	216.72
	13	1.53	.655	18.0	7.11	.126	257	65.38	6.10	330
6	14	1.39	.722	19.0	7.10	.117	267	88.46	a	219
	15	1.54	.650	19.0	7.10	.117	245	71.15	3.33	260
Ave		1.486	.675	18.666	7.10	.120	256.333	74.996	4.715	269.666
SD		.068	.032	.471	.004	.004	8.993	9.807	1.385	45.83
Var		.004	.001	.222	0.000	.000	80.888	96.179	1.918	2100.22
	13				Excessive Bacteria Growth					
7	14				"					
	15				"					
Ave										
SD										
Var										
	13	1.39	.722	19.5	7.10	.112	310	67.31	2.76	257
8	14	1.49	.670	19.5	7.11	.110	291	73.07	4.07	275
	15	1.44	.696	19.5	7.10	.116	300	51.92	3.33	238
Ave		1.440	.696	19.50	7.103	.112	300.333	64.100	3.386	256.666
SD		.001	.021	0.000	.004	.002	7.760	79.705	.536	15.107
Var		.040	.000	0.000	0.000	0.000	60.222	8.927	.287	228.222

[a] Missing data.

Table D-3. Continuous flow steady state data for chemostats numbers 9, 10, 11, and 12, at 20°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	13	1.96	.509	19.0	7.16	.162	445	101.92	a	285
9	14	2.22	.450	19.0	7.15	.152	536	101.92	4.73	153
	15	2.13	.471	19.0	7.13	.178	400	92.31	3.33	187
Ave		2.103	.476	19.00	7.146	.164	460.333	98.716	4.03	208.333
SD		.107	.024	0.000	.012	.010	56.57	4.530	.700	55.960
Var		.011	.000	0.000	0.000	0.000	3200.222	20.522	.490	3131.555
	13			Excessive Bacteria Growth						
10	14			"						
	15			"						
Ave										
SD										
Var										
	13			Excessive Bacteria Growth						
11	14			"						
	15			"						
Ave										
SD										
Var										
	13	1.79	.559	20.0	7.18	.138	401	82.69	2.76	137
12	14	2.00	.500	20.0	7.19	.152	518	92.31	4.07	275
	15	1.93	.518	20.0	7.17	.165	440	86.54	4.67	160
Ave		1.906	.525	20.000	7.180	.151	453.000	87.180	3.833	190.666
SD		.087	.024	0.000	.008	.011	48.641	3.953	.797	60.367
Var		.007	0.000	0.000	.000	0.000	2366.000	15.628	.636	3644.222

[a] Missing data.

Table D-4. Continuous flow steady state data for chemostats numbers 13, 14, 15, and 16, at 20°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	13				Excessive Bacteria Growth					
13	14				"					
	15				"					
Ave										
SD										
Var										
	13	1.75	.573	19.0	7.11	.148	412	86.54	4.07	112
14	14	2.02	.495	19.0	7.19	.143	450	86.54	a	202
	15	1.95	.514	19.0	7.18	.160	410	78.85	6.67	280
Ave		1.906	.527	19.000	7.160	.150	428.000	83.976	5.370	198.000
SD		.114	.033	0.000	.035	.007	18.402	3.625	1.300	68.644
Var		.013	.001	0.000	.001	0.000	338.666	13.141	1.690	4712.000
	13				Excessive Bacteria Growth					
15	14				"					
	15				"					
Ave										
SD										
Var										
	13				Excessive Bacteria Growth					
16	14				"					
	15				"					
Ave										
SD										
Var										

[a] Missing data.

Table D-5. Continuous flow steady state data for chemostats numbers 17, 18, 19, and 20, at 20°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	23	5.26	.190	19.5	7.22	.188	441	98.07	14.55	12
	24	4.59	.218	19.0	7.20	.178	430	a	a	
17	25	4.55	.220	18.5	7.21	.208	507	124.99	16.62	15
	26	4.61	.217	19.0	7.21	.208	440	a	a	
	27	3.98	.251	19.0	7.21	.198	430	111.54	18.31	12
Ave		4.598	.2192	19.00	7.21	.196	450.1	111.533	14.550	13.00
SD		.4056	.0193	.3162	.00632	.0166	29.083	10.990	18.310	1.414
Var		.1645	.0004	.1000	.00000	.000136	845.84	120.781	16.620	2.00
	23	4.46	.224	20.0	7.24	.258	486	128.84	16.62	4
	24	3.70	.270	19.5	7.21	.258	459	a	a	
18	25	4.59	.218	19.0	7.22	.262	438	90.38	15.49	7
	26	4.85	.206	20.0	7.22	.250	440	113.46	a	
	27	4.46	.224	19.0	7.22	.243	440	117.31	11.27	4
Ave		4.412	.2284	19.50	7.22	.254	452.6	112.50	14.46	5.000
SD		.3834	.0218	.4472	.00979	.0068	18.369	13.967	2.302	1.414
Var		.1470	.00047	.200	.0000	.0000	337.44	195.069	5.300	2.000
19	4	0.75	1.34	18.5	7.10	.045	112	29.60	1766.33	39
	5	0.74	1.35	18.5	7.10	.045	115	32.00	1614.74	41
Ave		.745	1.345	18.50	7.10	0.045	113.500	30.800	1690.535	40.000
SD		.005	0.005	0.000	0.000	0.000	1.500	1.200	75.795	1.000
Var		0.000	0.000	0.000	0.000	0.000	2.250	1.440	5744.88	1.000
20	4	0.71	1.40	18.5	7.10	.037	90	24.00	2467.33	32
	5	0.75	1.34	18.5	7.10	.044	92	28.80	2068.88	30
Ave		.730	1.370	18.5	7.10	0.040	91.00	26.40	2268.105	31.00
SD		.020	0.030	0.000	0.000	0.000	1.000	2.400	199.225	1.000
Var		0.000	0.000	0.000	0.000	0.003	1.000	5.760	39690.60	1.000

[a] Missing data.

Table D-6. Continuous flow steady state data for chemostats numbers 1, 2, 3, and 4, at 24°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	10	1.00	1.00	24.0	7.13	.099	225	51.92	a	164
1	11	1.02	.980	24.0	7.15	.101	230	53.30	4.71	129
	12	1.03	.971	24.0	7.13	.099	229	67.31	a	a
Ave		1.016	.983	24.000	7.136	.099	228.000	57.510	4.71	146.500
SD		.012	.012	.000	.009	.000	2.160	6.952	-	17.500
Var		.000	.000	.000	.000	.000	4.666	48.337	-	306.250
	10	1.06	.940	24.0	7.18	.095	210	59.15	2.45	398
2	11	1.02	.980	24.0	7.15	.101	204	65.00	0.34	246
	12	1.02	.980	24.0	7.15	.095	205	50.00	a	a
Ave		1.033	.966	24.000	7.160	.097	206.333	58.050	1.395	322.000
SD		.018	.018	.000	.014	.002	2.624	6.172	1.055	76.000
Var		.000	.000	.000	.000	.000	6.888	38.105	1.113	5776.000
	10	1.01	.990	24.0	7.18	.101	285	61.54	1.83	155
3	11	1.02	.981	24.0	7.14	.102	280	68.30	a	145
	12	1.01	.990	24.0	7.13	.090	260	55.77	4.64	a
Ave		1.013	.987	24.000	7.150	.097	275.000	61.870	3.235	150.000
SD		.004	.004	.000	.021	.005	10.801	5.120	1.405	5.000
Var		.000	.000	.000	.000	.000	116.666	26.221	1.974	25.000
	10	1.000	1.00	24.0	7.18	.094	234	69.23	2.14	212
4	11	1.00	1.00	24.0	7.16	.105	212	58.30	1.01	214
	12	1.01	.990	24.0	7.15	.097	210	61.54	2.50	a
Ave		1.003	.996	24.00	7.163	.098	218.666	63.023	1.883	213.000
SD		.004	.004	.000	.012	.004	10.873	4.583	.634	1.000
Var		.000	.000	.000	.000	.000	118.222	21.010	.402	1.000

[a] Missing data.

Table D-7. Continuous flow steady state data for chemostats numbers 5, 6, 7, and 8, at 24°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ Days^{-1}	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	10	1.68	.595	24.0	7.23	.157	540	120.00	1.83	285
5	11		Pump Malfunction					a	a	a
	12		"					a	a	a
Ave		1.68	.595	24.0	7.23	.157	540	120.00	1.83	285
SD		-	-	-	-	-	-	-	-	-
Var		-	-	-	-	-	-	-	-	-
	10	1.67	.600	24.0	7.25	.179	680	112.00	2.75	139
6	11	1.67	.600	24.0	7.25	.190	670	100.00	7.07	178
	12	1.67	.600	24.0	7.25	.169	696	103.33	a	a
Ave		1.670	.600	24.000	7.250	.179	682.00	105.110	4.910	158.500
SD		.000	.000	.000	.000	.008	10.708	5.058	2.160	19.500
Var		.000	.000	.000	.000	.000	114.666	25.584	4.665	380.250
	10	1.64	.610	24.5	7.26	.179	640	a	3.98	312
7	11	1.64	.610	24.5	7.24	.180	694	110.00	4.71	a
	12	1.63	.615	24.5	7.24	.174	648	113.33	3.21	
Ave		1.636	.611	24.5	7.246	.177	660.666	111.665	3.966	312.000
SD		.004	.002	0.000	.009	.002	23.795	1.665	.612	-
Var		.000	.000	0.000	.000	.000	566.222	2.772	.375	-
	10	1.54	.650	24.5	7.26	.165	640	136.00	3.06	176
8	11	1.54	.650	24.5	7.24	.172	638	113.30	a	145
	12	1.56	.640	24.5	7.22	.163	646	90.00	3.21	a
Ave		1.546	.646	24.5	7.240	.166	641.333	113.100	3.135	160.500
SD		.009	.004	0.000	.016	.003	3.399	18.779	.075	15.500
Var		.000	.000	0.000	.000	.000	11.555	352.686	.005	240.250

[a] Missing data.

Table D-8. Continuous flow steady state data for chemostats numbers 9, 10, 11, and 12, at 24°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ Days^{-1}	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	10	1.98	.505	23.5	7.25	.189	720	136.00	4.89	347
9	11	1.98	.505	23.5	7.25	.191	813	116.70	4.71	172
	12	1.98	.505	23.5	7.25	.181	680	110.00	5.35	a
Ave		1.980	.505	23.5	7.25	.187	737.666	120.900	4.982	259.500
SD		.000	.000	.000	.000	.004	55.715	11.022	.269	87.500
Var		.000	.000	.000	.000	.000	3104.222	121.486	.072	7656.250
	10	1.87	.535	24.0	7.25	.187	750	110.00	3.36	159
10	11	1.89	.530	24.0	7.26	.192	772	120.00	2.02	a
	12	1.90	.525	24.0	7.25	.181	691	100.00	3.21	a
Ave		1.886	.530	24.000	7.253	.186	737.666	110.000	2.863	159
SD		.012	.004	.000	.004	.004	34.198	8.164	.599	-
Var		.000	.000	.000	.000	.000	1169.555	66.666	.359	-
	10	1.87	.535	25.0	7.22	.180	736	123.33	1.54	355
11	11	2.00	.500	25.0	7.23	.182	740	100.00	9.09	360
	12	1.92	.520	25.0	7.24	.181	708	110.00	4.64	a
Ave		1.93	.518	25.000	7.230	.181	728.000	111.110	5.090	357.500
SD		.053	.014	.000	.008	.000	14.236	9.556	3.098	2.500
Var		.002	.000	.000	.000	.000	202.666	91.330	9.601	6.250
	10	1.90	.525	25.0	7.23	.183	740	126.67	a	121
12	11	1.89	.530	25.0	7.28	.188	748	110.00	6.73	159
	12	1.89	.530	25.0	7.25	.165	648	103.33	3.57	a
Ave		1.893	.528	25.000	7.253	.178	712.000	113.333	5.150	140.000
SD		.004	.002	.000	.020	.009	45.372	9.815	1.580	19.000
Var		.000	.000	.000	.000	.000	2058.666	96.348	2.496	361.000

[a] Missing data.

Table D-9. Continuous flow steady state data for chemostats numbers 13, 14, 15, and 16, at 24°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	10	1.92	.520	24.5	7.26	.187	740	130.00	4.89	101
13	11	1.96	.510	24.5	7.27	.183	712	110.00	a	284
	12	1.96	.510	24.5	7.26	.180	708	116.67	5.71	a
Ave		1.946	.513	24.5	7.263	.183	720.000	118.890	5.300	192.500
SD		.018	.004	.000	.004	.002	14.236	8.314	.410	91.500
Var		.000	.000	.000	.000	.000	202.666	69.130	.168	8372.250
	10	1.94	.515	24.5	7.26	.189	680	130.00	3.36	334
14	11	1.96	.510	24.5	7.23	.190	780	116.70	a	406
	12	2.00	.500	24.5	7.23	.183	788	106.67	5.36	a
Ave		1.966	.508	24.500	7.240	.187	749.333	117.790	4.360	370.000
SD		.024	.006	.000	.014	.003	49.134	9.555	1.000	36.000
Var		.000	.000	.000	.000	.000	244.222	117.790	1.000	1296.000
	10	1.81	.550	24.5	7.23	.184	752	116.67	2.45	a
15	11	1.89	.530	24.5	7.25	.190	783	116.70	2.02	15
	12	1.85	.540	24.5	7.24	.180	748	110.00	5.00	a
Ave		1.850	.540	24.500	7.240	.184	761.000	114.123	3.156	15
SD		032	.008	.000	.008	.004	244.666	2.945	1.315	-
Var		001	.000	.000	.000	.000		8.677	1.729	-
	10	1.80	.555	24.5	7.26	.188	790	120.00	4.89	140
16	11	1.89	.530	24.5	7.28	.198	814	113.30	5.05	85
	12	1.89	.530	24.5	7.27	.192	810	116.67	2.86	a
Ave		1.860	.538	24.500	7.270	.192	804.666	116.656	4.266	112.500
SD		.042	.011	.000	.008	.004	10.498	2.735	.996	27.500
Var		.001	.000	.000	.000	.000	110.222	7.481	.993	756.250

[a] Missing data.

Table D-10. Continuous flow steady state data for chemostats numbers 1, 2, 3, and 4, at 24°C without toxicant during Experiment number 2.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l
1	7	1.052	.950	23.0	7.11	.109	130	58.65	7.00
	8	1.063	.940	24.5	7.11	.114	149	76.92	4.20
Ave		1.058	.945	23.750	7.110	0.111	139.500	67.785	5.600
SD		.005	.005	.750	.000	.002	9.500	9.135	1.400
Var		.000	.000	.562	.000	.000	90.250	83.445	1.960
2	7	1.071	.934	23.0	7.14	.130	247	73.08	16.14
	8	1.075	.930	24.5	7.14	.132	246	79.81	13.83
Ave		1.073	.932	23.750	7.140	.131	246.500	76.445	14.985
SD		.002	.002	.750	.000	.001	.500	3.365	1.155
Var		.000	.000	.562	.000	.000	.250	11.323	1.334
3	7	1.364	.733	23.0	7.16	.137	223	72.11	15.62
	8	1.351	.740	24.0	7.18	.143	251	71.15	14.00
Ave		1.358	.737	23.500	7.170	.140	237.000	71.630	14.810
SD		.006	.003	.500	.010	.003	14.000	.480	.810
Var		.000	.000	.250	.000	.000	196.000	.230	.656
4	7	1.414	.707	23.0	7.19	.202	349	91.34	11.28
	8	1.400	.715	24.0	7.20	.171	346	86.53	7.00
Ave		1.407	.711	23.500	7.195	.186	347.500	88.94	9.140
SD		.007	.004	.500	.005	.015	1.500	2.405	2.140
Var		.000	.000	.250	.000	.000	2.250	5.785	4.579

Table D-11. Continuous flow steady state data for chemostats numbers 17, 18, 19, and 20, at 24°C without toxicant during Experiment number 2.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l
17	7	.870	1.150	23.5	7.13	.108	203	73.07	4.51
	8	.885	1.130	24.5	7.14	.110	187	69.23	6.13
Ave		0.878	1.14	24.000	7.135	.109	195.000	71.150	5.320
SD		.007	.010	.500	.005	.001	8.000	1.920	.810
Var		.000	.000	.250	.000	.000	64.000	3.686	.656
18	7	.889	1.125	23.5	7.12	.089	157	59.61	13.65
	8	.873	1.145	24.5	7.12	.093	140	63.46	15.40
Ave		.881	1.135	24.000	7.120	.091	148.500	61.54	14.525
SD		.008	.010	.500	.000	.002	8.500	1.925	.875
Var		.000	.000	.250	.000	.000	72.250	3.705	.765
19	7	.700	1.434	23.5	7.13	.104	129	61.54	4.00
	8	.717	1.395	24.0	7.12	.098	129	61.54	9.76
Ave		.709	1.414	23.750	7.125	.101	129.000	61.54	6.880
SD		.008	.019	.250	.005	.003	.000	.000	2.880
Var		.000	.000	.062	.000	.000	.000	.000	8.294
20	7	.662	1.510	23.5	7.11	.117	138	63.46	949.9
	8	.645	1.550	24.0	7.10	.115	137	63.46	735.0
Ave		.654	1.530	23.750	7.105	.116	138.500	63.460	842.450
SD		.008	.020	.250	.005	.001	.500	.000	107.450
Var		.000	.000	.062	.000	.000	.250	.000	11545.502

Table D-12. Continuous flow steady state data for chemostats numbers 1, 2, 3, and 4, at 27°C without toxicant during Experiment number 2.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l
1	14	1.075	.930	27.0	7.13	.107	175	61.54	a
	15	1.095	.913	26.5	7.10	.087	124	69.23	8.93
Ave		1.085	.922	26.750	7.115	.097	149.500	65.385	8.93
SD		.010	.008	.250	.015	.010	25.500	3.845	-
Var		.000	.000	.062	.000	.000	149.500	14.784	-
2	14	1.093	.915	27.0	7.17	.119	200	67.31	a
	15	1.095	.913	26.5	7.13	.118	192	76.92	15.48
Ave		1.094	.914	26.750	7.150	.118	196.000	72.115	15.48
SD		.001	.001	.250	.020	.000	4.000	4.805	-
Var		.000	.000	.062	.000	.000	16.000	23.088	-
3	14	1.418	.705	26.5	7.20	.162	449	80.77	7.40
	15	1.449	.690	26.0	7.18	.177	448	88.46	5.00
Ave		1.434	.698	26.250	7.190	.169	448.50	84.615	6.20
SD		.015	.007	.250	.010	.007	.500	3.845	1.200
Var		.000	.000	.062	.000	.000	.250	14.784	1.440
4	14	1.433	.698	26.5	7.20	.187	508	92.30	3.10
	15	1.449	.690	26.0	7.19	.193	520	100.00	2.64
Ave		1.441	.694	26.250	7.195	.190	514.00	96.150	2.870
SD		.008	.004	.250	.005	.003	6.000	3.850	.052
Var		.000	.000	.062	.000	.000	36.000	14.822	.230

[a] Missing data.

Table D-13. Continuous flow steady state data for chemostats numbers 17, 18, 19, and 20, at 27°C without toxicant during Experiment number 2.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l
17	14	.919	1.088	27.0	7.18	.122	254	75.00	1.00
	15	.933	1.072	27.0	7.14	.119	228	69.23	4.82
Ave		.926	1.080	27.000	7.160	.120	241.000	72.12	2.910
SD		.007	.008	.000	.020	.001	13.000	2.885	1.910
Var		.000	.000	.000	.000	.000	169.000	8.323	3.648
18	14	.871	1.148	27.0	7.17	.092	200	51.92	a
	15	.854	1.170	27.0	7.12	.076	184	52.89	a
Ave				Incomplete data analysis					
SD				"					
Var				"					
19	14	.745	1.343	26.5	7.14	.124	237	56.63	129.00
	15	.719	1.390	26.5	7.10	.106	160	67.31	264.20
Ave		.732	1.367	26.5	7.120	.115	198.500	61.970	196.60
SD		.013	.023	.000	.020	.009	38.500	5.340	67.600
Var		.000	.000	.000	.000	.000	1482.250	28.513	4569.760
20	14	.657	1.522	27.0	7.11	.106	226	65.38	808.40
	15	.660	1.515	27.0	7.11	.116	254	70.19	a
Ave		.659	1.519	27.000	7.110	0.111	240.000	67.785	808.40
SD		.001	.003	.000	.000	.006	14.000	2.405	-
Var		.000	.000	.000	.000	.000	196.000	5.784	-

[a] Missing data.

Table D-14. Continuous flow steady state data for chemostats numbers 1, 2, 3, and 4, at 28°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 .μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	12	.97	1.030	28.5	7.18	.120	277	78.85	18.00	18
1	13	1.10	.910	28.0	7.18	.115	275	78.84	16.00	24
	14	1.04	.960	28.5	7.18	.123	285	84.61	17.59	21
Ave		1.036	.966	28.333	7.180	.119	279.000	80.766	17.196	21.000
SD		.053	.049	.235	.000	.003	4.320	2.717	.862	2.449
Var		.002	.002	.055	.000	.000	18.666	7.385	.744	6.000
	12	.99	1.010	28.5	7.18	.100	198	73.08	11.60	24
2	13	.96	1.038	28.0	7.15	.095	182	63.46	8.00	21
	14	1.02	.980	28.5	7.15	.103	196	69.23	10.34	24
Ave		.990	1.009	28.333	7.160	.099	192.000	68.590	9.980	23.000
SD		.024	.023	.235	.014	.003	7.118	3.953	1.491	1.414
Var		.000	.000	.055	.000	.000	50.666	15.628	2.224	2.000
	12	1.02	.980	28.5	7.18	.121	265	80.77	4.00	26
3	13	1.02	.985	28.5	7.18	.118	275	73.08	10.00	31
	14	1.04	.960	28.0	7.18	.112	264	88.46	4.80	23
Ave		1.026	.975	28.333	7.180	.117	268.000	80.770	6.266	26.666
SD		.009	.010	.235	.000	.003	4.966	6.278	2.659	3.299
Var		.000	.000	.055	.000	.000	24.666	39.424	7.075	10.888
	12	1.00	1.00	28.5	7.18	.125	283	82.69	9.20	14
4	13	1.00	1.00	28.5	7.18	.130	276	75.00	5.17	16
	14	1.04	.960	28.0	7.18	.135	281	84.61	2.40	21
Ave		1.013	.986	28.333	7.180	.130	280.000	80.766	5.590	17.000
SD		.018	.018	.235	.000	.004	2.943	4.152	2.791	2.943
Var		.000	.000	.055	.000	.000	8.666	17.241	7.794	8.666

Table D-15. Continuous flow steady state data for chemostats numbers 5, 6, 7, and 8, at 28°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No/ml NO^{-5}
	12	1.41	.716	28.0	7.16	.120	258	82.69	9.00	47
5	13	1.52	.660	28.0	7.18	.120	324	73.08	12.40	48
	14	1.43	.700	28.0	7.18	.150	300	73.07	6.90	a
Ave		1.453	.690	28.000	7.173	.130	294.000	76.280	9.433	47.500
SD		.047	.021	.000	.009	.014	27.276	4.532	2.266	.500
Var		.002	.000	.000	.000	.000	744.000	20.544	5.135	.250
	12	1.45	.690	28.0	7.19	.140	394	84.61	9.00	48
6	13	1.54	.650	28.0	7.19	.125	324	82.69	3.60	58
	14	1.45	.690	28.0	7.19	.157	385	80.76	6.55	a
Ave		1.480	.676	28.000	7.190	.140	367.666	82.686	6.383	53.000
SD		.042	.018	.000	.000	.013	31.094	1.571	2.207	5.000
Var		.001	.000	.000	.000	.000	966.888	2.470	4.873	25.000
	12	1.54	.650	28.5	7.20	.125	328	84.61	11.45	24
7	13	1.48	.675	28.5	7.20	.137	318	84.61	10.00	27
	14	1.56	.640	28.0	7.20	.155	344	86.55	12.00	21
Ave		1.526	.645	28.333	7.200	.139	330.000	85.256	11.150	24.000
SD		.033	.026	.055	.000	.012	10.708	.914	.843	2.449
Var		.001	.000	.235	.000	.000	114.666	.836	.711	6.000
	12	1.52	.660	28.5	7.20	.098	205	73.08	10.00	34
8	13	1.64	.610	28.0	7.20	.110	229	71.15	7.59	27
	14	1.67	.600	28.0	7.20	.110	195	73.07	16.77	a
Ave		1.610	.623	28.166	7.200	.106	209.666	72.433	11.453	30.500
SD		.064	.026	.235	.000	.005	14.267	.907	3.886	3.500
Var		.004	.000	.055	.000	.000	203.555	.823	15.101	12.250

[a] Missing data.

Table D-16. Continuous flow steady state for chemostats numbers 9, 10, 11, and 12, at 28°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No./ml NO^{-5}
	12	2.30	.435	28.5	7.20	.143	279	100.00	6.14	87
9	13	2.22	.450	28.5	7.19	.145	255	96.15	10.80	88
	14	2.22	.450	28.5	7.20	.145	260	96.15	7.93	a
Ave		2.246	.445	28.5	7.196	.144	264.666	97.433	8.290	87.500
SD		.037	.007	.000	.004	.000	10.338	1.814	1.919	.500
Var		.001	.000	.000	.000	.000	106.888	3.293	3.684	.250
	12	2.13	.470	28.5	7.20	.170	483	101.92	11.86	83
10	13	2.06	.485	28.5	7.20	.170	475	98.07	4.40	99
	14	2.33	.430	28.0	7.20	.200	498	100.00	13.38	a
Ave		2.173	.461	28.333	7.200	.180	485.333	99.996	9.880	91.000
SD		.114	.023	.235	.000	.014	9.533	1.571	3.924	8.000
Var		.013	.000	.055	.000	.000	90.888	2.470	15.400	64.000
	12	2.13	.470	28.5	7.20	.187	493	101.92	7.60	130
11	13	2.11	.475	28.5	7.21	.174	507	101.92	8.62	103
	14	2.33	.430	28.0	7.21	.185	496	100.00	7.60	a
Ave		2.190	.458	28.333	7.206	.182	498.666	101.280	7.940	116.500
SD		.099	.020	.235	.004	.005	6.018	.905	.480	13.500
Var		.009	.000	.055	.000	.000	36.222	.819	.231	182.250
	12	2.04	.490	28.0	7.20	.156	345	84.61	12.67	67
12	13	1.98	.505	28.5	7.20	.129	389	86.53	12.06	57
	14	2.22	.450	28.0	7.20	.150	438	82.69	10.00	a
Ave		2.080	.481	28.166	7.200	.145	390.666	84.610	11.576	62.000
SD		.101	.023	.235	.000	.011	37.985	1.567	1.142	5.000
Var		.010	.000	.055	.000	.000	1442.888	2.457	1.304	25.000

[a] Missing data.

Table D-17. Continuous flow steady state data for chemostats numbers 13, 14, 15, and 16, at 28°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/.1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No./ml NO^{-5}
	12	2.04	.490	29.0	7.19	.185	468	98.07	11.04	109
13	13	1.94	.515	28.5	7.20	.148	467	92.30	13.45	174
	14	2.22	.450	28.0	7.20	.170	478	92.31	8.00	b
Ave		2.066	.485	28.500	7.196	.167	471.000	94.226	10.830	141.500
SD		.115	.026	.408	.004	.015	4.966	2.717	2.229	32.500
Var		.013	.000	.166	.000	.000	24.666	7.385	4.972	1056.250
	12	2.44	.410	29.0	7.18	.175	404	94.23	14.32	120
14	13	2.08	.480	28.5	7.20	.148	392	90.40	10.00	115
	14	2.22	.450	28.0	7.20	.175	501[a]	96.15	12.07	b
Ave		2.246	.446	28.500	7.193	.166	432.333	93.593	12.130	117.500
SD		.148	.028	.408	.009	.012	48.801	2.390	1.764	2.500
Var		.021	.000	.166	.000	.000	2381.555	5.713	3.112	6.250
	12	2.04	.490	28.5	7.19	.108	263	69.23	15.54	134
15	13	2.00	.500	28.5	7.20	.106	260	67.30	12.40	115
	14	2.06	.485	28.0	7.20	.102	261	71.15	10.69	b
Ave		2.033	.491	28.333	7.196	.105	261.333	69.226	12.874	
SD		.024	.006	.235	.004	.002	1.247	1.571	2.008	
Var		.000	.000	.055	.000	.000	1.555	2.470	4.034	
	12	2.04	.490	28.5	7.20	.174	460	94.23	13.50	83
16	13	2.08	.480	28.5	7.20	.170	453	98.07	14.83	116
	14	2.06	.485	28.0	7.20	.172	454	105.77	10.00	b
Ave		2.066	.485	28.333	7.200	.172	455.666	99.356	12.776	99.500
SD		.016	.004	.235	.000	.001	3.091	4.798	2.037	16.500
Var		.000	.000	.055	.000	.000	9.555	23.023	4.149	272.250

[a]Excessive variation for steady state.

[b]Missing data.

Table D-18. Continuous flow steady state data for chemostats numbers 17, 18, 19, and 20, at 28°C without toxicant.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/.1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No./ml NO^{-5}
	22	4.26	.235	29.0	7.29	.235	496	101.92	6.69	30
17	23	5.26	.190	28.0	7.28	.230	495	101.92	7.01	a
	24	4.48	.223	28.5	7.28	.232	535	a	7.02	15
	25	4.65	.215	28.0	7.30	.226	568	92.31	10.17	a
	26	4.52	.221	28.0	7.28	.222	566	111.54	10.14	16
Ave		4.634	.216	28.300	7.286	.229	532.000	101.922	8.206	20.333
SD		.337	.014	.400	.008	.004	32.018	6.798	1.595	6.847
Var		.113	.000	.160	.000	.000	1025.200	46.224	2.546	46.888
	22	4.34	.230	29.0	7.29	.202	500	101.92	9.15	24
18	23	5.12	.195	28.0	7.29	.202	450	96.15	10.17	a
	24	4.61	.217	28.5	7.30	.208	446	a	10.53	8
	25	4.76	.210	28.0	7.30	.198	447	80.76	12.28	a
	26	4.95	.202	28.5	7.28	.213	460	107.69	17.13	33
Ave		4.756	.210	28.400	7.292	.204	460.600	96.630	11.852	21.666
SD		.270	.012	.374	.007	.005	20.313	10.029	2.825	10.338
Var		.072	.000	.140	.000	.000	412.640	100.598	7.983	106.888
19	13	.63	1.580	28.5	7.11	.089	175	46.15	842.1	0.6
	14	.65	1.550	28.0	7.11	.090	177	44.23	699.3	0.7
Ave		.640	1.565	28.250	7.110	.0895	176.000	45.190	770.700	.650
SD		.010	.015	.250	.000	.000	1.000	.960	71.400	.050
Var		.000	.000	.062	.000	.000	1.000	.921	5097.960	.002
20	13	.70	1.430	28.5	7.12	.097	184	40.38	736.85	1.9
	14	.71	1.400	28.0	7.12	.092	195	42.31	741.1	1.8
Ave		.705	1.415	28.250	7.120	.094	189.500	41.295	738.915	1.850
SD		.005	.015	.250	.000	.002	5.500	1.015	2.125	.050
Var		.000	.000	.062	.000	.000	30.250	1.030	4.515	.002

[a]Missing data.

Table D-19. Continuous flow steady state data for chemostats numbers 1, 2, 3, and 4, at 28°C without toxicant during Experiment number 2.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1^a mg/l	S_1^b NH_4-N μg/l
1	22	1.105	.905	27.5	7.11	.117	200	-	-
	23	1.100	.910	28.5	7.10	.114	216	75.00	10.92
Ave		1.103	.908	28.000	7.105	.115	208.000	75.00	10.92
SD		.002	.002	.500	.005	.001	8.000	-	-
Var		.000	.000	.250	.000	.000	64.000	-	-
2	22	1.130	.885	27.5	7.13	.151	344	-	-
	23	1.117	.895	28.5	7.12	.154	314	81.41	11.85
Ave		1.124	.890	28.000	7.125	.152	329.000	81.41	11.85
SD		.006	.005	.500	.005	.001	15.000	-	-
Var		.000	.000	.250	.000	.000	225.000	-	-
3	22		Non-steady state						
	23								
Ave									
SD									
Var									
4	22	1.460	.685	28.0	7.18	.182	443	-	-
	23	1.460	.685	29.0	7.17	.189	501	95.51	7.03
Ave		1.460	.685	28.500	7.175	.185	472.000	95.51	7.03
SD		.000	.000	.500	.500	.003	29.000	-	-
Var		.000	.000	.250	.000	.000	841.000	-	-

[a]Average of 3 samples.

[b]Average of 2 samples.

Table D-20. Continuous flow steady state data for chemostats numbers 17, 18, 19, and 20, at 28°C without toxicant during Experiment number 2.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1^a mg/l	S_1^b NH_4-N μg/l
17	22		Non-steady state						
	23								
Ave									
SD									
Var									
18	22		Non-steady state						
	23								
Var									
SD									
Var									
19	22		Non-steady state						
	23								
Ave									
SD									
Var									
20	22		Non-steady state						
	23								
Ave									
SD									
Var									

[a]Average of 3 samples.

[b]Average of 2 samples.

Table D-21. Continuous flow steady state data for chemostats numbers 1, 2, 3, and 4, at 33°C without toxicant during Experiment number 2.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l
1	28	1.095	.913	32.0	7.09	.081	78	61.54	106.05
	29	1.076	.929	33.0	7.10	.072	68	56.74	38.38
Ave		1.085	.921	32.500	7.095	.076	73.000	59.140	72.215
SD		.009	.008	.500	.005	.004	5.000	2.400	33.835
Var		.000	.000	.250	.000	.000	25.000	5.760	1144.807
2	28	1.121	.892	32.0	7.10	.092	108	65.38	248.46
	29	1.044	.958	33.0	7.10	.096	72	67.31	246.00
Ave		1.082	.925	32.500	7.100	.094	90.000	66.345	247.23
SD		.038	.033	.500	.000	.002	18.000	.965	1.230
Var		.001	.001	.250	.000	.000	324.000	.931	1.512
3	28	1.403	.713	32.5	7.11	.093	117	64.42	190.58
	29	1.357	.737	33.5	7.10	.081	87	73.08	232.88
Ave		1.380	.725	33.000	7.105	.087	102.00	68.750	211.73
SD		.023	.012	.500	.005	.006	15.000	4.330	21.150
Var		.000	.000	.250	.000	.000	225.000	18.748	447.150
4	28	1.431	.699	32.5	7.11	.119	173	80.77	12.12
	29	1.458	.686	33.5	7.11	.116	107	75.00	14.60
Ave		1.440	.693	33.000	7.110	.117	140.000	77.885	13.360
SD		.013	.006	.500	.000	.001	33.000	2.885	1.240
Var		.000	.000	.250	.000	.000	1089.000	8.323	1.537

Table D-22. Continuous flow steady state data for chemostats numbers 17, 18, 19, and 20, at 33°C without toxicant during Experiment number 2.

Chemostat Number	Days of Operation	θ Days	μ $Days^{-1}$	Temp °C	pH	OD 750 μm	X_1 Cells/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l
17	28	.932	1.072	32.5	7.10	.090	155	67.31	169.68
	29	.959	1.043	32.5	7.10	.082	112	65.39	75.44
Ave		.945	1.058	32.500	7.100	.086	133.50	66.350	122.560
SD		.013	.014	.000	.000	.004	21.500	.960	47.120
Var		.000	.000	.000	.000	.000	462.250	.921	2220.294
18	28	.894	1.118	32.5	7.11	.101	189	67.31	69.69
	29	.897	1.115	32.5	7.12	.108	134	65.39	a
Ave		.895	1.117	32.500	7.115	.104	161.500	66.350	69.69
SD		.001	.001	.000	.005	.003	27.500	.960	-
Var		.000	.000	.000	.000	.000	756.250	.921	-
19	28	.805	1.242	32.5	7.09	.104	218	71.15	802.95
	29	.777	1.287	32.5	7.10	.115	182	76.92	a
Ave		.791	1.265	32.500	7.095	.109	200.00	74.035	802.95
SD		.014	.022	.000	.005	.005	18.000	2.885	-
Var		.000	.000	.000	.000	.000	324.000	8.323	-
20	28	.688	1.454	32.0	7.06	.062	114	51.92	1954.35
	29	.706	1.416	32.5	7.09	.077	122	51.92	1377.60
Ave		.697	1.435	32.250	7.075	.069	118.000	51.920	1665.98
SD		.009	.019	.250	.015	.007	4.000	.000	288.375
Var		.000	.000	.062	.000	.000	16.000	.000	83160.140

[a]Missing data.

Table D-23. Continuous flow kinetic data at 20°C without toxicant.

μ Days^{-1}	S_1 µg/l	X_1 Cells/ .1 mm^3	$\frac{1}{\mu}$ Days	S_1/μ 1/NH_4-N µg	S_1/μ Days µg/l	μ/S_1 1/µg Day
1.370	2268.11	91	0.73	0.00044	1655.55	0.00060
1.340	1690.54	114	0.75	0.00059	1261.60	0.00079
0.990	7.47	113	1.01	0.134	7.55	0.133
0.963	4.33	111	1.04	0.231	4.50	0.222
0.727	4.66	205	1.38	0.215	6.41	0.156
0.696	3.39	300	1.44	0.295	4.87	0.205
0.675	4.72	256	1.48	0.212	6.99	0.143
0.527	5.37	428	1.90	0.186	10.19	0.098
0.525	3.83	453	1.90	0.190	7.30	0.137
0.476	4.03	460	2.10	0.248	8.47	0.118

Table D-24. Continuous flow kinetic data at 24°C without toxicant.

μ Days^{-1}	S_1 µg/l	X_1 Cells/ .1 mm^3	$\frac{1}{\mu}$ Days	S_1/μ 1/NH_4-N µg	S_1/μ Days µg/l	μ/S_1 1/µg Day
1.530	842.45	138	0.65	0.0012	550.33	0.0018
1.410	6.88	129	0.71	0.145	4.88	0.205
1.140	14.53	149	0.88	0.069	12.75	0.078
1.140	5.32	195	0.88	0.188	4.67	0.214
0.996	1.88	219	1.00	0.532	1.89	0.530
0.987	3.24	275	1.01	0.309	3.28	0.305
0.983	4.71	278	1.02	0.212	4.79	0.209
0.966	1.40	206	1.04	0.714	1.45	0.690
0.945	5.60	140	1.06	0.179	5.93	0.169
0.932	14.99	247	1.07	0.067	16.08	0.062
0.737	14.81	237	1.37	0.068	20.09	0.050
0.711	9.14	348	1.41	0.109	12.86	0.078
0.646	3.14	641	1.55	0.318	4.86	0.206
0.611	3.97	661	1.64	0.252	6.49	0.154
0.600	4.91	682	1.67	0.204	8.18	0.122
0.595	1.83	540	1.68	0.546	3.07	0.325
0.540	3.16	761	1.85	0.316	5.85	0.171
0.538	4.27	805	1.86	0.234	7.94	0.126
0.530	2.86	738	1.89	0.350	5.40	0.186
0.528	5.15	712	1.89	0.194	9.75	0.102
0.518	5.09	728	1.93	0.196	9.83	0.102
0.513	5.30	720	1.95	0.189	10.33	0.097
0.508	4.36	749	1.97	0.229	8.58	0.117
0.505	4.98	738	1.98	0.201	9.86	0.101

Table D-25. Continuous flow kinetic data at 27°C without toxicant.

μ Days^{-1}	S_1 µg/l	X_1 Cells/.1 mm^3	$\frac{1}{\mu}$ Days	S_1/μ 1/NH_4-N µg	S_1/μ Days µg/l	μ/S_1 1/µg Day
1.519	808.40	240	.658	.0012	532.19	.0019
1.367	196.60	199	.732	.0050	143.82	.0070
1.080	2.91	241	.926	.344	2.69	.371
.922	8.93	150	1.085	.112	9.69	.103
.914	15.48	196	1.094	.065	16.94	.059
.698	6.20	449	1.432	.161	8.88	.113
.694	2.87	514	1.441	.348	4.14	.242

Table D-26. Continuous flow kinetic data at 28°C without toxicant.

μ Days^{-1}	S_1 µg/l	X_1 Cells/.1 mm^3	$\frac{1}{\mu}$ Days	S_1/μ 1/NH_4-N µg	S_1/μ Days µg/l	μ/S_1 1/µg Day
1.565	770.70	176	0.64	0.0013	492.46	.0020
1.415	738.92	190	0.71	0.0014	522.20	0.0019
1.009	9.98	192	0.99	0.100	9.89	.101
0.986	5.59	280	1.01	.179	5.67	.176
0.975	6.27	268	1.03	.159	6.43	.156
0.966	17.20	279	1.04	.058	17.81	.056
0.908	10.92	208	1.10	.092	12.03	.083
0.895	11.85	329	1.12	.084	13.24	.076
0.690	9.43	294	1.45	.106	13.67	.073
0.685	7.03	472	1.46	.142	10.26	.097
0.676	6.38	366	1.48	.157	9.44	.106
0.645	11.15	330	1.55	.090	17.29	.058
0.623	11.45	210	1.61	.087	18.38	.054
0.491	12.87	261	2.04	.078	26.21	.038
0.485	12.78	455	2.06	.078	26.35	.038
0.485	10.83	471	2.06	.092	22.33	.045
0.481	11.58	391	2.08	.086	24.07	.042
0.461	9.88	485	2.17	.101	24.43	.047
0.458	9.94	499	2.18	.101	21.70	.046
0.446	12.13	432	2.24	.082	27.20	.037
0.445	8.29	264	2.25	.121	18.63	.054
0.216	8.21	532	4.63	0.122	38.01	.026
0.210	11.85	461	4.76	0.084	56.43	.018

Table D-27. Continuous flow kinetic data at 33°C without toxicant.

μ Days^{-1}	S_1 µg/l	X_1 Cells/.1 mm^3	$\frac{1}{\mu}$ Days	S_1/μ 1/NH_4-N µg	S_1/μ Days µg/l	μ/S_1 1/µg Day
1.435	1665.98	118	.697	.00060	1160.96	.00086
1.265	802.95	200	.791	.00124	634.74	.00157
1.117	69.69	162	.895	.0143	62.39	.016
1.058	122.56	134	.945	.0082	115.84	.0086
.925	247.33	90	1.044	.0040	258.17	.0039
.921	72.22	73	1.086	.0138	78.41	.0127
.725	211.73	102	1.379	.0047	292.04	.0034
.693	13.36	140	1.443	.0748	19.28	.052

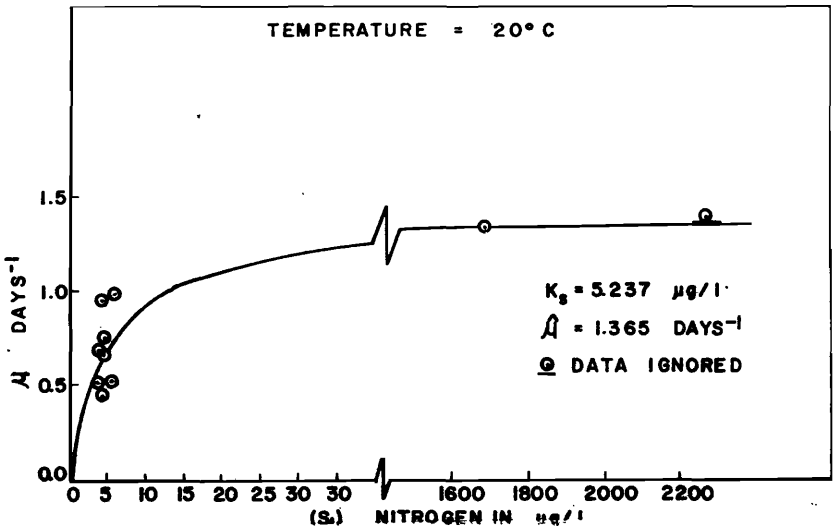

Figure D-1. Michaelis-Menten (Monod) kinetic model at 20°C without toxicant.

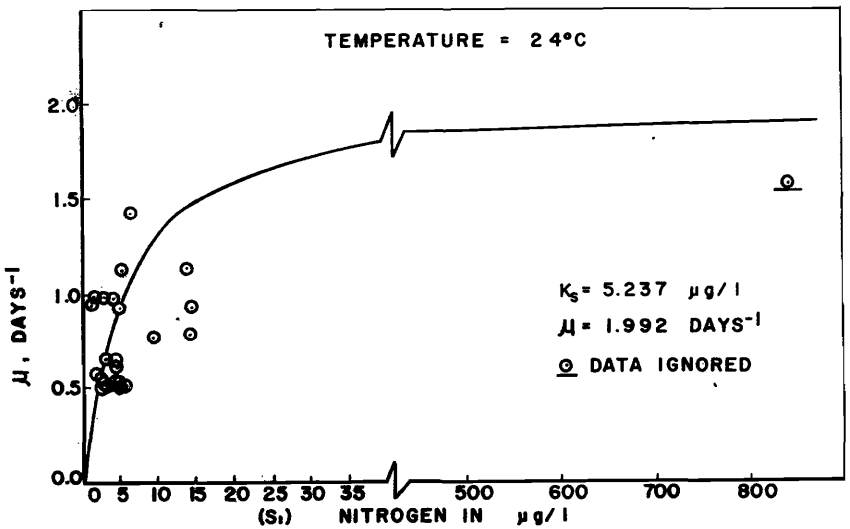

Figure D-2. Michaelis-Menten (Monod) kinetic model at 24°C without toxicant.

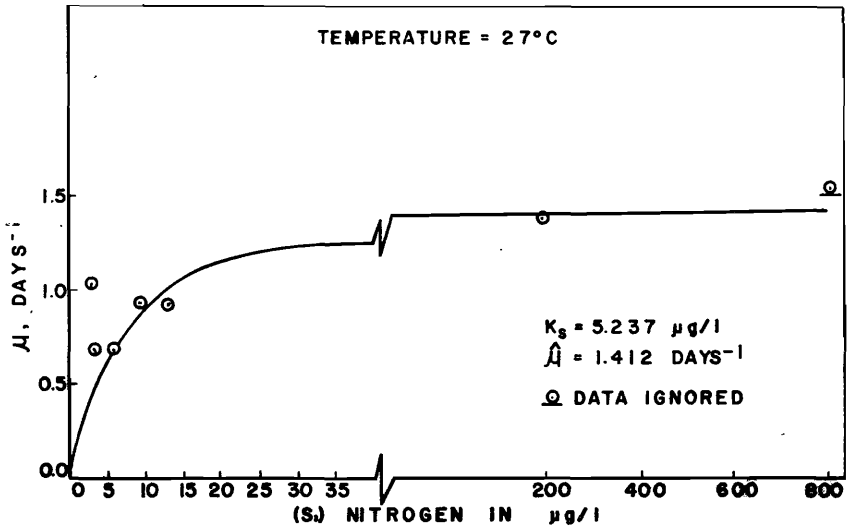

Figure D-3. Michaelis-Menten (Monod) kinetic model at 27°C without toxicant.

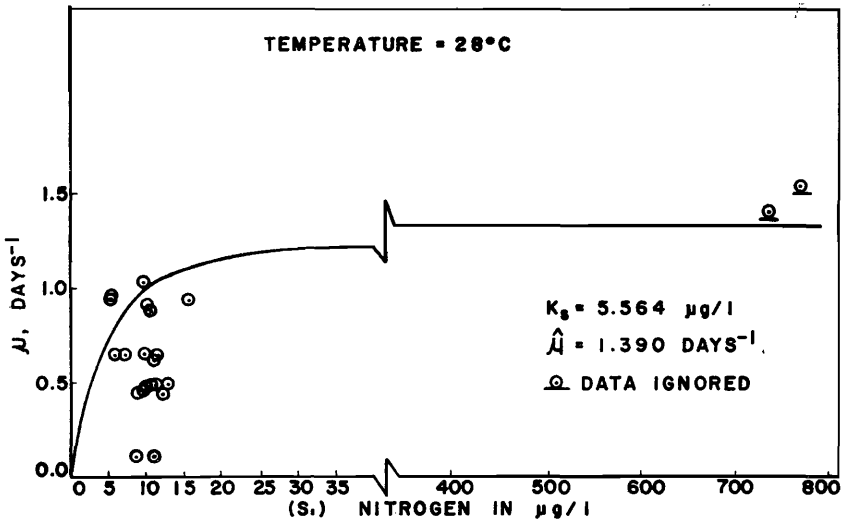

Figure D-4. Michaelis-Menten (Monod) kinetic model at 28°C without toxicant.

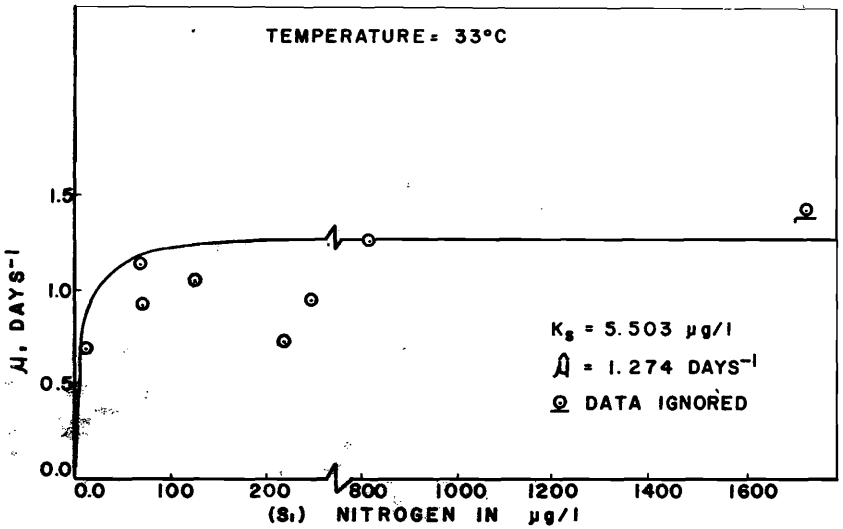

Figure D-5. Michaelis-Menten (Monod) kinetic model at 33°C without toxicant.

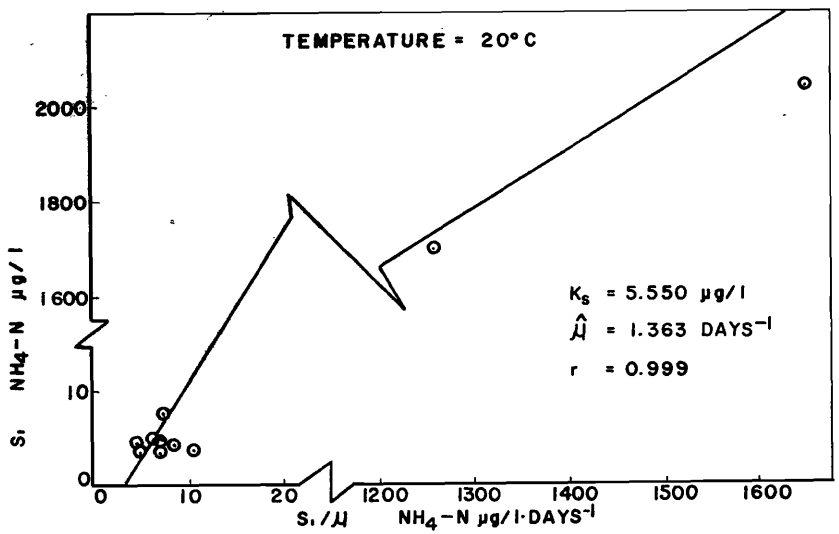

Figure D-6. Linear transformation of Michaelis-Menten (Monod) equation, $S_1 = (S_1/\mu)\hat{\mu} - K_s$.

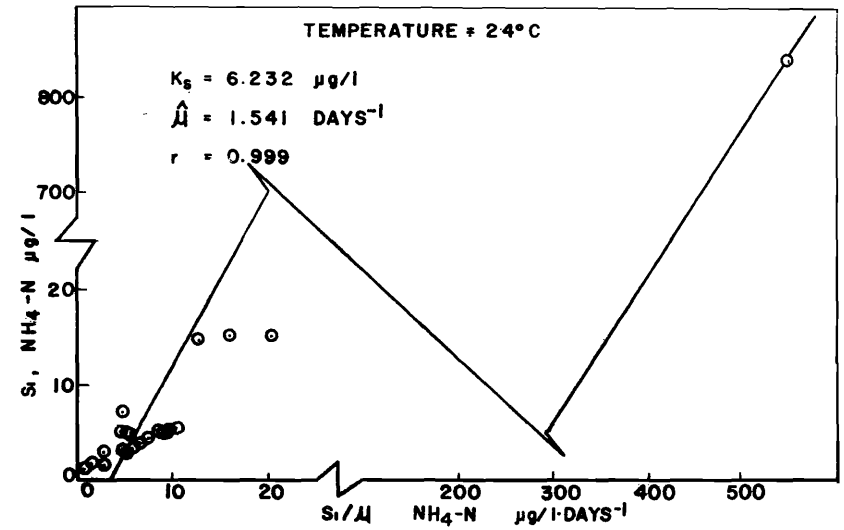

Figure D-7. Michaelis-Menten (Monod) kinetic model at 24°C without toxicant.

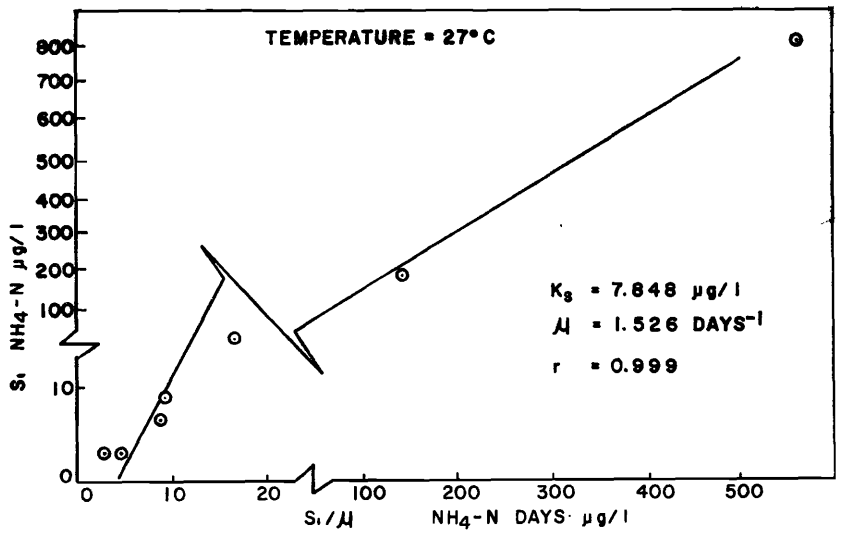

Figure D-8. Linear transformation of Michaelis-Menten (Monod) equation, $S_1 = (S_1/\mu)\hat{\mu} - K_s$.

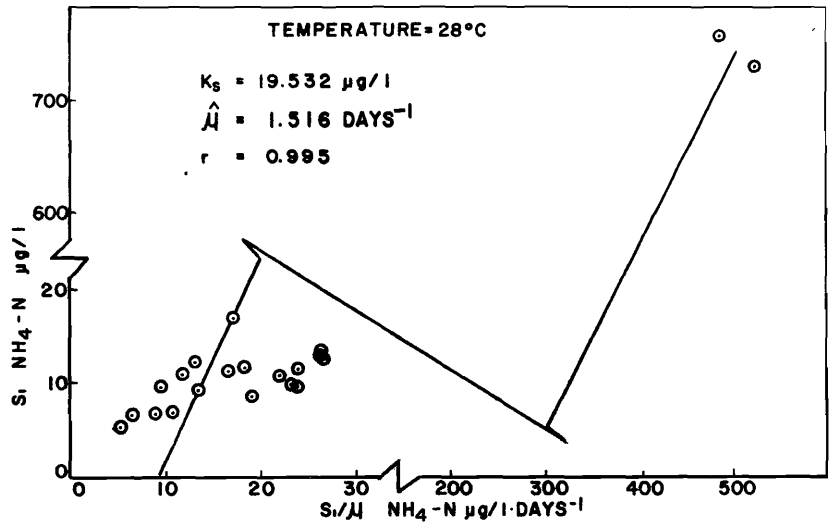

Figure D-9. Linear transformation of Michaelis-Menten (Monod) equation, $S_1 = (S_1/\mu)\hat{\mu} - K_s$.

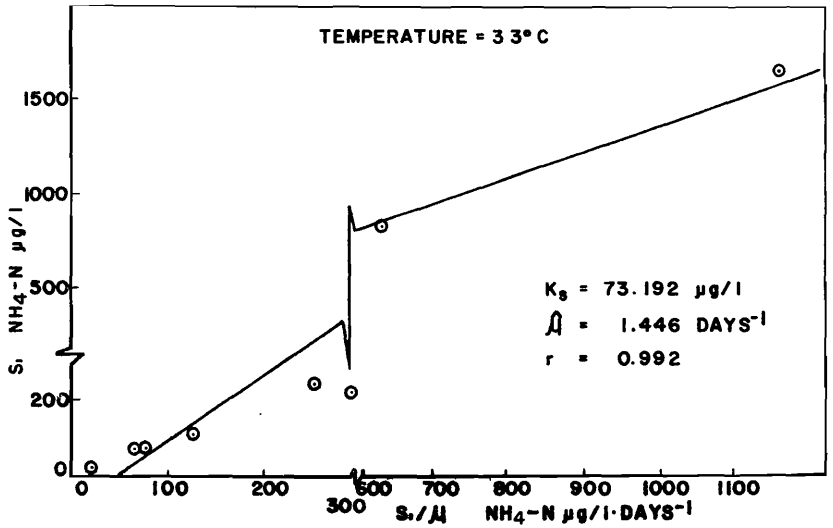

Figure D-10. Linear transformation of Michaelis-Menten (Monod– equation, $S_1 = (S_1/\mu)\hat{\mu} - K_s$.

Appendix E

Continuous Flow Kinetic Data with Toxicant

Table E-1. Continuous flow steady state data for chemostats numbers 1, 2, 3, and 4, at 20°C with toxicant.

Chemo-stat No.	I_o ϕ mg/l	Days of Oper.	θ Days	μ^{-1} days^{-1}	Temp °C	pH	OD 750 μm	X_1 cell/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bac. No./ ml x10^{-6}	I_1^a ϕ mg/l
1	0.0	33	1.02	.982	18.0	7.06	.109	192	73.08	b	60	0.0
		34	1.10	.915	17.5	7.04	.097	185	69.23	8.46	55	
		35	1.11	.900	17.5	7.03	.140	190	b	8.45	15	
Ave			1.076	.932	17.666	7.043	.115	189.00	71.155	8.455	43.333	
SD			.040	.035	.235	.012	.018	2.943	3.705	.005	20.138	
Var			.001	.001	.055	0.000	.000	8.666	1.925	.000	405.138	
2	20.0	33	1.00	1.00	18.0	7.00	.076	96	54.67	2.42	109	2.4
		34	1.08	.930	17.5	7.00	.052	94	42.00	b	83	
		35	1.09	.920	17.5	7.01	.067	97	49.33	1.41	15	
Ave			1.056	.950	17.666	7.003	.065	95.666	48.000	1.915	69.000	
SD			.040	.035	.235	.004	.009	1.247	6.062	.505	39.631	
Var			.001	.001	.055	.000	.000	1.555	36.752	.255	1570.666	
3	40.0	33	1.04	.966	19.0	6.99	.060	24	49.33	3.63	108	2.4
		34	1.10	.905	18.0	6.99	.059	25	44.00	6.77	170	
		35	1.11	.900	18.0	6.99	.072	25	43.99	b	b	
Ave			1.083	.923	18.333	6.990	.063	24.666	43.773	5.200	139.000	
SD			.030	.030	.471	.000	.005	.471	2.514	1.570	31.000	
Var			0.000	.000	.222	.000	.000	.222	6.324	2.464	961.000	
4	30.0	33	1.02	.982	19.0	6.99	.060	45	50.00	3.03	102	1.2
		34	1.07	.935	18.0	6.99	.050	43	b	5.76	91	
		35	1.09	.920	18.0	6.99	.070	46	56.00	7.04	15	
Ave			1.060	.945	18.333	6.990	.060	44.666	53.000	5.276	69.333	
SD			.029	.026	.471	.000	.008	1.247	3.000	1.672	38.681	
Var			0.000	.000	.222	.000	.000	1.555	9.000	2.796	1496.222	

[a]Average of at least 3 samples.

[b]Missing data.

Table E-2. Continuous flow steady state data for chemostats numbers 5, 6, 7, and 8, at 20°C with toxicant.

Chemo-stat No.	I_o ϕ mg/l	Days of Oper.	θ Days	μ^{-1} days^{-1}	Temp °C	pH	OD 750 μm	X_1 cell / .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bac. No./ ml x10^{-6}	I_1^a ϕ mg/l
5	20.0	33	1.41	.711	18.5	7.01	.075	100	61.53	9.99	97	2.5
		34	1.49	.670	18.0	7.00	.062	92	42.67	4.06	108	
		35	1.52	.660	18.5	7.00	.069	96	54.67	b	b	
Ave			1.473	.680	18.333	7.003	.068	96.000	53.003	7.025	102.50	
SD			.046	.022	.235	.004	.005	3.265	7.845	2.965	5.500	
Var			.002	.000	.055	.000	.000	10.666	61.555	8.791	30.250	
6	30.0	33	1.33	.754	18.5	7.01	.074	68	45.33	5.45	148	2.0
		34	1.38	.725	18.0	7.00	.058	62	40.00	2.71	126	
		35	1.41	.710	18.5	7.00	.060	63	42.67	4.23	b	
Ave			1.373	.726	18.333	7.003	.064	64.333	42.660	4.130	137.000	
SD			.032	.014	.235	.004	.007	2.624	2.175	1.120	11.000	
Var			.001	.000	.055	.000	.000	6.888	4.734	1.256	121.000	
7		33										
		34										
		35										
Ave				Excessive Bacteria Growth								
SD												
Var												
8	40.0	33	1.36	.737	19.0	7.000	.046	21	41.33	9.08	96	1.8
		34	1.45	.690	19.5	7.000	.046	21	37.33	3.38	113	
		35	1.45	.690	19.0	7.000	.062	28	48.00	5.91		
Ave			1.420	.705	19.166	7.000	.051	23.333	42.220	6.123	104.500	
SD			.042	.022	.235	0.000	.007	3.299	4.401	2.331	8.500	
Var			.001	.000	.055	0.000	.000	10.888	19.370	5.437	72.250	

[a]Average of at least 3 samples.

[b]Missing data.

Table E-3. Continuous flow steady state data for chemostats numbers 9, 10, 11, and 12, at 20°C with toxicant.

Chemo-stat. No.	I_o ϕ mg/l	Days of Oper.	θ Days	μ $days^{-1}$	Temp °C	pH	OD 750 μ m	X_1 cell/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bac. No./ $mlx10^{-6}$	I_1^a ϕ mg/l
9	20.0	33	1.86	.539	18.5	7.08	.137	184	b	7.57	121	1.8
		34	1.89	.530	18.5	7.02	.133	187	57.33	3.38	125	
		35	2.10	.475	18.5	7.02	.141	190	64.00	3.38	b	
Ave			1.950	.514	18.500	7.040	.137	187.00	60.665	4.776	123.000	
SD			.106	.028	0.000	.028	.000	2.449	3.335	1.975	2.000	
Var			.011	.000	0.000	.000	.003	6.000	11.122	3.901	4.000	
10	0.0	33	1.67	.598	18.5	7.14	.188	428	105.76	6.06	110	0.0
		34	1.88	.530	18.5	7.10	.180	439	92.31	3.38	94	
		35	1.92	.520	18.5	7.10	.182	428	b	3.66	b	
Ave			1.823	.549	18.500	7.113	.183	431.666	99.035	4.366	102.000	
SD			.109	.034	.000	.013	.003	5.185	6.725	1.202	8.000	
Var			.012	.001	.000	.000	.000	26.888	45.225	1.446	64.000	
11		33										
		34										
		35			Excessive Bacteria Growth							
Ave												
SD												
Var												
12	30.0	33	1.85	.540	19.5	7.01	.082	73	58.67	b	147	1.8
		34	1.96	.510	19.0	7.01	.070	79	48.00	3.40	118	
		35	2.13	.470	19.0	7.01	.084	80	53.33	4.08	b	
			1.988	.506	19.166	7.010	.078	77.333	53.333	3.740	132.500	
Ave			.115	.028	.235	.000	.006	3.091	4.356	.340	14.500	
SD			.013	.000	.055	.000	.000	9.555	18.974	.115	210.250	
Var												

[a] Average of at least 3 replicates.

[b] Missing data.

Table E-4. Continuous flow steady state data for chemostats number 13, 14, 15, and 16, at 20°C with toxicant.

Chemo stat No.	I_o ϕ mg/l	Days of Oper.	θ Days	μ $days^{-1}$	Temp. °C	pH	OD 750 μm	X_1 cell/ .1 mm^3	X_1 mg/l	S_1 NH_4-N μg/l	Total Bacteria No./ $mlx10^{-6}$	I_1^a ϕ mg/l
13		33			Excessive Bacteria Growth							
		34										
		35										
Ave												
SD												
Var												
14	40.0	33	1.96	.511	19.0	7.01	.054	27	38.76	9.08	119	1.7
		34	1.82	.550	19.0	7.00	.052	26	44.00	6.77	123	
		35	1.90	.525	18.0	7.01	.062	28	46.67	10.99	b	
Ave			1.893	.528	18.666	7.006	.056	27.000	43.143	8.946	121.000	
SD			.057	.016	.471	.004	.004	.816	3.285	1.725	2.000	
Var			.003	.000	.222	.000	.000	.666	10.794	2.976	4.000	
15		33			Excessive Bacteria Growth							
		34										
		35										
Ave												
SD												
Var												
16		33			Excessive Bacteria Growth							
		34										
		35										
Ave												
SD												
Var												

[a] Average of at least 3 replicates.

[b] Missing data.

Table E-5. Continuous flow steady state data for chemostat numbers 1, 2, 3, and 4, at 24°C with toxicant.

Chemo-stat No.	I_o ϕ mg/l	Days of Oper.	θ Days	μ days^{-1}	Temp. °C	pH	OD 750 µm	X_1 cell/ .1 mm^3	X_1 mg/l	S_1 NH_4-N µg/l	Total Bacteria No./ ml x 10^{-6}	I_1 [a] ϕ mg/l
1	60.0	33	1.04	.958	24.0	7.03	.090	80	55.77	2.92	86	14.0
		34	1.03	.970	24.0	7.03	.078	83	55.77	b	61	
		35	1.06	.947	24.0	7.03	.090	85		1.22	81	
Ave			1.043	.967	24.000	7.03	.086	82.666	55.77	2.070	76.000	
SD			.012	.015	.000	.000	.005	2.054	.000	.850	10.801	
Var			.000	.000	.000	.000	.000	4.222	.000	.722	116.666	
2	40.0	33	1.15	.868	24.0	7.03	.065	50	53.85	6.14	59	2.0
		34	1.01	.995	24.5	7.05	.060	53	38.46	2.50	98	
		35	1.01	.995	24.0	7.05	.040	56	44.23	2.44	69	
Ave			1.056	.952	24.166	7.043	.055	53.000	45.513	3.693	75.333	
SD			.065	.059	.235	.009	.010	2.449	6.348	1.730	16.539	
Var			.004	.003	.055	.000	.000	6.000	40.298	3.693	273.555	
3	20.0	33	1.03	.970	24.0	7.10	.115	230	88.46	9.36	17	2.0
		34	1.00	.996	24.5	7.10	.143	254	90.38	12.50	69	
		35	.88	1.135	24.5	7.09	.123	200	80.77	6.10	24	
Ave			.970	1.033	24.333	7.096	.123	228.000	86.536	9.320	36.666	
SD			.064	.072	.235	.004	.015	22.090	4.152	2.612	23.041	
Var			.004	.005	.055	.000	.000	488.00	17.241	6.827	530.888	
4	0.00	33	1.00	1.001	24.0	7.13	.111	200	57.69	b	33	0.0
		34	.99	1.008	24.5	7.13	.115	236	65.38	2.25	26	
		35	.95	1.058	24.0	7.13	.093	200	67.31	4.39	16	
Ave			.980	1.022	24.166	7.13	.106	212.000	63.460	3.305	25.000	
SD			.021	.025	.235	.000	.009	16.970	4.155	1.055	6.976	
Var			.000	.000	.055	.000	.000	288.000	17.267	1.113	48.666	

[a] Average of at least 3 replicates.

[b] Missing data.

Table E-6. Continuous flow steady state data for chemostat numbers 5, 6, 7, and 8, at 24°C with toxicant.

Chemo-stat No.	I_o ϕ mg/l	Days of Oper.	θ Days	μ days^{-1}	Temp. °C	pH	OD 750 µm	X_1 cell/ .1mm^3	X_1 mg/l	S_1 NH_4-N µg/l	Total Bacteria No./ mlx10^{-6}	I_1 [a] ϕ mg/l
5	60.0	34	1.68	.594	24.0	7.07	.095	63	63.46	5.56	35	18.0
		35	1.64	.611	24.0	7.07	.075	68	69.23	b	72	
		36	1.62	.619	24.0	7.07	.075	71	63.46	4.88	72	
Ave			1.646	.608	24.000	7.07	.081	67.333	65.383	5.220	59.666	
SD			.024	.010	.000	.000	.009	3.299	2.720	.340	17.441	
Var			.000	.000	.000	.000	.000	10.888	7.390	.115	304.222	
6	40.0	34	1.81	.553	24.0	7.09	.098	70	57.69	2.25	67	2.0
		35	1.78	.562	24.0	7.09	.90	100	65.38	2.20	92	
		36	1.74	.574	24.0	7.09	.088	60	44.23	b	91	
Ave			1.776	.563	24.000	7.09	.092	76.666	55.716	2.025	83.333	
SD			.028	.008	.000	.000	.004	16.996	8.686	.025	11.556	
Var			.000	.000	.000	.000	.000	288.888	75.447	.000	133.555	
7	20.0	34	1.75	.570	24.0	7.17	.200	332	143.33	7.31	76	2.0
		35	1.72	.581	25.0	7.16	.198	340	143.33	9.50	74	
		36	1.68	.594	25.0	7.16	.170	300	130.00	4.63	84	
Ave			1.716	.581	24.666	7.163	.189	324.000	138.886	7.146	78.000	
SD			.028	.009	.471	.004	.013	17.281	6.283	1.991	4.320	
Var			.000	.000	.222	.000	.000	298.666	39.486	3.966	18.666	
8	0.0	34	1.61	.622	24.5	7.20	.180	600	126.67	b	36	0.0
		35	1.52	.657	25.0	7.20	.182	600	130.00	6.75	31	
		36	1.56	.641	25.0	7.20	.175	608	120.00	6.10	b	
Ave			1.563	.640	24.833	7.200	.179	602.666	125.333	6.425	33.500	
SD			.036	.014	.236	.000	.002	3.771	4.109	.325	2.500	
Var			.000	.000	.055	.000	.000	14.222	16.888	.105	6.250	

[a] Average of at least 3 replicates.

[b] Missing data.

Table E-7. Continuous flow steady state data for chemostats numbers 9, 10, 11, and 12, at 24°C with toxicant.

Chemo-stat No.	I_o ϕ mg/l	Days of Oper.	θ Days	μ $days^{-1}$	Temp. °C	pH	OD 750 μm	X_1 cell/ .1mm^3	X_1 mg/l	S_1 NH_4-N $\mu g/l$	Total Bacteria No./ ml x 10^{-6}	I_1[a] ϕ mg/l
9	60.0	34	2.07	.484	23.0	7.07	.059	25	50.00	11.40	83	19.0
		35	2.06	.485	23.0	7.07	.057	20	57.69	b	86	
		36	1.98	.504	23.0	7.0	.050	25	57.69	2.20	b	
Ave			2.036	.491	23.000	7.073	.055	23.333	55.126	6.800	84.500	
SD			.040	.009	.000	.004	.003	2.357	3.625	4.600	1.500	
Var			.001	.000	.000	.000	.000	5.555	13.141	21.160	2.250	
10	40.0	34	2.06	.485	23.0	7.10	.048	25	53.85	2.92	98	9.5
		35	2.07	.484	24.0	7.09	.060	38	57.69	2.75	103	
		36	2.01	.498	23.0	7.09	.055	25	50.00	2.20	b	
Ave			2.046	.489	23.333	7.093	.054	29.33	53.846	2.623	100.500	
SD			.026	.006	.471	.004	.004	6.128	3.139	.307	2.500	
Var			.000	.000	.222	.000	.000	37.555	9.856	.094	6.250	
11	20.0	34	2.06	.485	25.0	7.11	.179	256	126.67	11.11	69	0.0
		35	2.04	.490	25.0	7.11	.168	258	100.00	7.00	73	
		36	1.97	.507	25.0	7.11	.150	250	130.00	9.51	b	
Ave			2.023	.494	25.000	7.110	.165	254.666	118.890	9.206	71.00	
SD			.038	.009	.000	.000	.000	3.399	13.426	1.691	2.00	
Var			.001	.000	.000	.000	.011	11.555	180.264	2.861	4.000	
12	0.0	34	2.02	.495	25.0	7.20	.202	532	130.00	13.16	25	0.0
		35	1.96	.511	25.0	7.17	.218	564	120.00	b	16	
		36	1.90	.525	25.0	7.17	.175	506	120.00	5.12	b	
Ave			1.960	.518	25.000	7.180	.198	534.000	123.333	9.140	20.500	
SD			.048	.023	.000	.014	.017	23.720	4.714	4.020	4.500	
Var			.002	.000	.000	.000	.000	562.666	22.222	16.160	20.250	

[a]Average of at least 3 replicates.

[b]Missing data.

Table E-8. Continuous flow steady state data for chemostats numbers 13, 14, 15, and 16, at 24°C with toxicant.

Chemo-stat number	I_o ϕ mg/l	Days of Oper.	θ Days	μ $days^{-1}$	Temp. °C	pH	OD 750 μm	X_1 cell/ .1 mm^3	X_1 mg/l	S_1 NH_4-N $\mu g/l$	Total Bacteria No./ ml x 10^{-6}	I_1[a] ϕ mg/l
13	60.0	34	2.11	.475	26.0	7.29	.530	892	320.00	1154.0	72	2.0
	R.F.	35	2.04	.490	26.0	7.29	.540	740	266.67	950.0	72	
		36	2.00	.501	26.0	7.24	.650	1080	393.33	b	b	
Ave			2.050	.488	26.000	7.276	.573	904.000	326.666	1052.000	72.000	
SD			.045	.010	.000	.026	.054	139.063	51.923	102.000	0.000	
Var			.002	.000	.000	.000	.002	19338.666	2696.014	10404.000	.000	
14	40.0	34	2.20	.454	25.5	7.29	.562	1120	306.67	38.01	104	3.0
	R.F.	35	2.13	.469	25.5	7.30	.540	1120	253.33	b	77	
		36	2.10	.477	25.0	7.29	.560	1296	300.00	19.51	b	
Ave			2.143	.466	25.333	7.293	.554	1112.000	286.666	28.760	90.500	
SD			.041	.009	.235	.004	.009	11.313	23.729	9.250	13.500	
Var			.001	.000	.055	.000	.000	128.000	563.081	85.562	182.250	
15	20.0	34	2.00	.500	25.0	7.30	.480	1168	286.67	28.66	75	0.0
	R.F.	35	2.02	.494	25.0	7.30	.485	1080	266.67	b	94	
		36	1.96	.510	25.0	7.31	.495	1144	340.00	19.02	b	
Ave			1.993	.501	25.000	7.303	.486	1130.666	297.780	23.840	84.500	
SD			.024	.006	.000	.004	.006	37.142	30.950	4.820	9.500	
Var			.000	.000	.000	.000	.000	1379.555	957.930	23.232	90.250	
16	0.0	34	1.89	.530	25.0	7.22	.202	672	143.33	10.23	31	0.0
		35	1.90	.525	25.0	7.22	.200	712	140.00	11.75	15	
		36	1.83	.546	25.5	7.24	.208	750	136.67	4.87	b	
Ave			1.873	.533	25.166	7.226	.203	711.333	140.000	8.950	23.000	
SD			.030	.008	.235	.009	.003	31.846	2.718	2.950	8.000	
Var			.000	.000	.055	.000	.000	1014.222	7.392	8.708	64.000	

R.F. = oil refinery waste.

[a]Average of at least 3 replicates.

[b]Missing data.

Table E-9. Continuous flow steady state data for chemostats numbers 1, 2, 3, and 4, at 28°C with toxicant.

Chemostat No.	I_o φ mg/l	Days of Oper.	θ days	μ $days^{-1}$	Temp °C	pH	OD 750 μm	X_1 cell/ $.1mm^3$	X_1 mg/l	S_1[b] NH_4-N μg/l	Total Bacteria No./ ml x10-6	I_1[a] φ mg/l
1	20.0	24	1.305	.766	28.5	7.10	.100	94	c	2.46	204	3.33
		25	1.265	.790	28.5	7.08	.087	93	46.15	4.21	15	
		26	1.300	.769	28.5	7.02	.097	93	51.92	c	138	
Ave			1.290	.775	28.500	7.066	.094	93.333	49.035	3.335	119.000	
SD			.017	.010	.000	.033	.005	.471	2.885	.875	78.319	
Var			.000	.000	.000	.001	.000	.222	8.323	.765	6134.000	
2	0.0	24	.980	1.020	29.0	7.18	.110	172	75.00	7.04	62	0.000
		25	1.052	.950	28.5	7.14	.068	116	53.85	4.91	c	
		26	1.084	.922	28.5	7.12	.110	166	71.15	c	32	
Ave			1.038	.964	28.666	7.146	.096	151.333	66.666	5.975	47.000	
SD			.043	.041	.235	.024	.019	25.104	9.198	1.065	15.000	
Var			.001	.001	.055	.000	.000	630.222	84.603	1.134	225.000	
3	40.0	24	1.063	.940	29.0	7.10	.070	20	71.15	10.56	114	6.81
		25	1.086	.920	28.0	7.09	.059	20	50.00	7.71	94	
		26	1.089	.918	28.5	7.05	.068	21	61.54	c	127	
Ave			1.079	.926	28.500	7.080	.065	20.333	60.896	9.235	111.666	
SD			.011	.009	.408	.021	.004	.471	8.646	1.525	13.572	
Var			.000	.000	.166	.000	.000	.222	74.760	2.325	184.222	
4	30.0	24	1.042	.959	29.0	7.12	.095	100	76.92	6.34	150	2.76
		25	1.069	.935	28.0	7.10	.089	106	61.53	8.07	285	
		26	1.062	.941	28.5	7.05	.083	99	75.00		300	
Ave			1.057	.945	28.500	7.090	.089	101.666	71.150	7.205	245.000	
SD			.011	.010	.408	.029	.004	3.091	6.847	.865	67.453	
Var			.000	.000	.166	.000	.000	9.555	46.886	.748	4550.000	

[a]Average of at least 3 replicates.

[b]Data for two days only.

[c]Missing data.

Table E-10. Continuous flow steady state data for chemostats numbers 5, 6, 7, and 8, at 28°C with toxicant.

Chemostat No.	I_o φ mg/l	Days of Oper.	θ days	μ $days^{-1}$	Temp. °C	pH	OD 750 μm	X_1 cell/ $.1mm^3$	X_1 mg/l	S_1[b] NH_4-N μg/l	Total Bacteria No./ ml x10-6	I_1[a] φ mg/l
5	0.0	24	1.402	.713	28.0	7.19	.122	170	78.84	8.81	150	0.0
		25	1.470	.680	27.5	7.16	.104	171	c	8.77	c	
		26	1.428	.700	28.0	7.12	.094	162	75.500	c	41	
Ave			1.433	.697	27.833	7.156	.106	167.666	76.920	8.790	95.500	
SD			.028	.013	.235	.028	.011	4.027	1.920	.020	54.500	
Var			.000	.000	.055	.000	.000	16.222	3.686	.000	2970.250	
6	20.0	24	1.499	.667	28.0	7.18	.080	67	59.61	3.52	147	4.28
		25	1.587	.630	27.5	7.14	.074	66	48.00	1.75	94	
		26	1.552	.644	28.0	7.08	.065	63	57.69	c	201	
Ave			1.546	.647	27.833	7.133	.073	65.333	55.100	2.635	147.333	
SD			.036	.015	.235	.041	.006	1.699	5.081	.885	43.683	
Var			.001	.000	.055	.001	.000	2.888	25.819	.783	1908.222	
7	30.0	24	1.488	.672	28.5	7.18	.080	58	76.92	6.69	95	6.81
		25	1.562	.640	28.0	7.12	.071	56	67.31	10.53	285	
		26	1.524	.656	29.0	7.08	.070	56	73.00	c	118	
Ave			1.524	.656	28.500	7.126	.073	56.666	72.410	8.610	166.00	
SD			.030	.013	.408	.041	.004	.942	3.945	1.920	84.667	
Var			.000	.000	.166	.001	.000	.888	15.566	3.686	7168.666	
8	40.0	24	1.567	.638	28.5	7.13	.067	21	73.07	11.27	76	5.69
		25	1.639	.610	28.0	7.12	.062	20	61.54	10.53	291	
		26	1.569	.637	29.0	7.09	.063	22	67.31	c	228	
Ave			1.591	.628	28.500	7.113	.064	21.000	67.306	10.900	198.333	
SD			.033	.013	.408	.016	.002	.816	4.707	.370	90.245	
Var			.000	.000	.166	.000	.000	.666	22.156	.136	8144.222	

[a]Average of at least 3 replicates.

[b]Data for 2 days only.

[c]Missing data.

Table E-11. Continuous flow steady state data for chemostats numbers 9, 10, 11, and 12, at 23°C with toxicant.

Chemo-stats No.	I_o ϕ mg/l	Days of Oper.	θ days	μ $days^{-1}$	Temp. °C	pH	OD 750 µm	X_1 cell/ $.1mm^3$	X_1 mg/l	S_1[b] NH_4-N µg/l	Total Bacteria No./ ml x 10^{-6}	I_1[a] ϕ mg/l
9	0.0	24	2.212	.452	29.0	7.22	.177	358	96.15	5.63	60	0.0
		25	2.061	.485	28.0	7.21	.163	351	90.38	6.67	c	
		26	2.288	.437	29.0	7.19	.167	367	86.54	c	79	
Ave			2.186	.458	28.666	7.206	.169	358.666	91.023	6.150	69.500	
SD			.093	.020	.471	.012	.005	6.548	3.949	.520	9.500	
Var			.008	.000	.222	.000	.000	42.888	15.598	.270	90.250	
10	20.0	24	1.930	.518	29.0	7.20	.151	194	90.00	13.68	177	7.35
		25	2.173	.460	28.0	7.18	.150	194	82.69	13.64	185	
		26	2.392	.418	29.0	7.19	.134	183	75.00	c	104	
Ave			2.165	.465	28.666	7.190	.145	190.333	82.563	13.660	155.333	
SD			.188	.040	.471	.008	.007	5.185	6.124	.020	36.444	
Var			.035	.001	.222	.000	.000	26.888	37.508	.000	1328.222	
11	30.0	24	2.247	.445	28.5	7.18	.094	64	69.23	9.51	147	5.36
		25	2.272	.440	28.0	7.14	.087	59	69.23	8.74	196	
		26	2.415	.414	28.5	7.09	.080	57	63.46	c	224	
Ave			2.311	.433	28.333	7.136	.087	60.000	67.306	9.125	189.000	
SD			.074	.013	.235	.036	.005	2.943	2.820	.385	31.822	
Var			.005	.000	.055	.001	.000	8.666	7.398	.148	1012.666	
12	20.0	24	1.996	.501	28.5	7.28	.256	360	124.99	192.9	174	8.85
	R.F.	25	2.127	.470	28.0	7.29	.272	324	153.33	174.8	149	
		26	2.044	.489	28.5	7.28	.280	401	163.33	c	126	
Ave			2.055	.486	28.333	7.283	.269	361.666	147.216	183.850	149.666	
SD			.054	.012	.235	.004	.009	31.457	16.238	9.050	19.601	
Var			.002	.000	.055	.000	.000	989.555	263.679	81.902	384.222	

[a]Average of at least 3 replicates.

[b]Data for 2 days only.

[c]Missing data.

R.F. = oil refinery waste.

Table E-12. Continuous flow steady state data for chemostats numbers 13, 14, 15, and 16, at 28°C with toxicant.

Chemo-stats No.	I_o ϕ mg/l	Days of Oper.	θ days	μ $days^{-1}$	Temp °C	pH	OD 750 µm	X_1 cell/ $.1mm^3$	X_1 mg/l	S_1[b] NH_4-N µg/l	Total Bacteria No./ ml x 10^{-6}	I_1[a] ϕ mg/l
13	30.0	24	2.212	.452	29.0	7.33	.380	800	173.08	10.91	295	8.35
	R.F.	25	2.247	.445	28.0	7.33	.395	817	146.67	10.14	180	
		26	2.288	.437	28.5	7.30	.370	804	203.33	c	276	
Ave			2.249	.444	28.500	7.320	.381	807.000	174.026	10.525	250.333	
SD			.031	.006	.408	.014	.010	7.257	22.732	.385	50.334	
Var			.000	.000	.166	.000	.000	52.666	516.787	.148	2533.555	
14	40.0	24	2.155	.464	29.0	7.34	.455	968	136.67	17.54	300	13.45
	R.F.	25	2.380	.420	28.0	7.32	.519	1050	240.00	17.13	270	
		26	2.242	.446	28.5	7.32	.490	1002	273.33	c	276	
Ave			2.259	.443	28.500	7.326	.488	1006.666	216.666	17.335	282.000	
SD			.092	.018	.408	.009	.026	33.638	58.179	.205	12.961	
Var			.008	.000	.166	.000	.000	1131.555	3384.881	.042	168.000	
15	0.0	24	1.949	.513	28.5	7.28	.140	288	63.46	9.86	60	0.0
		25	2.325	.430	28.0	7.25	.140	286	69.23	19.58	83	
		26	2.242	.446	28.5	7.19	.143	288	82.69	c	117	
Ave			2.172	.463	28.333	7.240	.141	287.333	71.793	14.720	96.666	
SD			.161	.035	.235	.037	.001	.942	8.057	4.860	23.414	
Var			.026	.000	.055	.001	.000	.888	64.917	23.619	548.222	
16	40.0	24	2.136	.468	28.5	7.14	.095	37	75.00	14.78	74	41.54
		25	2.380	.420	28.0	7.14	.072	38	c	27.71	136	
		26	2.392	.418	28.0	7.10	.071	37	63.46	c	95	
Ave			2.302	.435	28.166	7.126	.079	37.333	69.230	21.245	101.666	
SD			.117	.023	.235	.018	.011	.471	5.770	6.465	25.746	
Var			.013	.000	.055	.000	.000	.222	33.292	41.796	662.888	

[a]Average of at least 3 replicates.

[b]Data for 2 days only.

[c]Missing data.

R.F. = oil refinery waste.

Appendix F

Figures of Continuous Flow Kinetic Data

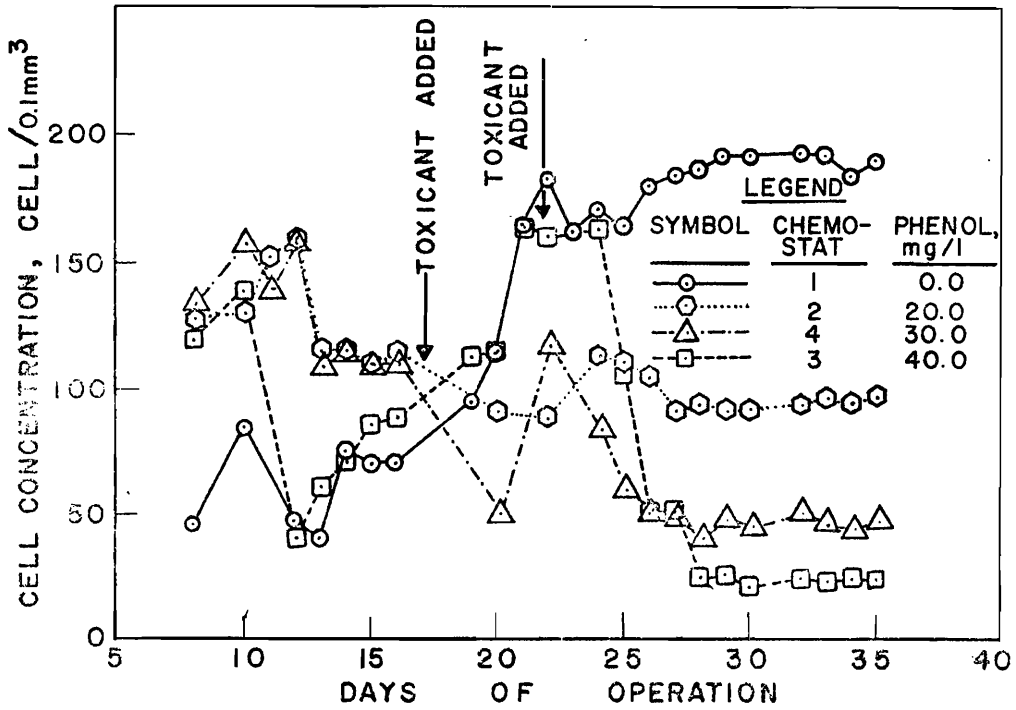

Figure F-1. Cell concentration versus time for chemostats numbers 1, 2, 3, and 4 with a 1 day residence time at 20°C (Experiment number 1).

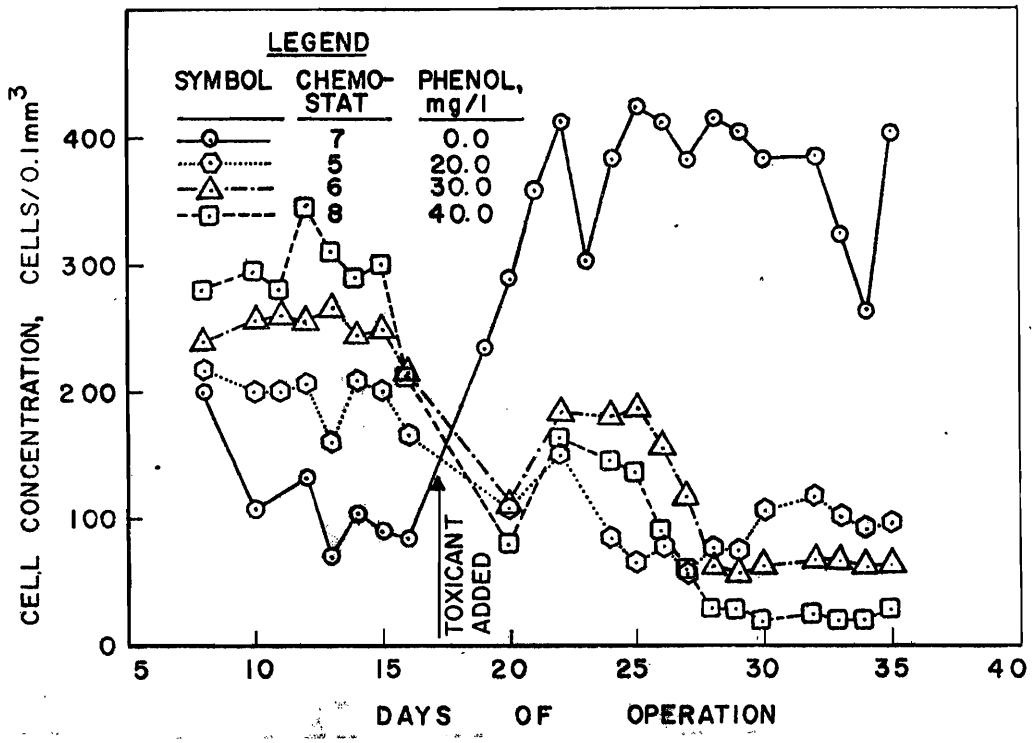

Figure F-2. Cell concentration versus time for chemostats numbers 5, 6, 7, and 8 with a 1.5 day residence time at 20°C (Experiment number 1).

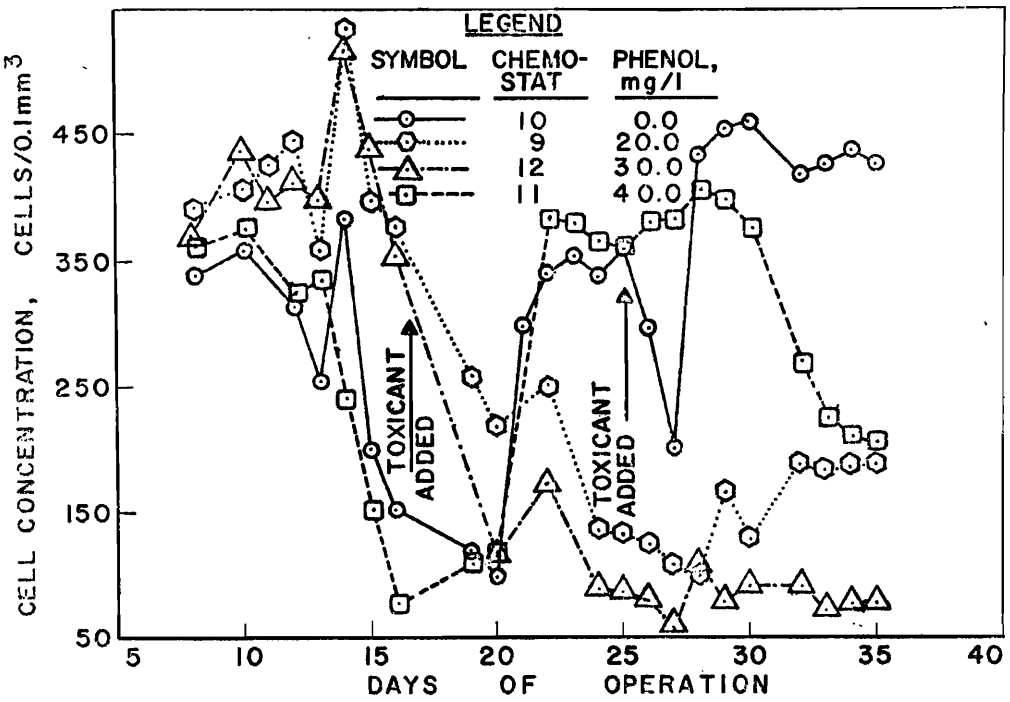

Figure F-3. Cell concentration versus time for chemostats numbers 9, 10, 11, and 12 with a 2 day residence time at 20°C (Experiment number 1).

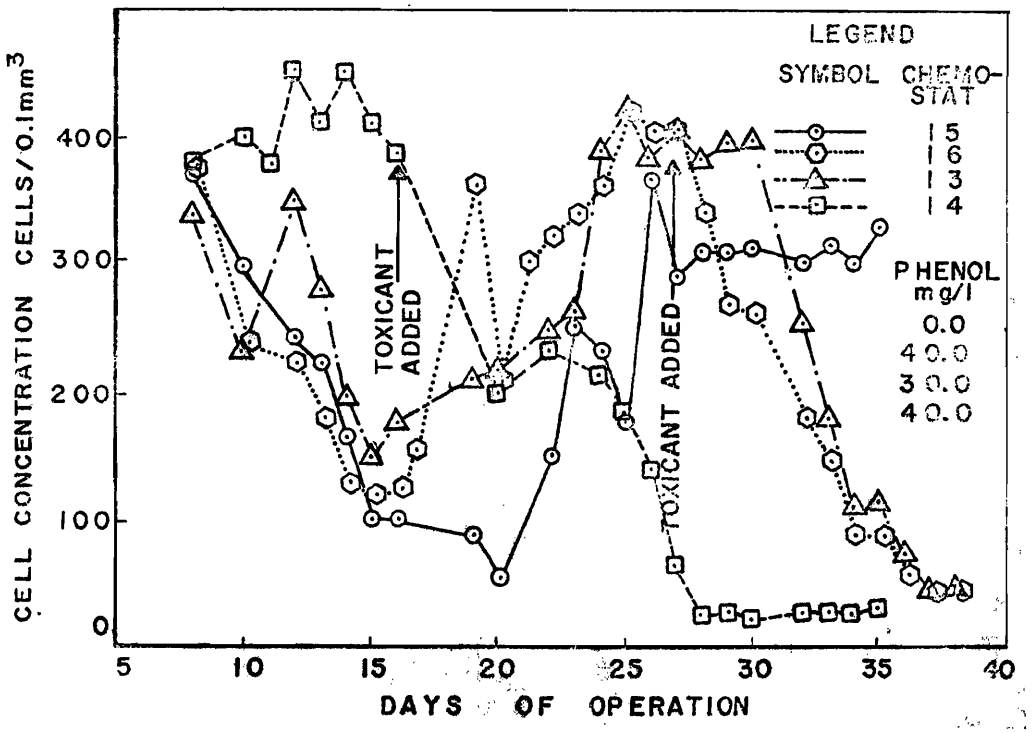

Figure F-4. Cell concentration versus time for chemostats numbers 13, 14, 15, and 16 with a 2 day residence time at 20°C (Experiment number 1).

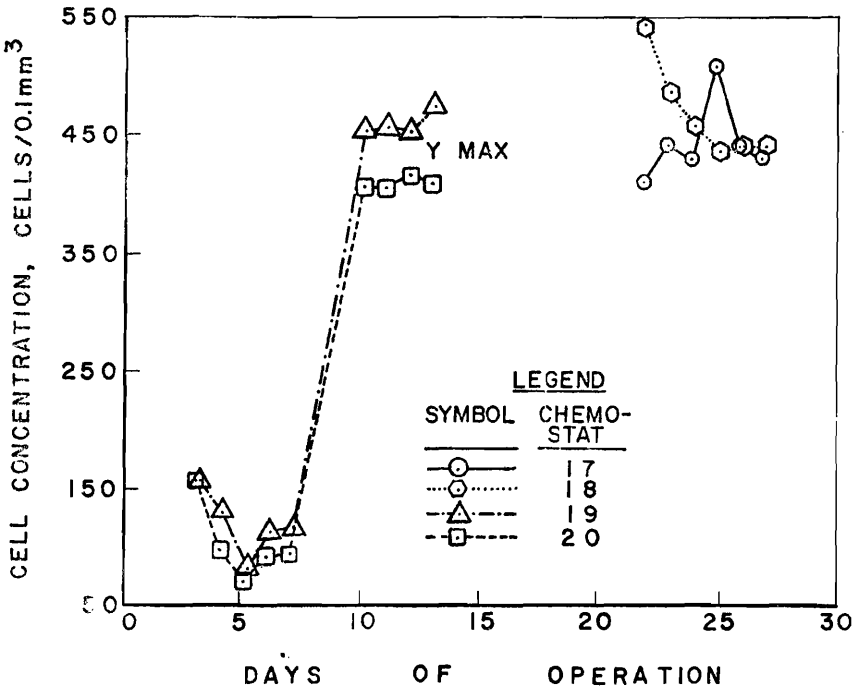

Figure F-5. Cell concentration versus time for chemostats numbers 19 and 20 with a 0.74 day residence time and chemostats numbers 17 and 18 with a 4 day residence time at 20°C (Experiment number 1; N = 4.2 mg/l).

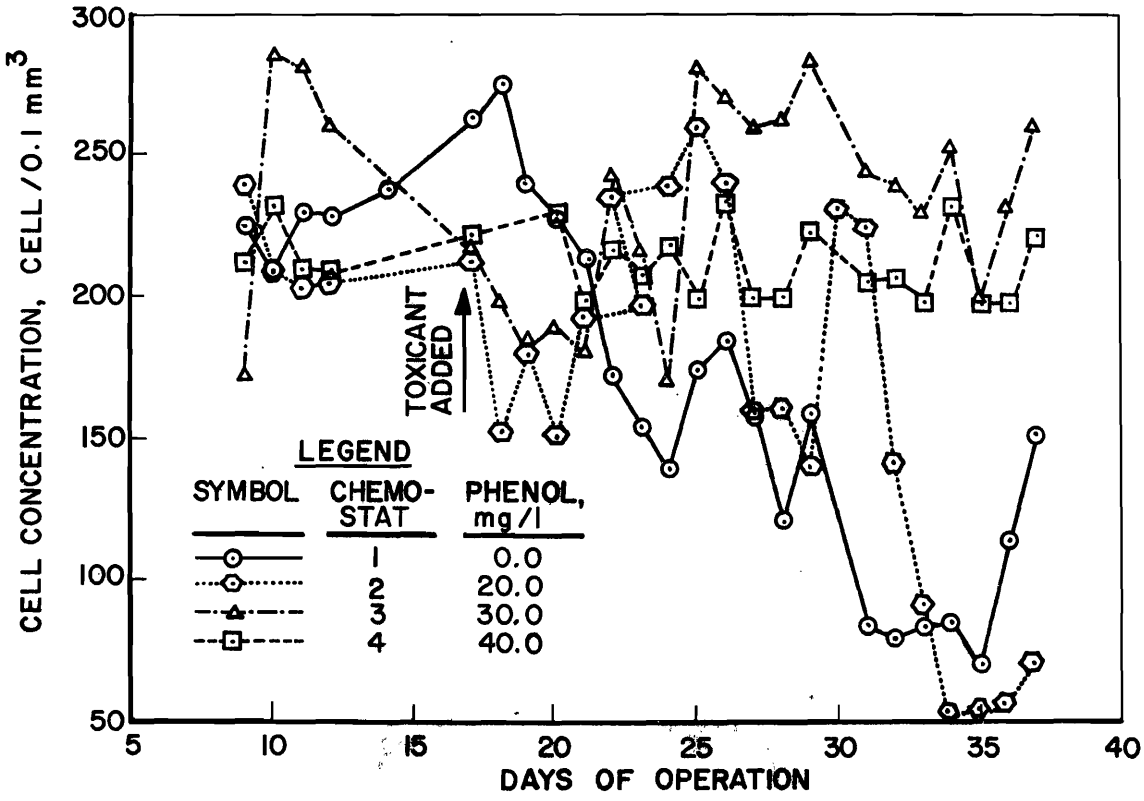

Figure F-6. Cell concentration versus time for chemostats numbers 1, 2, 3, and 4, with a 1 day residence time at 24°C (Experiment number 1).

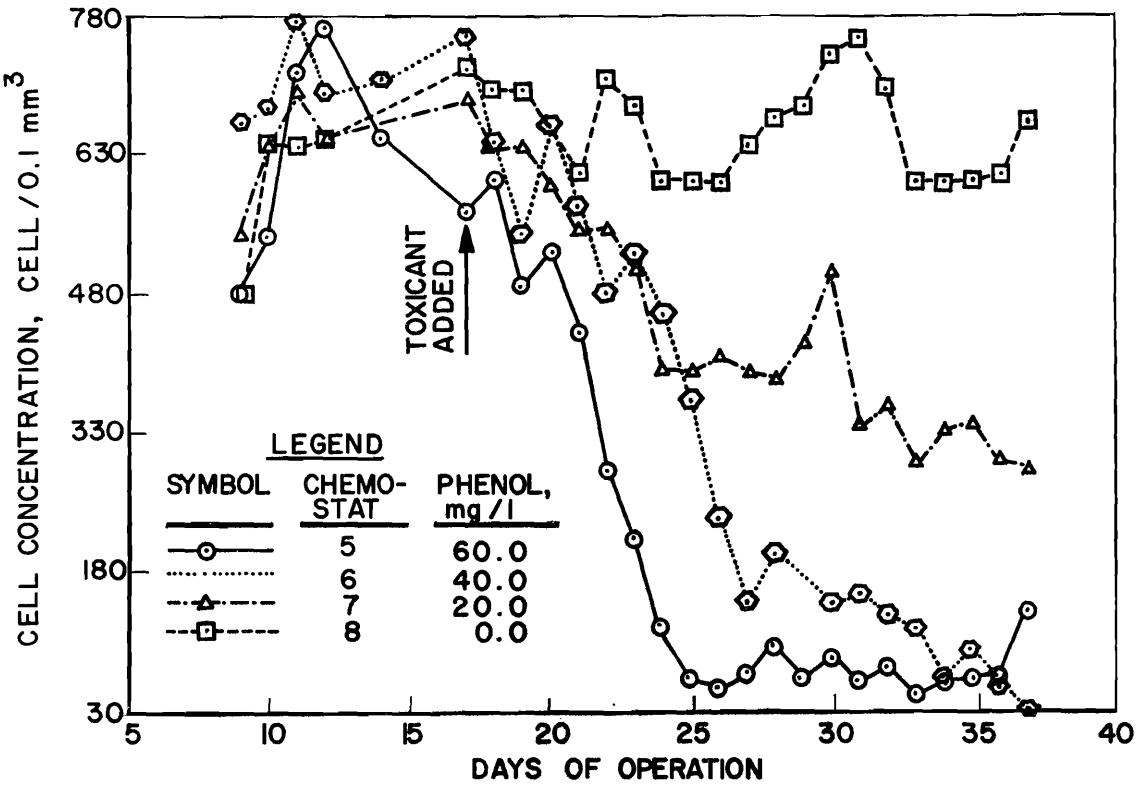

Figure F-7. Cell concentration versus time for chemostats numbers 5, 6, 7, and 8 with a 1.5 day residence time at 24°C (Experiment number 1).

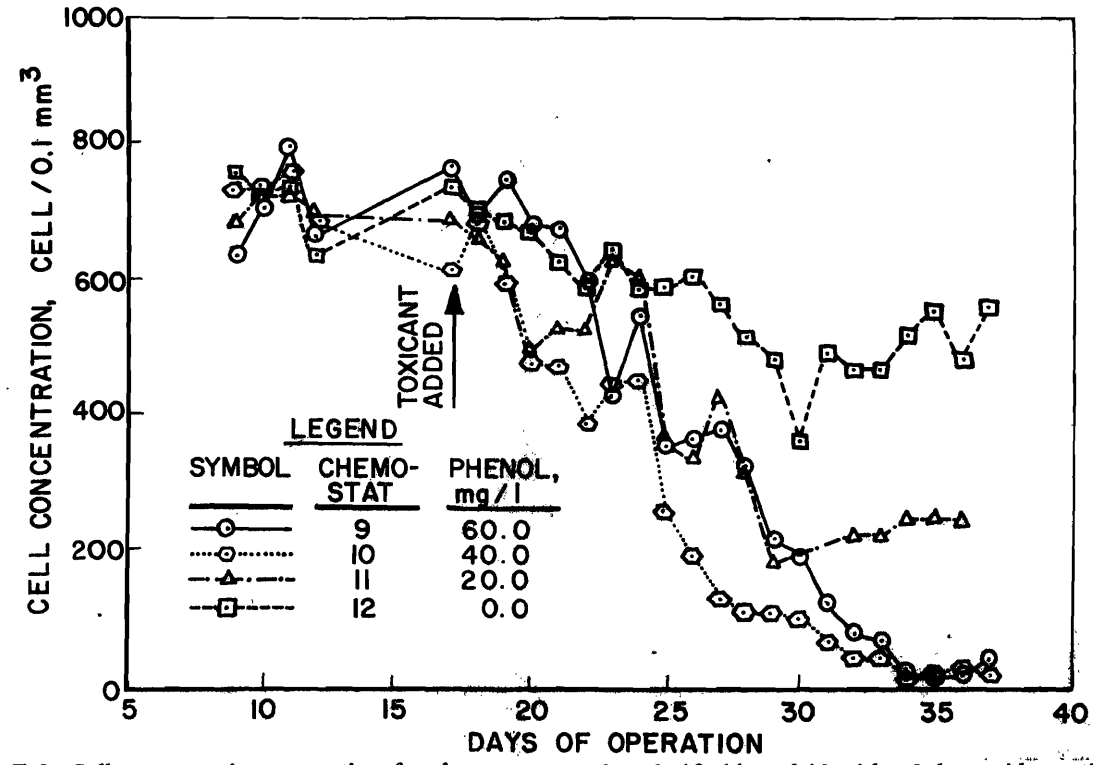

Figure F-8. Cell concentration versus time for chemostats numbers 9, 10, 11, and 12 with a 2 day residence time at 24°C (Experiment number 1).

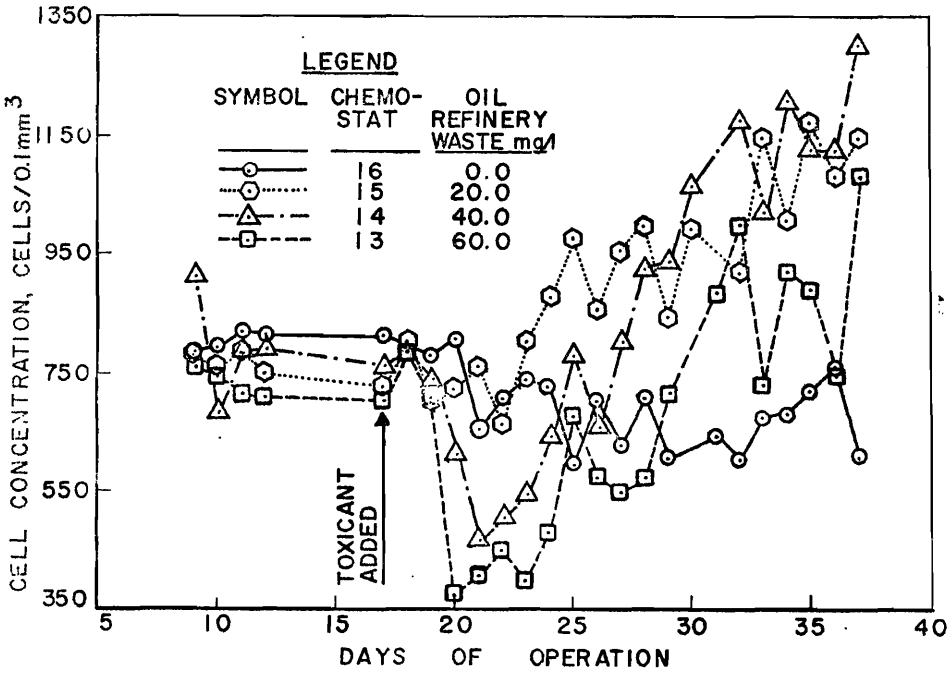

Figure F-9. Cell concentration versus time for chemostats numbers 13, 14, 15, 16, with a 2 day residence time at 24°C (Experiment number 1).

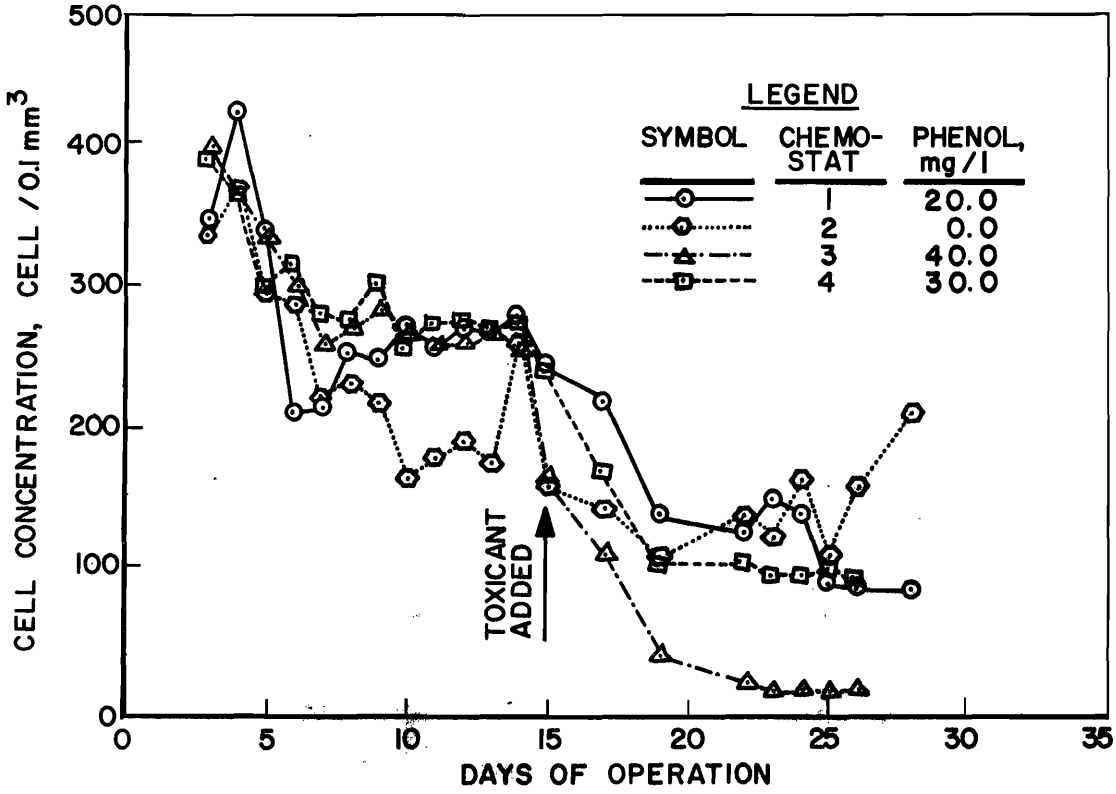

Figure F-10. Cell concentration versus time for chemostats numbers 1, 2, 3, and 4, with a 1 day residence time at 28°C (Experiment number 1).

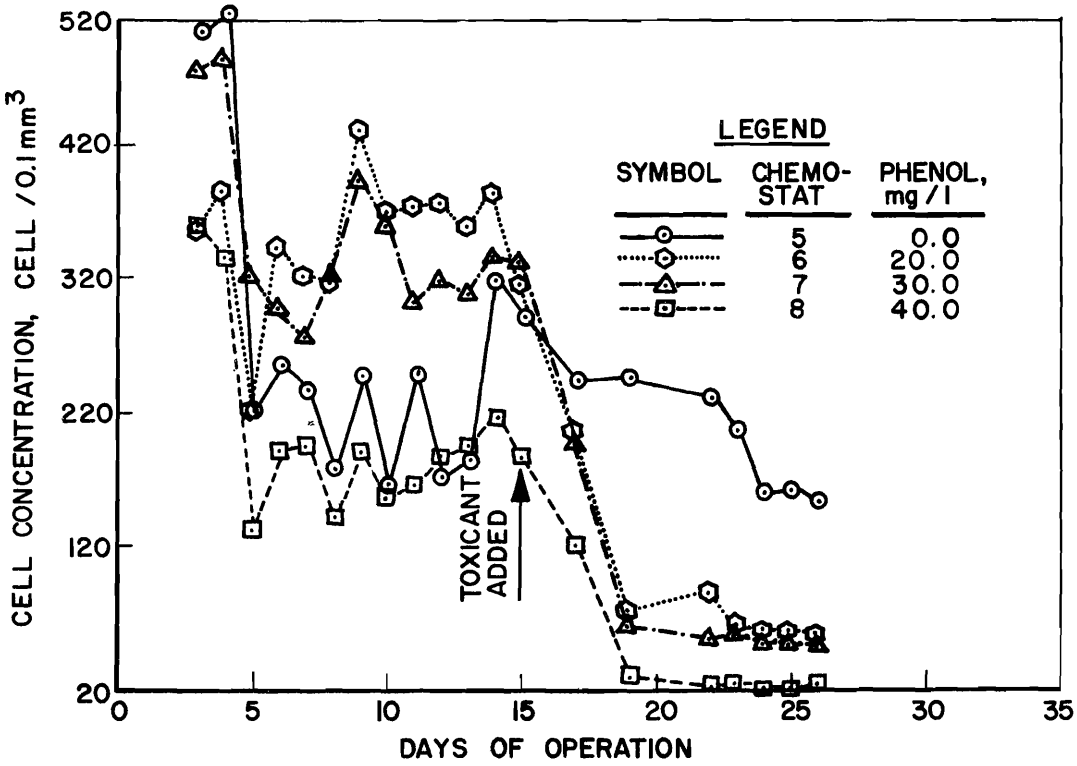

Figure F-11. Cell concentration versus time for chemostats numbers 5, 6, 7, and 8 with a 1.5 day residence time at 28°C (Experiment number 1).

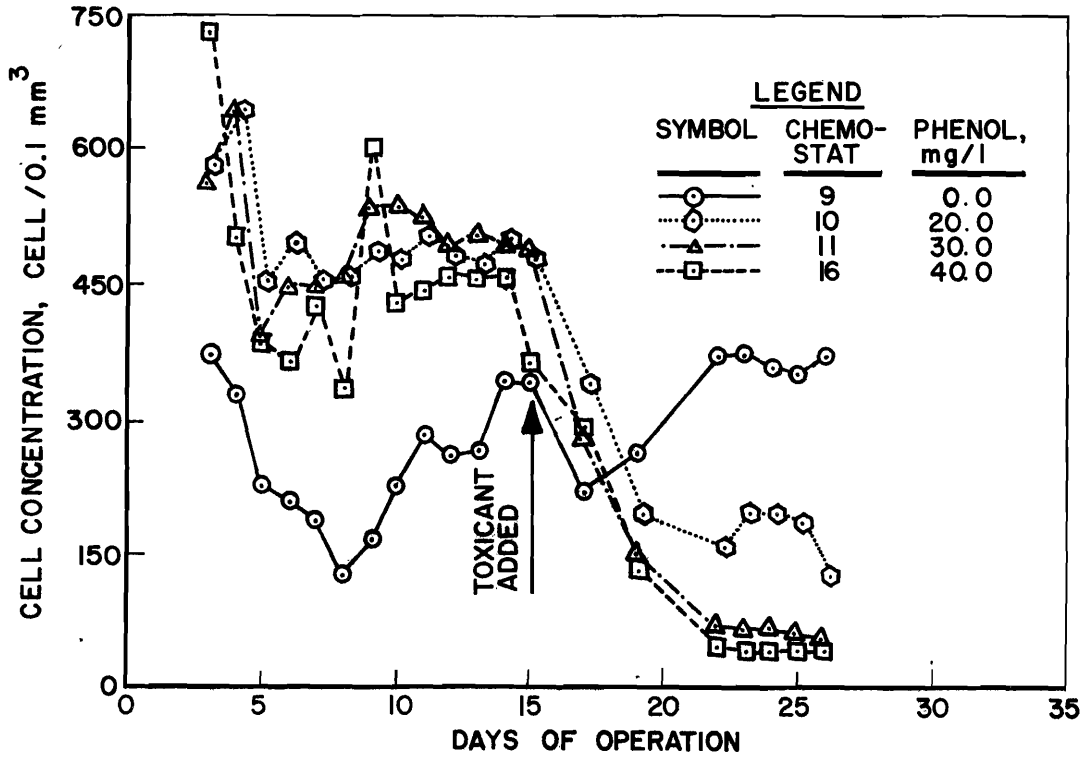

Figure F-12. Cell concentration versus time for chemostats numbers 9, 10, 11, and 16 with a 2 day residence time at 28°C (Experiment number 1).

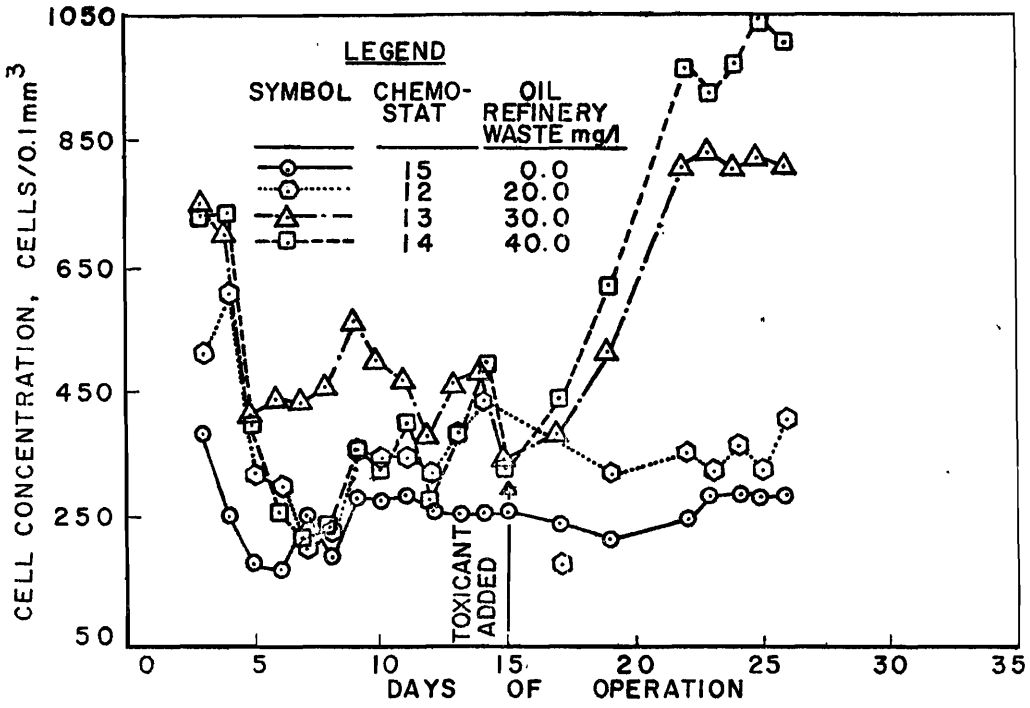

Figure F-13. Cell concentration versus time for chemostats numbers 12, 13, 14, and 15 with a 2 day residence time at 28°C (Experiment number 1).

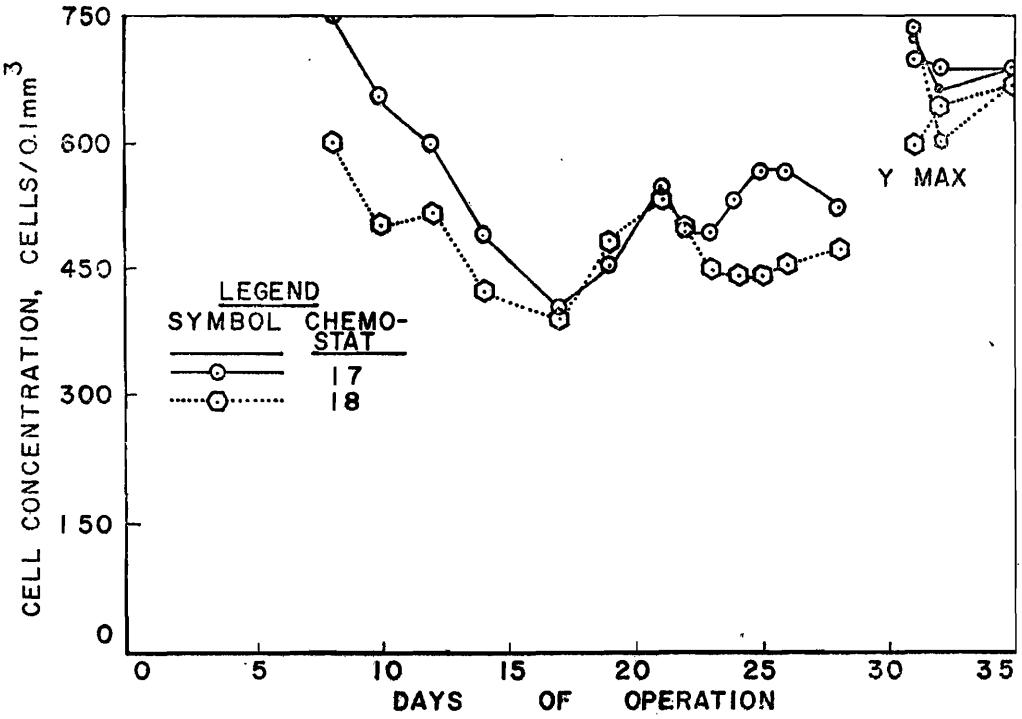

Figure F-14. Cell concentration versus time for chemostats numbers 17 and 18 with a 4.7 day residence time at 28°C (Experiment number 1, N = 4.2 mg/l).

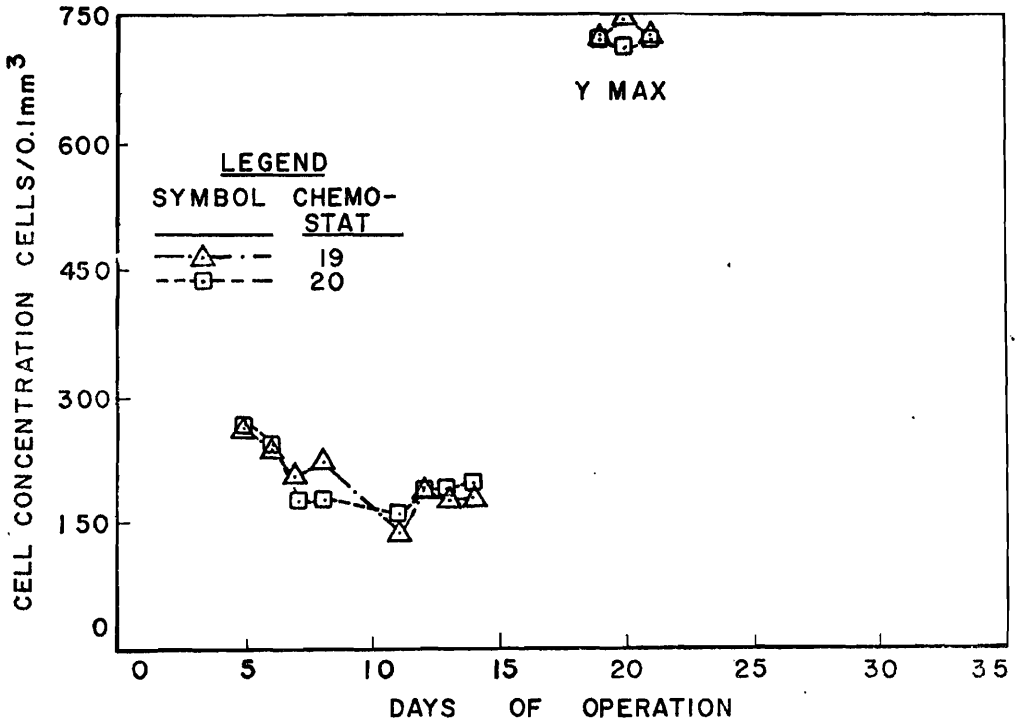

Figure F-15. Cell concentration versus time for chemostats numbers 19 and 20 with a 0.67 day residence time at 28°C (Experiment number 1, N = 4.2 mg/l).

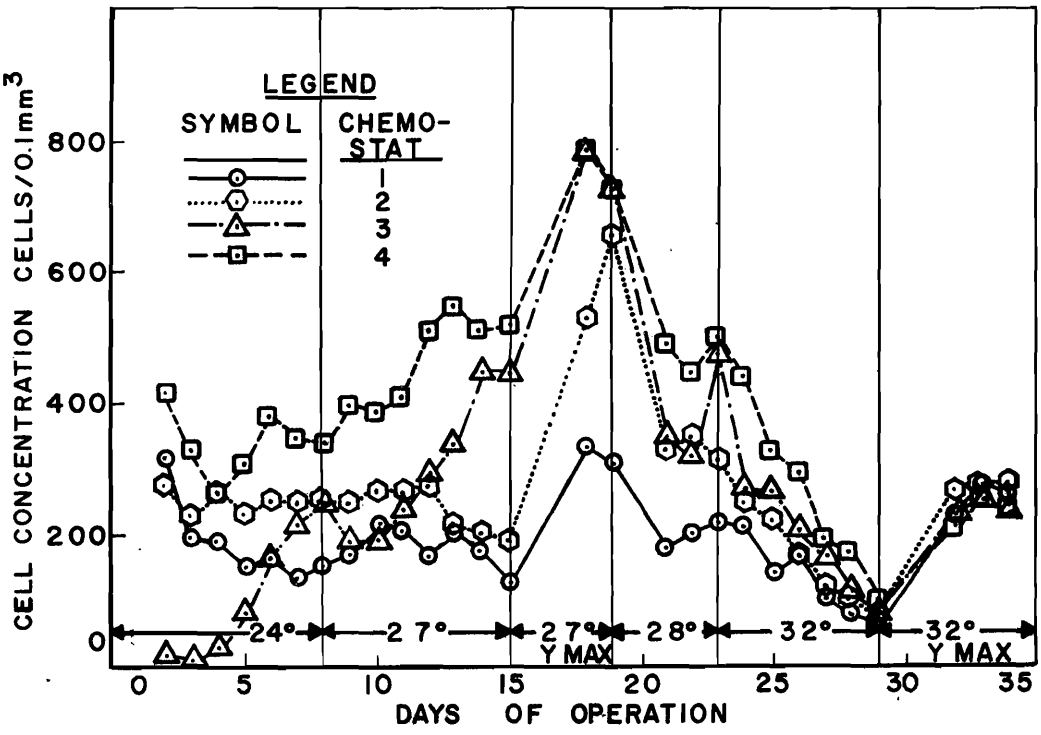

Figure F-16. Cell concentration versus time for chemostates numbers 1 and 2 (θ = 1 day) and chemostats numbers 3 and 4 (θ = 1.4 days) with N = 4.2 mg/l at various temperatures (Experiment number 2).

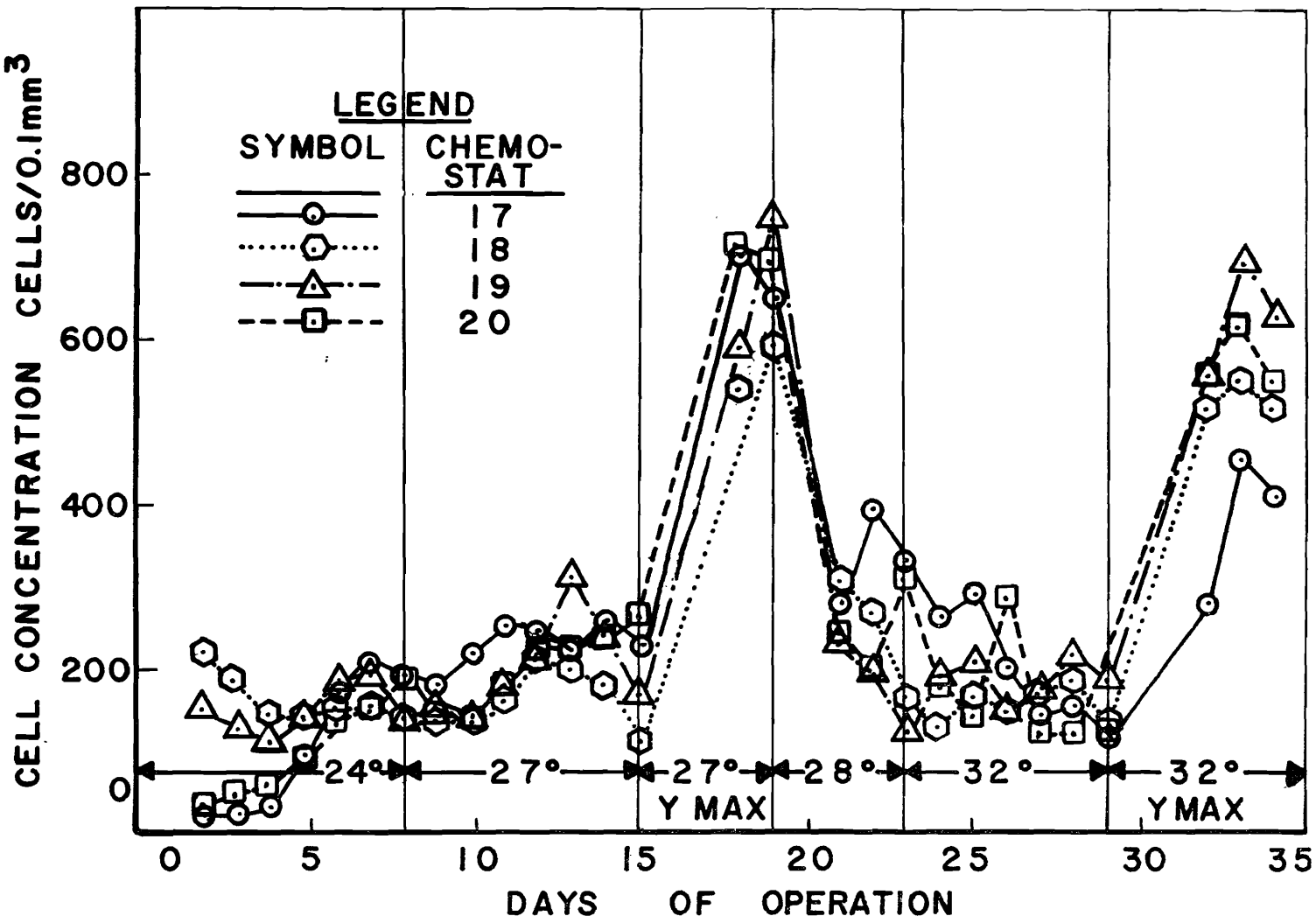

Figure F-17. Cell concentration versus time for chemostats numbers 17 and 18 (θ = 0.9 day) and chemostats numbers 19 and 20 (θ = 0.7 day) with N = 4.2 mg/l at various temperatures (Experiment number 2).

Appendix G

Computer Program for Linear Regression Analysis

Fortran IV Program for Linear Regression Analysis for Burroughs 6700 Computer

I. OBJECTIVE

The object of this program is to determine the linear regression equation $Y = AX + B$ from a least squares fit. The program also indicates the confidence interval for A and B, and correlation coefficient. It also determines an 'F' value for comparison of slopes between different regression equations.

II. INPUT

m = the number of data sets to be evaluated. It is punched on a separate data card in an I5 format.

n = the number of data points per data set. It is punched on a separate data card in an I5 format and must appear at the beginning of each respective data set.

X(n) and Y(n) = a pair of data points. They are punched one pair to a data card in 2F10.5 format. The value for X is punched in the first 10 columns, and the value for Y is punched in the second 10 columns.

III. OUTPUT

m, n, X(n), Y(n) = the data input.

A = the intercept of the regression equations.

B = the regression coefficient or slope of the regression equation.

R = the correlation coefficient

XBAR = the mean of the X values for a given data set.

YBAR = the mean of the Y values for a given data set.

SDB(I) = the standard error of the regression coefficient B. It is used to determine the appropriate confidence interval for B; i.e.

$$\text{Confidence interval} = B \pm t_{(a)\,(dF)} \cdot SDB(I)$$

in which

a = significance level

dF = degrees of freedom

t = student 'T' distribution

SDY(I) = the standard error of the intercept A. It is used to determine the appropriate confidence interval for A; i.e.

$$\text{Confidence interval} = A \pm t_{[a]\,[dF]}\ SDY(I)$$

in which

a = significance level

dF = degrees of freedom

t = student 'T' distribution

CTXY = the correction term used to determine the sum of squares on X and Y.

SSI = the sum of squares for each data set.

SMWIN = mean sum of squares within each data set.

SSCOM = sum of squares in common with each data set.

SSREG = sum of squares due to regression.

F = critical value of 'F' for 'F test' to determine if the slopes of the regression equations which have been developed are the same.

IDFN = the degrees of freedom in the numerator of the 'F test.'

IDFD = the degrees of freedom in the denominator of the 'F test.'

IV. F TEST

If the F value calculated by the computer is less than the critical F value obtained from the F-distribution in a statistical table, the slopes of the regression equations which have been developed are the same at the desired confidence level.

Computer program used for linear regression analysis

```
    B 6 7 0 0   F O R T R A N   C O M P I L A T I O N   M A R K
$SET BCD

FILE  5=INPUT
      C
       DIMENSION  X(20,70),Y(20,70),XSUM(20),YSUM(20),XYSUM(20),XXSUM(20)
      1,YYSUM(20),N(20),XBAR(20),YBAR(20),CTX(20),CTY(20),CTXY(20)
      2,XDSQ(20),YDSQ(20),XYDSQ(20),B(20),R(20),SS(20)
      3,A(20),YX(20),SDB(20),SDY(20)
       READ(5,50) M
   50 FORMAT(2I5)
      DO 5 I=1,M
      WRITE(6,55)
   55 FORMAT (1H  // 1H )
      READ(5,50) N(I)
      WRITE(6,52) M,N(I)
      DO 10 J=1,N(I)
      READ(5,51)  X(I,J),Y(I,J)
   51 FORMAT(F10.5,F10.5)
   52 FORMAT(1H ,'M=',I5,5X,'N=',I5 // 1H ,11X,'X',14X,'Y' / 1H )
      WRITE(6,53) X(I,J),Y(I,J)
   53 FORMAT(1H ,F15.5,F15.5)
   10 CONTINUE
      WRITE(6,54)
   54 FORMAT(1H // 1H )
    5 CONTINUE
      NSUM1=0
      NSUM2=0
      TXYDSQ=0.0
      TXDSQ=0.0
      TYDSQ=0.0
      SSWIN=0.0
      DO 15 I=1,M
      XSUM(I)=0.0
      YSUM(I)=0.0
      XYSUM(I)=0.0
      XXSUM(I)=0.0
      YYSUM(I)=0.0
      DO 20 J=1,N(I)
      XSUM(I)=XSUM(I)+X(I,J)
      YSUM(I)=YSUM(I)+Y(I,J)
      XYSUM(I)=XYSUM(I)+X(I,J)*Y(I,J)
      XXSUM(I)=XXSUM(I)+X(I,J)*X(I,J)
      YYSUM(I)=YYSUM(I)+Y(I,J)*Y(I,J)
```

```
   20 CONTINUE
      XBAR(I)=XSUM(I)/N(I)
      YBAR(I)=YSUM(I)/N(I)
      CTX(I)=XSUM(I)*XSUM(I)/N(I)
      CTY(I)=YSUM(I)*YSUM(I)/N(I)
      CTXY(I)=XSUM(I)*YSUM(I)/N(I)
      XDSQ(I)=XXSUM(I)-CTX(I)
      YDSQ(I)=YYSUM(I)-CTY(I)
      XYDSQ(I)=XYSUM(I)-CTXY(I)
      B(I)=XYDSQ(I)/XDSQ(I)
      A(I)=YBAR(I)-B(I)*XBAR(I)
      R(I)=XYDSQ(I)/SQRT(XDSQ(I)*YDSQ(I))
      SS(I)=YDSQ(I)-(XYDSQ(I)*XYDSQ(I)/XDSQ(I))
    C
      YX(I)=SS(I)/(N(I)-2)
      SDB(I)=SQRT(YX(I)/(XDSQ(I)))
      SDY(I)=SQRT(YX(I)*((1/N(I))+(XBAR(I)*XBAR(I)/XDSQ(I))))
    C
      SSWIN=SSWIN+SS(I)
      TYDSQ=TYDSQ+YDSQ(I)
      TXDSQ=TXDSQ+XDSQ(I)
      TXYDSQ=TXYDSQ+XYDSQ(I)
      NSUM2=NSUM2+N(I)-2
      NSUM1=NSUM1+N(I)-1
   15 CONTINUE
      SMWIN=SSWIN/NSUM2
      SSCOM=TYDSQ-(TXYDSQ*TXYDSQ/TXDSQ)
      NSUM1=NSUM1-1
C
C   NSUM1 HAS BEEN CHANGED TO CALCULATE MSREG
C
      SSREG=SSCOM-SSWIN
      SMREG=SSREG/(NSUM1-NSUM2)
      F=SMREG/SMWIN
      IDFN=NSUM1-NSUM2
      IDFD=NSUM2
      WRITE(6,56)
   56 FORMAT(1H1 // 1H ,8X,'A',12X,'B',12X,'R',10X,'XBAR',8X,'YBAR',8X,
     1'SDB',9X,'SDY',9X,'CTXY'8X,'SSI',7X,'NSUM2',3X,'NSUM1')
      DO 25 I=1,M
      WRITE(6,57)A(I),B(I),R(I),XBAR(I),YBAR(I),SDB(I),SDY(I),CTXY(I)
     1,SS(I),NSUM2,NSUM1
   57 FORMAT(1H / 1H ,9E12.5,2I8)
   25 CONTINUE
      WRITE(6,58) SMWIN,SSCOM,SSREG,SMREG
   58 FORMAT(1H // 1H ,'SMWIN = ',E13.5,'   SSCOM = ',E13.5,'   SSREG ='
     1,E13.5,'   SMREG = 'E13.5)
      WRITE(6,59) F,IDFN,IDFD
   59 FORMAT(1H , 'F = ',F7.3,'   IDFN =',I6,'   IDFD =',I6)
      CALL EXIT
      END
```