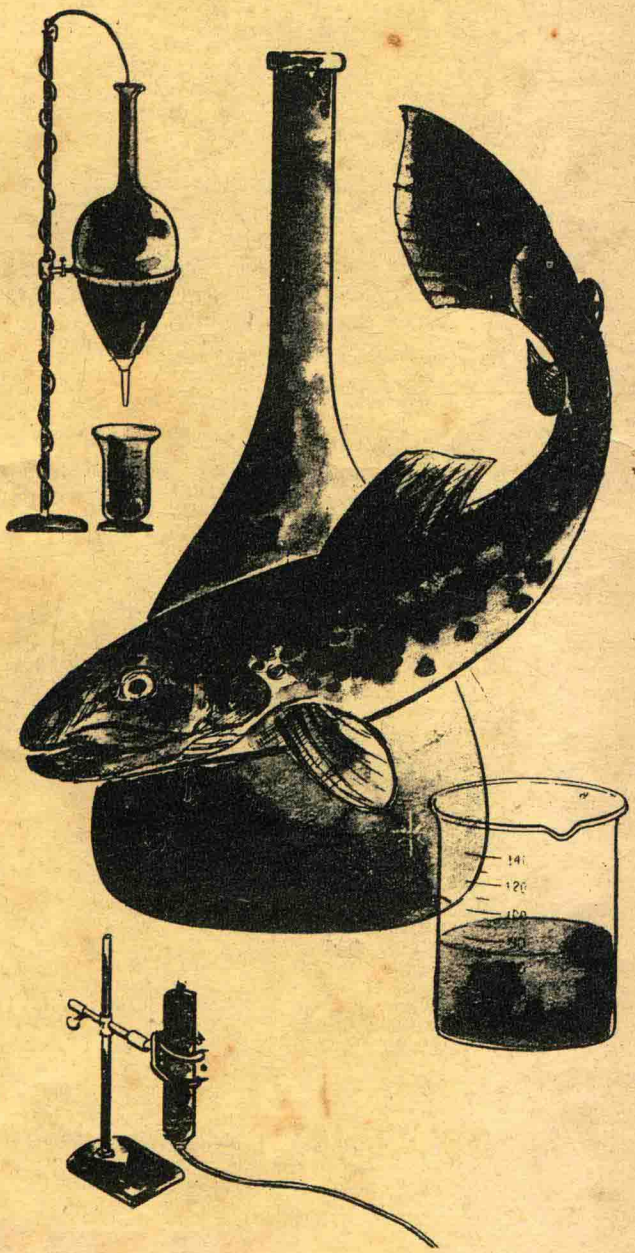
A CONTINUOUS FLOW KINETIC MODEL TO PREDICT THE EFFECTS OF TEMPERATURE ON THE TOXICITY OF WASTE TO ALGAE

James H. Reynolds
E. Joe Middlebrooks
Donald B. Porcella
William J. Grenney



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College of Engineering
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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 °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.

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

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

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

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