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**Heavy-Metal Toxicity Phenomena
in Laboratory-Scale ANFLOW
Bioreactors**

A. L. Rivera

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HEAVY-METAL TOXICITY PHENOMENA IN LABORATORY-SCALE ANFLOW BIOREACTORS

A. L. Rivera

This report was prepared as a dissertation and submitted to the Faculty of the Graduate School of Northwestern University in partial fulfillment of the degree of Doctor of Philosophy in the Department of Environmental Engineering.

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HEAVY-METAL TOXICITY PHENOMENA IN LABORATORY-SCALE ANFLOW BIOREACTORS

A. L. Rivera

ABSTRACT

Oak Ridge National Laboratory (ORNL) has developed an energy-conserving wastewater treatment system based on an anaerobic, upflow (ANFLOW) bioreactor. Since many applications of the ANFLOW process could involve the treatment of wastewaters containing heavy metals, the potentially toxic effects of these metals on the biological processes occurring in ANFLOW columns (primarily acetogenesis and methanogenesis) were investigated.

Both step and pulse inputs of zinc ranging from 100 to 1000 mg/L were added to synthetic wastewaters being treated in ANFLOW columns with 0.057-m³ volumes. Column responses were used to develop descriptive models for toxicity phenomena in such systems. It was found that an inhibition function could be defined and used to modify a model based on plugflow with axial dispersion and first-order kinetics for soluble substrate removal. The inhibitory effects of zinc on soluble substrate removal were found to be predominantly associated with its sorption by biosolids. Sorption initially occurred in the lower regions of the column, but was gradually observed in higher regions as the sorption capacity of the lower regions was exhausted. Sorption phenomena could be described with the Freundlich equation.

Sorption processes were accompanied by shifts of biological processes to regions higher in the columns. A regenerative process was observed when feeding of wastewaters without zinc was resumed. It was postulated that regeneration could be based on sloughing of layers of biofilms, or other biosolids involved in zinc sorption, followed by continued growth of lower layers of biofilms not involved in heavy-metal sorption.

1. INTRODUCTION

Oak Ridge National Laboratory (ORNL) has developed an energy-conserving wastewater treatment system based on an anaerobic, upflow

(ANFLOW) bioreactor that utilizes films of bacteria fixed to inert, stationary packing material. The treatment process is based on passing wastewaters upward through the bioreactor for continuous treatment by gravitational settling, biophysical filtration, and biological decomposition. The ANFLOW process has been demonstrated using municipal sewage with a pilot plant bioreactor designed to process $19 \text{ m}^3/\text{d}$ (5000 gpd).¹

Conceptual designs for total treatment systems processing up to $3800 \text{ m}^3/\text{d}$ (1.0 million gpd) of wastewater were developed based on the performance of the pilot-plant bioreactor. These systems were compared to activated-sludge treatment systems under secondary treatment requirements and were found to consume as little as 30% of the energy required by the activated sludge systems. The economic advantages of the process result from the elimination of operating energy requirements associated with the aeration of aerobic-based processes and with the significant decrease of sludge-handling costs required with conventional activated-sludge treatment systems. Methane produced by anaerobic fermentation processes occurring during the biological decomposition of carbonaceous wastes also represented a significant and recoverable energy production term as wastewater flow rates approaches $3800 \text{ m}^3/\text{d}$.

To support commercialization of the process, ORNL is continuing process development efforts with both laboratory studies and a $190\text{-m}^3/\text{d}$ (50,000-gpd) pilot study based on the results of the previous $19\text{-m}^3/\text{d}$ pilot system. Since many applications of the ANFLOW process could involve the treatment of wastewaters containing heavy metals, the need to define potentially toxic effects of these metals on the biological processes occurring in ANFLOW columns was identified as a required support-

ing research study. It was anticipated that the information generated from such a study would be required for both future design exercise and for planning approaches to operation and control of such systems.

The research program which was developed for use in the above study had the following four general objectives:

1. to investigate the expression of toxicity within the ANFLOW column (i.e., under what conditions and on what biological reactions do effects occur),
2. to define the mechanisms (i.e., sorption or precipitation) by which heavy metals are removed from the liquid phase, and to consider the ultimate fate of these metals within the column,
3. to develop models of the ANFLOW system which reflect toxicity effects, and
4. to investigate regenerative mechanisms by which the ANFLOW column can resume normal operation after exposure to toxic heavy metals.

The research program was designed to emphasize the development of methods, models, and definitions of mechanisms rather than to conduct a survey of varying effects among several metals.

2. GENERAL BACKGROUND

2.1 The ANFLOW Process

2.1.1 Studies with Synthetic Wastewaters

Early anaerobic column investigations were conducted by Young and McCarty² using dilute synthetic wastewater with low suspended solids.

At 25°C and with a 36-h detention time, greater than 90% of the chemical oxygen demand (COD) of the wastewater was removed with the anaerobic, upflow bioreactor. The solids accumulation rate was reported to be a function of the influent characteristics. It was suggested that the performance of the bioreactor might improve if a high-void-fraction packing was used. Additionally, it was reported that the off-gases of these bioreactors were 75% methane.

El-Shafie and Bloodgood³ investigated anaerobic, upflow bioreactors operated in series. With synthetic feeds again being used, these researchers found that the percent removal of organics was constant regardless of the organic load applied to the system. Fisher⁴ also reported satisfactory treatment of a dilute synthetic waste (60 to 80% COD removal at 32°C) for anaerobic, upflow bioreactor operated with detention times varying from 0.5 to 2 d. One bioreactor in this study which received concentrated synthetic feed was reported to fail because of unspecified inhibition effects.

2.1.2 Studies with Industrial Wastewaters

The use of anaerobic, upflow bioreactors for industrial wastewater treatment has generally involved warm (30 to 37°C) waste streams with high carbon content. As a result, anaerobic biomass reaction rates have been increased as has been the quantity of methane-rich off-gas produced.

Industrial wastewaters investigated have contained both soluble and insoluble components. Soluble wastes are generated from the organic chemicals and textiles industries, for example, while food processing

and pharmaceutical industries discharge significant quantities of raw wastewater solids. Plummer⁵ evaluated bench-scale anaerobic, upflow bioreactors for treatment of a food processing waste. Four bioreactors were packed with a mixture of Raschig rings and berl saddles. A 94% removal of biological oxygen demand (BOD) was achieved with this waste. Although bacterial growth rates were high, no column plugging occurred. Solids were retained in the column for periods ranging from 4 to 163 d. Plummer suggested that hydraulic loading would be limited by biomass washout. It was reported that the effluent suspended solids separated readily by gravity settling.

Jennett and Dennis⁶ operated a 15.2-cm (6-in.) diam anaerobic, upflow column to treat a high-strength, low-solids pharmaceutical waste. At 35°C the column reduced the raw waste COD (1250 to 16,000 mg/L) by 94 to 98%. The filter operated over 6 months without sludge removal while operating at a COD loading rate of 0.22 to 3.528 kg COD/d-m³ (13.8 to 220 lb COD/1000 3 ft-d).

Results from pilot-scale anaerobic filters, 1.22 and 2.44-m bed depths packed with 3.8-cm (1.5-in.) rock, were reported by Pailthrop⁷ for primary clarifier supernatant from a potato processing plant. The supernatant BOD and suspended solids values were 1700 and 650 mg/L, respectively. At 0.96 kg BOD/d-m³ (60 lb BOD/1000 cu ft-d) and 20°C, the 2.44-m (8-ft) upflow column produced 75% BOD removal. The performance of the 1.22- and 2.44-m columns were similar.

Results for the first commercial size anaerobic, upflow bioreactors were reported by Taylor⁸ and separately by Richter and Machie.⁹ Three,

6.1-m (20-ft) high, 9.1-m (30-ft) diam anaerobic filters were packed with 2.5- to 7.5-cm (1- to 3-in.) rock for pretreatment of wheat starch-gluten plant wastewater for discharge to a city treatment facility. The raw wastewater BOD and suspended solids values were 7290 and 4000 mg/L, respectively. COD removal ranged from 75 to 80% for a raw waste at 29.4°C (85°F). Economic comparisons between this system and an aerobic system showed that the use of anaerobic technology reduced costs by 50 to 80%.

2.1.3 Studies with Municipal Wastewaters

Early investigation of upflow contact processes coupled with anaerobic treatment of municipal wastewaters demonstrated that such approaches could be used to provide some gasification of the wastes and some solids removal.¹⁰⁻¹² These processes depended primarily on filtering phenomena but did provide a basis for pursuing the development of anaerobic bioreactors.

Coulter¹⁰ and Pretorius¹³ investigated anaerobic treatment of sewage using multistage systems that included upflow clarifiers followed by packed-bed columns. The Coulter system included an unstirred contactor with a subsequent upflow rock filter, whereas Pretorius used a mixed contactor digester with a settling chamber and sludge return stone-sand upflow filter. Using medium-strength sewage, Pretorius obtained a 90% reduction of COD in 24 h at a temperature of 20°C; 60 to 65% of incoming solids accumulated in the digester and had to be removed periodically. The major part of the gasification was found to occur in the biophysical filter. More recent work by Pretorius¹⁴ also demonstrated

that since the anaerobic nature of his first stage, as opposed to aerobic processes, produced electron donors, an efficient denitrifying stage could be added to extend the treatment possible with the overall process.

Griffith and Compere¹⁵ extended the work of Young and McCarty with detailed laboratory studies using columns with volumes of 0.057 m^3 (2 ft^3), high void-volume packing, and synthetic feeds. These studies were conducted with low-strength wastes at 20°C . Their work was given impetus by early nitrogen starvation tests conducted on columns; these tests showed that the decay coefficients of the columns were lower than would be expected from calculations and suggested the presence of a very stable biomass in the columns. These researchers reported reductions in BOD of greater than 80% for residence times of approximately 8 h. Griffith and Compere also performed studies with these columns in the field using dilute raw sewage with total organic carbon (TOC) concentrations of 30 to 100 mg/L at temperatures in the range 15 to 25°C . Results of these studies showed that the columns were capable of adapting to the low-temperature, low-strength feeds while retaining their ability to significantly reduce the pollutant concentration and to generate off-gas with a methane content approaching 70%. They also showed that the columns were capable of reducing the total suspended solids (TSS) content by more than 70%. The implied advantages of this flow process over conventional anaerobic digestion processes, which could require 10 to 30 d of holdup for equivalent treatment, were very significant.

The research projects described above demonstrated that upflow anaerobic, fixed-film bioreactors could clearly provide efficient treatment of high- or medium-strength wastewaters with off-gases having high methane contents. The residence times required were significantly lower than those required for conventional technology, and operating energy requirements were also anticipated to be greatly lowered. Sludge removal requirements would be related to the rate of solids accumulation in the bioreactor. Data indicated that such bioreactors would also efficiently treat low-strength, low-temperature wastewaters. The potential for developing a needed, near-term technology for wastewater treatment based on an anaerobic, fixed-film bioreactor led ORNL to a pilot-plant development and demonstration project for realistic evaluation of a total process.

2.2 Overview of Anaerobic Digestion Processes

The anaerobic digestion of complex organic wastes is normally considered to consist of reactions occurring in series as illustrated in Fig. 2.1. In the first stage (commonly known as waste conversion), the complex organics are changed in form by a group of facultative and anaerobic bacteria commonly termed the acid formers. Complex materials such as fats, proteins, and carbohydrates are hydrolyzed, fermented and biologically converted to simple organic materials. For the most part, the end products of this first-stage conversion are organic fatty acids (Table 2.1). Acid-forming bacteria bring about these initial conversions to obtain the small amounts of energy released for growth, and a small portion of the waste is converted to cells. Although no waste

Figure 2.1. Anaerobic digestion of organic wastes.

stabilization occurs during the first stage of treatment, this stage is required to convert the organic matter into a form suitable for the second stage of treatment.

Waste stabilization occurs in the second stage of methane fermentation. During this stage, the organic acids are converted into the gaseous end products, carbon dioxide and methane by special bacteria termed methanogens, or methane formers. These methanogens are strictly anaerobic, and even small quantities of oxygen are harmful to them.

Table 2.1. Common Volatile Acid Intermediates

Acid	Chemical Formula
Formic acid	HCOOH
Acetic acid	CH ₃ COOH
Propionic acid	CH ₃ CH ₂ COOH
Butyric acid	CH ₃ CH ₂ CH ₂ COOH
Valeric acid	CH ₃ CH ₂ CH ₂ CH ₂ COOH
Isovaleric acid	(CH ₃) ₂ CHCH ₂ COOH
Caproic acid	CH ₃ CH ₂ CH ₂ CH ₂ CH ₂ COOH

There are several different groups of bacteria among the methane formers, and each group is characterized by its ability to ferment a relatively limited number of organic compounds. In the complete fermentation of complex materials, several different groups of methane-producing bacteria are required.

The most important methane formers use acetic and propionic acids as substrates. They grow quite slowly, require an optimum pH range of 6.8

to 7.4, and sludge retention times of four days or longer are required for their growth.¹⁶ These bacteria carry out the major portion of waste stabilization. Their slow growth and low rate of acid utilization is normally considered to represent the rate-limiting step around which anaerobic treatment processes should be designed.

As there are different methane-forming bacteria, there are also different acid-forming bacteria. Waste stabilization requires a balanced interaction among all these organisms. The establishment and maintenance of this balance are normally controlled through monitoring of the concentration of volatile acids, a varying (i.e., dynamic) parameter. The volatile acids are the short-chain organic acids listed in Table 2.1. These acids are considered to be the most common intermediate compounds of importance in anaerobic treatment, and most of the methane formed from this process results from fermentation of these acids by methane bacteria.

The two major volatile acid intermediates formed in anaerobic treatment are acetic and propionic acid.¹⁷⁻¹⁹ Acetic acid is the most important volatile acid formed from the decomposition of complex organics and is the source of approximately 70% of the methane produced during anaerobic treatment.²⁰

Most of the remaining methane produced during anaerobic treatment is formed from the reduction of carbon dioxide. Here, hydrogen, which is removed from organic compounds by enzymes, reduces carbon dioxide to methane gas. The carbon dioxide functions as a hydrogen or electron acceptor, just as oxygen in aerobic treatment. These two major mechanisms of methane formation can be represented as the following:

1. Acetic acid cleavage:

2. Carbon dioxide reduction:

2.3 Overview of Toxicity Phenomena in Anaerobic Processes

No previous data have been published on the performance of ANFLOW bioreactors in the treatment of wastewaters containing toxic concentrations of heavy metals (Ni, Cu, Zn, Cd, Hg, and Pb are among the most common heavy metals of significance in municipal-industrial waste water treatment). Most previously published research on the toxic effects of heavy metals in anaerobic processes has emphasized suspended-growth processes such as anaerobic digester systems rather than fixed-film systems such as ANFLOW.

Early investigations conducted in the 1920s and 1930s identified four different behaviors resulting from the effects of cations on anaerobic processes: toxicity, stimulation, synergism, and antagonism — all of them concentration dependent. These will be discussed below. In general, it was found that all cations produce toxicity in any organism at some level, but the relative toxicity of the cations varied when compared on a weight-weight basis. The toxicity of heavy metals were found to depend on concentration in the soluble form rather than on the total concentration in the system. Mechanisms of heavy-metal removal under aerobic conditons have been studied. Four distinct processes were enumerated by Cheng:²¹ (1) adsorption, (2) incorporation into cells, (3)

precipitation and subsequent entrapment of the precipitate in the bio-floc, and finally (4) chelation of soluble metal species by organic and inorganic ligands. These four processes can also be considered to be the major mechanisms for heavy-metal removal under anaerobic conditions. In the case of an ANFLOW bioreactor, the entrapment of precipitates can also occur in the fixed-films.

The term toxic is relative, and the concentration at which a material becomes toxic or inhibitory may vary from a fraction of a mg/L to several thousands mg/L. Figure 2.2 indicates the general effects that result from the addition of most substances to biological systems. At very low concentrations, stimulation of activity usually occurs. This stimulatory concentration may range from only a fraction of a mg/L for heavy-metal salts to more than 100 mg/L for sodium or calcium salts.²² As the concentration is increased above the stimulatory concentration, the rate of biological activity begins to decrease. A point is then reached where inhibition is apparent and the rate of biological activity is less than that achieved in the absence of the material. Finally, at some higher concentration, the biological activity approaches zero. Heavy metals are considered to be toxic because they can be present in wastes at a concentration of a few milligrams per liter, which is several orders of magnitude above their range of activity stimulation.²³ Their potential to induce toxicity in anaerobic treatment processes depends on their fate within the bioreactor.

Microorganisms usually have the ability to adapt or acclimate to some extent to inhibitory concentrations of most materials. The extent

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Figure 2.2. General effects of salts or other materials on biological reactions.

of adaptation is relative, and in some cases the activity after acclimation may approach that obtained in the absence of the inhibitory material, but in other cases, the activity following acclimation may be much less than was present prior to inhibition.

Toxic concentrations reported in the literature, even for the same metal, vary widely. Explanations for these variations, under anaerobic conditions, may involve the many different factors, such as pH, alkalinity, concentrations of inorganic and/or organic complexing agents, and the presence of other heavy metals (synergistic/antagonistic effects) which modify the toxic effects of heavy metals. A primary effect of these factors is to limit the availability of heavy metals by precipitation, or by keeping them in solution (solubilization).

As would be expected then, the toxicity of a heavy metal in anaerobic digestion processes depends upon the various chemical forms which that metal may assume under anaerobic conditions and near-neutral pH values. For instance, heavy metals in the precipitated form are of little consequence to biological systems in terms of toxicity. Lawrence and McCarty²⁴ fed sulfates of copper, zinc, nickel, and iron to laboratory digesters and found little evidence of effects on digester performance in terms of reduced gas production. When metal chloride salts were substituted for the sulfates, thereby eliminating the sulfide precursor, marked reduction in digester performance was observed. It was concluded that the toxicity of heavy metals depends on their concentration in the soluble form and not on their total concentration in the system.

Lawrence and McCarty,²⁴ when investigating the role of sulfides in preventing heavy metal toxicity, also found that gas production was the initial parameter affected by heavy-metal toxicity. The volatile-acid concentration began to rise only after the gas production was noticeably reduced. They also noted that when their digesters began to fail, the percentage of methane decreased significantly.

Hayes and Theis²⁵ showed that toxic effects occurred when a large buildup of metals exists in close association to the cell mass. Metals included in the study included cadmium, chromium(VI), chromium(III), copper, lead, nickel, and zinc. The digesters were fed sludge from an activated-sludge treatment plant; these aerobic sludges were incubated with solution metals before being introduced into the anaerobic environment. Treatment times in the anaerobic digesters ranged from 5 to 20 d. This study concluded that extracellular heavy metals accounted for less than 1% of the total digester metals for all studies; soluble metals (those passing a 0.22- μ m filter) were also insignificant, except for nickel for which 1 to 3% of the total was found in the filtrate. The major portions of the heavy metals were distributed between the precipitated and intracellular components of the digesters, with 30 to 60% of the total (depending upon the metal) found in the intracellular fraction of the digested sludge.

Metal uptake curves generated were similar to adsorption isotherms with the point of nonlinearity coinciding with the onset of toxic conditions. Toxic effects were found to coincide with the near maximum uptake of metals by bacteria. After establishment of stable baseline parameters, the digesters were dosed with the particular heavy metal in a pulse and stepwise fashion. Among the parameters used as indicators

for digester response to heavy-metal loadings were: gas production rate, gas composition (% CH₄), pH, alkalinity, volatile acids, and total organic carbon (TOC). Normally, step feeding was accomplished at heavy-metal concentrations ranging from 10 to 100 mg/L, whereas pulse (slug) feeding was performed at heavy-metal concentrations from a few hundreds to several thousands mg/L. The order of decreasing toxicity on a weight-weight or molar basis was Ni>Cu>Zn. Control strategies for minimization of the impact of heavy metals in anaerobic digestion will be those which result in a reduced degree of metal association with the biomass. In this study the inhibitory level of a heavy metal was defined as that level at which a decrease in gas production first became evident. Toxic levels were taken to be the concentrations at which total gas production was reduced by 70% from baseline (see Table 2.2).

Table 2.2. Heavy-Metal Toxicity Limits for Anaerobic Digestion^a

Heavy Metal	Step-Fed		Pulse-Fed
	Inhibiting Concentration (mg/L)	Toxic Limit (mg/L)	Toxic Limit (mg/L)
Cr(III)	130	260	<200
Cr(VI)	110	420	<180
Cu	40	70	<50
Ni	10	30	>30
Cd	-	>20	>10
Pb	340	>340	>250
Zn	400	600	<1700

^aAs presented by Hayes and Thesis in "The Distribution of Heavy Metals in Anaerobic Digestion", J. Water Pollut. Control Fed., 1972, pp. 61-72.

Two major sets of processes are known to be capable of removing sufficient heavy metals from solution to prevent inhibition of anaerobic digestion. One involves the interaction of the metal with the hydrogen sulfide/sulfide system and the other its interaction with the carbon dioxide/carbonate system.²⁶ These two processes can be illustrated as:

sulfide

carbonate

Hydrogen sulfide is an end-product of anaerobic metabolism and its precursors include the sulfate ion ($\text{SO}_4^{=2-}$). The activity of carbonate ions present in anaerobic treatment is controlled by the pH and the dissolved carbon dioxide.

Table 2.3 shows the solubility products of some metal sulfides and carbonates.²⁷ The relative values of the solubility products for sulfides and carbonates emphasizes both the high insolubility of the metal sulfides and the fact that sulfide is a stronger precipitant than carbonate.

3. DEVELOPMENT OF MODELS OF THE ANFLOW SYSTEM WHICH REFLECT TOXICITY EFFECTS

3.1 General Perspective

A description of the ANFLOW System under normal conditions can be developed using nonideal flow models and first-order kinetics for the

Table 2.3. Solubility Products of Some Metal Salts at 18°C^a

Salt	Solubility Product	Solubility ^b (mg/L)
Cu ₂ S	2×10^{-47}	3×10^{-11}
CuS	8.5×10^{-45}	9×10^{-18}
PbS	3.4×10^{-28}	4×10^{-9}
CoS	3.0×10^{-26}	2×10^{-8}
NiS	1.4×10^{-24}	1×10^{-7}
ZnS	1.2×10^{-23}	3×10^{-7}
FeS	3.7×10^{-19}	5×10^{-5}
CdCO ₃	5×10^{-12}	0.4
ZnCO ₃	6×10^{-11}	1.0
FeCO ₃	3×10^{-11}	0.6
PbCO ₃	1.5×10^{-13}	0.1

^aHandbook of Chemistry and Physics, 61st edition, 1980-1981, CRC Press Inc.

^bCalculated from the solubility product.

removal of soluble COD. A more accurate description should reflect the growth phenomena in the system by incorporating a model such as that of Monod.^{16,27,28} This model is well known and has been discussed in relation to anaerobic systems by Kugelman²⁹ and others. However, the use of this model would require the evaluation of yield constants (grams biomass produced per gram substrate removed), specific growth rates, specific decay rates, and reaction rate constants. Techniques for evaluating these terms in mixed-culture, mixed-substrate, fixed-film systems such as ANFLOW have not been developed and were beyond the scope of this work. The kinetic parameters, for instance, always depend on environmental conditions, the kind of waste being treated, and the concentration of the waste, among other things.

It was therefore necessary to use an alternative approach to develop a model of the ANFLOW system, particularly one which could be used to follow inhibition and toxicity effects. A plug-flow model with superimposed axial-dispersion and first-order kinetics served to describe soluble COD removal. By a novel approach, variations in characteristic first-order rate constants were used to monitor the local and global toxicity phenomena resulting from the introduction of a heavy-metal toxicant into the system. The development of this model is described below.

3.2 Kinetic Model for Removal of Soluble COD

3.2.1 Lumped-Parameter Model for Global Analysis

The performance of a bioreactor depends not only on the relevant intrinsic kinetics of the reaction processes, but also on the physical

processes occurring on the reactor. The physical processes, such as mass transfer occurring within a multiphase system such as ANFLOW (liquid, gas, and solid or biological phases) depend very significantly upon the mixing characteristics in the phases involved.

The two extremes of mixing in a flow reactor are plug flow and complete mixing. In the first case, there is no axial mixing, and all elements of fluid within the reactor have identical velocities and residence times. For example, the flow in a packed-bed reactor with very large ratio of reactor length to tube (and packing) diameter can be approximated by a plug-flow model. The contrasting model for complete mixing would be applied for instance to very vigorously stirred batch reactors.

The nature of mixing in the majority of actual reactors falls intermediate to these two extremes. The deviations may be caused by factors such as nonuniform velocity profiles, by velocity fluctuations due to molecular or turbulent diffusion, by short-circuiting, bypassing, and channeling of fluid, by the presence of stagnant regions of fluid caused by the reactor shape and intervals, or by the recycling of fluid within the reactor as a result of agitation. Furthermore in an ANFLOW bioreactor, mixing is caused by the rising gas bubbles ($\text{CH}_4 + \text{CO}_2$) produced from the anaerobic stabilization of the organic waste.

The mixing characteristics of a reactor can be quantitatively evaluated by fitting appropriate models to the residence-time distribution (RTD) data. Many types of models can be used to characterize nonideal flow patterns within vessels. The simplest models (c.g., axial dispersion model, or simple dispersion model, or the tanks-in-series model)

contain only a single parameter correlating the RTD data. More complex models use two, three, or even four parameters to correlate RTD data.

The axial dispersion model, used in this work, characterizes the backmixing by a single one-dimensional Fick's-law-type diffusion equation. The constant of proportionality in this equation is commonly known as the axial dispersion coefficient. The assumption that all the mixing processes follow a Fick's-law-type diffusion equation, regardless of the actual mechanism, becomes, of course, increasingly inapplicable with large degrees of mixing. However, since the model characterizes the degree of mixing (or residence time distribution data) by a single parameter, its simplicity has made it one of the most widely-used models.

Using the axial dispersion model, the continuity equation governing the behavior of an ANFLOW bioreactor can be derived by considering a reactor of length L , through which a fluid is flowing at a constant and uniform velocity u . The reactants are mixed in the axial direction (direction of flow) as a result of a concentration gradient and diffusion and eddy dispersion. The reactants are assumed perfectly mixed in the radial direction, and the rate of reaction is $r(C)$. An elemental volume normal to the direction of flow is illustrated in Fig. 3.1.

The basic material balance for a reactant with concentration C in the elemental volume V shown in Fig. 3.1 is given by:

$$\text{Input} = \text{Output} + \text{Disappearance} + \text{Accumulation by Reaction} \quad (3-1)$$

Figure 3.1. Elemental volume in an ANFLOW bioreactor.

or

$$\begin{aligned} & (\text{Out-In})_{\text{bulk flow}} + (\text{Out-In})_{\text{axial dispersion}} + \\ & \text{Disappearance by Reaction} + \text{Accumulation} = 0 \end{aligned} \quad (3-2)$$

The individual terms in equation (3-2) in mol/time or mg/time are as follows,

1. Entering by bulk flow

$$\begin{aligned} & = (\text{mass/volume}) \times (\text{volumetric flow rate}) \\ & = (\text{mass/volume}) \times (\text{flow velocity}) \times (\text{cross-sectional area } A) \\ & = C_{\text{In}} uA \end{aligned} \quad (3-3)$$

2. Similarly, leaving by bulk flow

$$= C_{\text{Out}} uA \quad (3-4)$$

3. Entering by axial dispersion, from the dispersion model

$$V \frac{\partial C}{\partial t} \text{In} = - \frac{DA\Delta C}{\Delta l} \text{In} ; \quad (3-5)$$

where D is the dispersion coefficient.

4. Similarly, leaving by axial dispersion

$$V \frac{\partial C}{\partial t} \text{Out} = - \frac{DA\Delta C}{\Delta l} \text{Out} \quad (3-6)$$

5. Disappearance by reaction

$$= V[-r(C)] = A\Delta l [-r(C)] \quad (3-7)$$

6. Accumulation

$$= V \frac{\partial C}{\partial t} = A\Delta l \frac{\partial C}{\partial t} \quad (3-8)$$

After substituting the individual terms, equation (3-2) becomes

$$u[C_{\text{Out}} - C_{\text{In}}]A - D\left[\left(\frac{\Delta C}{\Delta l}\right)_{\text{Out}} - \left(\frac{\Delta C}{\Delta l}\right)_{\text{In}}\right]A + A\Delta l [-r(C)] + A\Delta l \frac{\Delta C}{\partial t} = 0 \quad (3-9)$$

dividing by $\Delta \ell$ and taking limits as $\Delta \ell \rightarrow 0$ equation (3-9) becomes

$$u \frac{\partial C}{\partial \ell} - D \frac{\partial^2 C}{\partial \ell^2} + [-r(C)] + \frac{\partial C}{\partial t} = 0 \quad (3-10)$$

In dimensionless form, by letting

$$Z = \ell/L$$

Equation (3-10) becomes

$$(D/uL) \frac{\partial^2 C}{\partial Z^2} - \frac{\partial C}{\partial Z} + (L/u)r(C) - \frac{\partial C}{\partial t} = 0 \quad (3-11)$$

The dispersion characteristics of the bioreactor can be obtained, as generally recommended, by injecting a conservative tracer at $Z = 0$ and monitoring its temporal concentration at $Z = 1$. If C is the concentration of a conservative tracer, the effluent response curve to a pulse input may be obtained from the solution to equation (3-11), where $r(C) = 0$, and the axial dispersion coefficient and the average fluid velocity are assumed to be constant over the length of the reactor. This solution has been given by Thomas and McKee³¹ and is of the form:

$$E(\Theta) = \frac{C}{C_T} = \sum_{i=1}^{\infty} \mu_i \left[\frac{\alpha \sin \mu_i + \mu_i \cos \mu_i}{\alpha^2 + 2\alpha + \mu_i^2} \right] \exp \left[\alpha - \frac{(\alpha^2 + \mu_i^2)\Theta}{2\alpha} \right], \quad (3-12)$$

where: C = Concentration of tracer in effluent, mg/L

C_T = mass of tracer injected divided by the bioreactor volume, mg/L

μ_i = a root to the equation

$$\mu_i = \cot^{-1}[(\mu_i/\alpha - \alpha/\mu_i)/2] \quad (3-13)$$

or

$$\mu_i^2 - 2\mu_i \alpha \frac{\cos \mu_i}{\sin \mu_i} - \alpha^2 = 0 \quad (3-14)$$

$$\Theta = t/\bar{t}, \text{ reduced time} \quad (3-15)$$

$$\alpha = 0.5[1/(D/uL)] \quad (3-16)$$

$$E(\Theta) = \text{exit age distribution so that } \int_0^{\infty} E(\Theta) d\Theta = 1 \quad (3-17)$$

Figure 3.2 illustrates the solution of equation (3-12). The dimensionless group (D/uL) , characterizes the vessel response to a tracer and is called the reactor dispersion number. It varies from zero for ideal plug flow to infinity for ideal mixed reactors and is the reciprocal of the Peclet number for mass transfer. To characterize the flow between the extremes represented by these two ideals, a curve is selected from the family of theoretical curves that most closely fits an experimental observation. The fit of experimental data is based on equating the variances of the two curves about the center of gravity of the distribution. This center of gravity is also defined as the mean residence time for the bioreactor and is calculated as:

$$\bar{t} = \frac{\int_0^{\infty} t C dt}{\int_0^{\infty} C dt} . \quad (3-18)$$

The variance about the centroid is

$$\sigma_t^2 = \frac{\int_0^{\infty} (t - \bar{t})^2 C dt}{\int_0^{\infty} C dt} . \quad (3-19)$$

The relationship between the dispersion number (D/uL) and the variance of the theoretical curve has been obtained by Van der Laan.³² These relationships are difficult to obtain and depend on the vessel inlet and outlet conditions. For a closed vessel,

$$\sigma_t^2 / \bar{t}^2 = 2 [(D/uL) - (D/uL)^2 (1 - \exp(-1/(D/uL)))]. \quad (3-20)$$

Thus by measuring the response to a conservative pulse tracer, $\bar{t}$ and σ_t^2 can be used to calculate a (D/uL) group which matches the variance between the dispersion model theoretical curve and the observed data. Although the above method can give a simple evaluation of the reactor dispersion number, the tailing in the residence time distribution curve

Figure 3.2. Solutions to equation (3-12) for different reactor dispersion numbers.

can cause significant inaccuracy in the evaluation of the reactor dispersion number.

The time of peak exit concentration approach is another way of determining the reactor dispersion number. This method suggested by the work of Levenspiel and Smith³³ and developed by Timpany³⁴ relates the reactor dispersion number (D/uL) with the time of peak exit concentration ($\theta_p = t_p/\bar{t}$). The time of peak exit concentration can be obtained from equation (3-12) by setting the differential of $E(\theta)$ equal to zero and solving for the $\theta_p = t_p/\bar{t}$ value associated with each (D/uL). Timpany developed a set of empirical relationships that approximate this solution and they are in the form:

$$(D/uL) = 0.2 (\theta_p)^{-1.34}, \quad (3-21)$$

where

$$0.03 < \theta_p < 0.3, \text{ and}$$

$$(D/uL) = 4.027 (10)^{-2.09} (\theta_p), \quad (3-22)$$

where

$$0.3 < \theta_p < 0.8.$$

After the mixing characteristics of an ANFLOW bioreactor have been fitted to the dispersion model and a dispersion number obtained, it is possible to calculate the steady-state response of this same bioreactor to an input of a soluble organic waste. The steady-state equation is obtained from equation (3-11) where

$$r(C) = KC^n, \quad (3-23)$$

$$\frac{\partial C}{\partial t} = 0, \quad (3-24)$$

$$f = C_e/C_o, \quad (3-25)$$

C = soluble Chemical Oxygen Demand (COD),

f = remaining soluble COD fraction,

C_0 = influent soluble COD,

C_e = effluent soluble COD.

$$(D/uL) \frac{d^2f}{dz^2} - \frac{df}{dz} K(L/u)C_0^{n-1} f^n = 0, \quad (3-26)$$

assuming first-order rate kinetics ($n = 1$), equation (3-26) becomes

$$(D/uL) \frac{d^2f}{dz^2} - \frac{df}{dz} - K(L/u)f = 0. \quad (3-27)$$

Equation (3-27) has been solved analytically by Wehner and Wilhelm³⁵ for a reactor with arbitrary entrance and exit conditions. The resulting expression, evaluated at $Z = 1$, is as follows:

$$f = (C_e/C_0) = \frac{4a \exp(1/2d)}{(1+a)^2 \exp(a/2d) - (1-a)^2 \exp(-a/2d)}, \quad (3-28)$$

where: $a = (1 + 4K\bar{t}d)^{1/2}$

$\bar{t} = L/u$

$d = (D/uL)$.

Equation (3-28) relates the soluble COD fraction f to functions of mixing levels, hydraulic residence times and first-order reaction rate constants. Figure 3.3 shows the relationship between (f) and $(K\bar{t})$ for different reactor dispersion numbers (d) .

Equation (3-28) reduces to the ideal plug flow and mixed reactors when $d = 0$ and $d = \infty$,

$$f = e^{-K\bar{t}} \text{ for } d = 0, \quad (3-29)$$

$$f = \frac{1}{1 + K\bar{t}} \text{ for } d = \infty. \quad (3-30)$$

Figure 3.3. Remaining soluble chemical oxygen demand fraction as function of $K\bar{t}$ for different dispersion numbers as predicted by equation (3-28).

Equation (3-28) describes the soluble COD removal in an ANFLOW bioreactor with three parameters (i.e., a hydraulic residence time ($\bar{t}$), a characteristic dispersion number (d) and a characteristic first-order rate constant (K)). The first-order rate constant can be seen as an indicator of biological activity (in the ANFLOW bioreactor, mainly by the methane formers). Thus K could be used to characterize the biochemical phenomena and $\bar{t}$ and d to characterize the physical phenomena.

The set of variables that can modify the performance of an ANFLOW bioreactor can be grouped in two major categories: The ones that affect K and the ones that affect $\bar{t}$ or d (see Table 3.1).

Table 3.1. Factors That Could Modify the Characteristic Parameters of an ANFLOW Bioreactor

Parameter	Modifying Factor
K	Organic loading rate
	Temperature
	Availability of nutrients
	Inhibitory substances
	pH/Alkalinity
	Kind of waste
$\bar{t}, d$	Hydraulic loading rate

Equation (3-28) was used to study the toxicity phenomena on laboratory-scale ANFLOW bioreactors by determining the variations of K with time, where $\bar{t}$ and d were assumed to be constants during a toxicity experiment. This black-box approach, or lumped-parameter model, describes the effects of heavy metals or any other inhibitory agent on the

overall performance of an ANFLOW bioreactor for soluble COD removal (see Figure 3.4).

3.2.2. Regional Model for Local Analyses

A second approach, which in this report will be called the regional model, divides an ANFLOW bioreactor into regions of different biological activity. Each region is characterized by a first-order rate constant whose magnitude is proportional to the biological activity of that region. The biological activity is measured by the soluble COD removal of the region.

This regional approach is simply the use of equation (3-28) to describe the performance of different regions of an ANFLOW bioreactor (see Figure 3.4). Mathematically it is expressed as follows:

$$f(K_o, \bar{t}_o, d_o) = \prod_{i=1}^N f_i(K_i, \bar{t}_i, d_i) \quad (3-31)$$

Where the subscript n refers to the overall system, and

$i = 1, 2, 3 \dots N$, number of regions

$$L_o = \sum_{i=1}^N L_i \quad (3-32)$$

$$\bar{t}_o = \sum_{i=1}^N \bar{t}_i \quad (3-33)$$

$(\text{diameter})_o = (\text{diameter})_i$ for a cylindrical vessel

Based on experimental observations from this study, an ANFLOW bioreactor was divided into three regions ($N = 3$). For this particular case, equation (3-31) becomes

$$f(K_o, \bar{t}_o, d_o) = \prod_{i=1}^3 f_i(K_i, \bar{t}_i, d_i) \quad (3-34)$$

Figure 3.4. Kinetic models used to study the phenomena of toxicity on laboratory-scale ANFLOW bioreactors: (a) global model, (b) regional or local model.

It is important to mention that N in equation (3-31) is arbitrary and depends on the particular application of the ANFLOW system and is best determined for each application from actual data.

The use of the regional model to study the phenomena of toxicity in ANFLOW bioreactors involves determining K_0 , K_1 , K_2 , K_3 during a toxicity experiment, assuming the average residence times and the dispersion numbers remain constant. However, the use of the regional model requires that $\bar{t}_1$, $\bar{t}_2$, $\bar{t}_3$, d_1 , d_2 , d_3 be known. Because of a lack of experimental data for these parameters and since the axial-dispersion coefficient and the average fluid velocity were assumed to be constants, the following equations:

$$d_1 = (L_0/L_1)d_0 \quad (3-35)$$

$$\bar{t}_1 = (L_1/L_0)\bar{t}_0, \quad (3-36)$$

which result from mathematical simplifications, were used to estimate the dispersion number and the average residence time for each region.

4. RESEARCH OBJECTIVES AND SCOPE

The successful stabilization of organic wastes by anaerobic treatment processes has been shown to depend on maintaining an environment favorable to the organisms involved. Toxicity-producing materials such as heavy metals are known to cause retardation or complete cessation of the internal processes on which the performance of these waste treatment systems depends. It is generally accepted that basic knowledge on the phenomena of toxicity from heavy metals or any other toxicant is very important and should be considered in the development, operation, and

control of any biological waste treatment process. This is particularly important in anaerobic waste treatment processes since the key group of bacteria, the methane formers, are very sensitive to heavy metals and other environmental stresses.

The fixed-film, packed-bed ANFLOW bioreactor is being developed for application in the treatment of municipal and/or industrial organic-wastes. In the development of this process, research needs were identified for the gathering of fundamental information on the mechanistics of toxicity from heavy metals. This basic information is considered to be of particular significance when the ANFLOW process is used for the treatment of organic wastes from industrial sources.

It was the main objective of this research project to investigate the phenomena of toxicity in laboratory-scale ANFLOW bioreactors. Specific goals to be accomplished during the project can be summarized as follows: (1) to study the phenomena of toxicity in laboratory-scale ANFLOW bioreactors by monitoring their performance in the treatment of a synthetic waste to which a heavy metal was added in both pulse and step fashions, (2) to develop a model with a lump parameter that could describe bioreactor performance in terms of soluble substrate removal and also reflect the inhibitory effects of heavy metals, (3) to study the incorporation of heavy metals into biomasses in ANFLOW bioreactors, (4) to investigate the removal of heavy metal in a fixed-film packed-bed bioreactor under anaerobic conditions, (5) to identify the operational parameters that would indicate the onset of unbalanced conditions associated with inhibition or toxicity and the need for early control

action, and (6) to identify the existence of any regenerative mechanism as a response from toxicity.

In order to achieve these research objectives, an experimental program was developed. One of the first steps in the experimental program was the selection of the heavy metal to be studied. Common heavy metals produced by industrial sources, such as the metal plating industry among others, include nickel, copper, and zinc.³⁶⁻⁴⁰ These metals have received much general attention with respect to their toxic effects on biological treatment processes.^{21,24,41-44} Zinc was the heavy metal chosen because it was considered to be suitable if there was a need for using radioactive tracers during the experimental work. Zinc has a radioisotope, Zn-65, with physical characteristics suitable for tracer studies. Among the desired characteristics are the ease of detection and a relatively long half-life. Zinc-65 emits a gamma-ray of 1.115 MeV and has a half-life of 245 days.⁴⁵

The experimental approach used in this research study was the induction of inhibition or toxicity using zinc as the toxicant. To induce toxicity efficiently, zinc was introduced as zinc chloride thereby eliminating the sulphate ion precursor of hydrogen sulfide. In a chloride form this heavy metal has a higher potential of inducing toxicity. Zinc was introduced in both pulse (slug) and step (continuous) fashions. A pulse or slug involved high heavy-metal concentrations for a time period of about one hydraulic residence time. A step, or continuous change, involved the gradual increase in heavy-metal concentration — each concentration maintained for much longer time (several residence times).

Since there is no single parameter which will always indicate the onset of unbalanced conditions, several parameters must be monitored for evaluating bioreactor performance. The variables used to evaluate reactor performance were:

- 1) Bioreactor Effluent:
 - a) Soluble COD
 - b) pH/Alkalinity
 - c) Soluble zinc
- 2) Bioreactor Off-Gas:
 - a) Gas production rate
 - b) Gas composition
- 3) Bioreactor Profiles:
 - a) Acetic and propionic acids
 - b) Soluble COD
 - c) Soluble zinc (step only)
 - d) Sorption factors (step only)

The dispersion model with first-order kinetics was used to characterize the global and regional or local performance characteristics of ANFLOW bioreactors in terms of soluble COD removal. The first-order rate constant defined by this model was used to study the kinetic aspects of the global and regional toxicity phenomena from heavy metals on this system.

CHAPTER 5. DESCRIPTION OF EXPERIMENTAL PROGRAM

5.1 Experimental System

5.1.1 Laboratory-Scale Anflow Bioreactors

The bioreactors, designed and built by ORNL to perform basic supportive research for pilot-scale processes, consisted of plexiglas cylinders 1.98 m long and 0.19 m in diameter (6.5 ft long and 8 in. in diameter) as shown in Figures 5.1 and 5.2. The packed-bed, 1.83 m long (6 ft), consisted of 2.54-cm (1-in.) Raschig rings randomly dispersed within the column. The influent is introduced at the bottom of the column and is evenly distributed across the bottom cross-sectional area by means of a diffuser plate. The effluent, taken at the upper end of the packed bed, is discharged to the drain system.

Eleven sample ports are located along the bioreactor at 15.24-cm (6-in.) intervals. The effluent was taken as sample port number 12. Samples were taken from different sections of the bioreactor to determine regional differences in substrate removal rates, heavy-metal removal, volatile acid production and utilization, and sorption factors.

Three bioreactors were involved in the experimental phase of this project. The same control bioreactor was used for all toxicity experiments. The other two, identified as T-1 and T-2, were subject to induced toxicity in both pulse and step fashions respectively.

5.1.2 Experimental Setup

The basic components of the experimental setup used during this research project (see Figure 5.3) included a feed supply system, feed

Figure 5.1. ANFLOW bioreactor designed and built by Oak Ridge National Laboratory to perform basic supportive research for pilot-scale processes.

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Figure 5.2. Schematic diagram of the ANFLOW bioreactor.

Figure 5.3. Schematic diagram of the basic components of the experimental setup used during the experimental program.

pump, ANFLOW bioreactor, and a wet-test meter to measure the off-gas production from the bioreactor.

Two different feed supply systems were used during the experimental work (see Figure 5.4). The first one, used during startup, steady-state approach and first toxicity experiment, included a feed tank with a capacity of about 1000 L and a flow and temperature ($T = 25^{\circ}\text{C}$)-controlled surge tank. With this procedure the feed solution (~600 L) lasted for about 1 week. It is important to point out that this synthetic feed solution was also used for another research project on the ammonia production on these systems. Operational problems associated with keeping the heavy metal from precipitating and the decrease of the soluble COD from 100% to about 80 to 85%, caused by bacterial growth in the feed lines, led to the development of an alternative feed-supply system. It was decided then to make the feed solution on a daily basis and used a 55-gal drum as the feed-supply system with no temperature control (see Figure 5.4(a)). This feed-supply system was used for the rest of the experimental work mainly toxicity experiments No. 2 and No. 3.

The feed-supply systems were common to all bioreactors, identified as test-1 (T-1), test-2 (T-2), and control bioreactor.

5.2 Overview of Experimental Program Development

This research project was developed in phases as shown in Figure 5.5. The most relevant phases were:

1. synthetic substrate development,

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(a)

(b)

Figure 5.4. Schematic diagrams of the feed supply systems used during the experimental program: (a) during startup, steady-state and toxicity experiment No. 1, (b) during toxicity experiments No. 2 and No. 3.

Figure 5.5. Schematic diagram of the main phases of the experimental program.

2. startup and steady-state operation,
3. residence time distribution, and
4. toxicity experiments.

A brief discussion of each phase is found in Sects. 5.2.1 to 5.2.4 respectively.

5.2.1 Synthetic Substrate Development

The toxicity phenomena studies were carried out using two specially developed substrates. This procedure was adopted to establish basic experimental conditions for the evaluation of heavy-metals effects. The absence of complicating factors in the substrate allow the establishment of basic heavy-metal effects. The relationship for toxicity obtained in this study will be those due to the presence of the heavy metal under study. It would be a rare situation to have a waste with characteristics similar to the synthetic substrates to be used. Most wastes are rather complicated solutions and could contain various substances which could produce alterations in the toxic effects of heavy metals. Furthermore, the presence of different heavy metals could result in synergistic or antagonistic effects that would enhance or prevent toxic effects.

Since organic wastes frequently are composed of fats, carbohydrates, or proteins in various combinations, a complex protein-carbohydrate mixture was initially selected as the synthetic substrate for the ANFLOW bioreactor toxicity studies. Nutrient broth, an almost pure protein, and glucose, a pure carbohydrate were combined in equal COD quantities to make up the first concentrated feed. Sufficient nitrogen and phosphorus should be available in the nutrient broth for the resulting

anaerobic growth, and no additional substances should be required. This substrate has been used by Yound and McCarty⁴⁶ when evaluating the anaerobic filter for waste treatment.

Four solutions containing different quantities of nutrient broth were dissolved in distilled water and analyzed for their COD content. The same procedure was followed for glucose (dextrose). It was found that the COD content per gram of substance was 937 ± 66 mg for nutrient broth and 1097 ± 41 mg for glucose. Sodium bicarbonate was added to help buffer the system during the startup and inhibitory operations.

It was found, during the first toxicity experiment, that zinc precipitated from solution (probably as carbonate and hydroxide forms). It became necessary to develop a second synthetic substrate with a substance that would keep the heavy metal in a soluble form during the experiment, especially under continuous operation. This substance shall complex the heavy metal and be biodegradable. Sodium citrate was the substance chosen because it (1) had been used to complex heavy metals,²¹ (2) was suspected to be biodegradable, and (3) was in good supply.

It was experimentally found that 3 g per L of sodium citrate was sufficient to complex and keep in a soluble form 1000 mg per L of zinc. The COD-equivalent of 3 g of sodium citrate were incorporated in the synthetic substrate. In order to keep the same feed strength at 3000 mg per L COD, the nutrient broth and glucose amounts were decreased, but their relative COD proportions were maintained as in the first synthetic substrate. Table 5.1 summarizes the synthetic substrates used in the experimental work.

Table 5.1. Summary of the Two Synthetic Substrates Used
During the Experimental Program

Synthetic Substrate	COD (mg/L)	Component	COD/g of Substance	COD Substrate in (mg/L)	Grams of Substance Required to Make COD
1	3000	Nutrient broth	937 ± 66^1	1500	1.6009
		Glucose (dextrose)	1097 ± 41^2	1500	1.3674
2	3000	Nutrient broth	937 ± 66	771	0.8228
		Glucose (dextrose)	1097 ± 41	771	0.7028
		Sodium citrate	486 ± 11^3	1458	3.0

The bicarbonate alkalinity of both synthetic substrates was 2000 mg/L as CaCO_3 (40 meq/L), made by dissolving 3.36 grams of sodium bicarbonate (NaHCO_3) per liter of solution.

¹Average of four samples.

²Average of four samples.

³Average of six samples.

5.2.2 Startup and Steady-State Operation

The laboratory-scale ANFLOW bioreactors used in this study have been seeded with digester sludge and operated for some time. At the time of starting the experimental work, it was about 3 months since the last time they were fed. The start-up transition to steady-state lasted about 2-3 weeks. They were then operated at steady-state for about 2 months to obtain baseline data on their performance.

5.2.3 Residence Time Distribution Experiments

A residence time distribution experiment was performed on each of the three bioreactors used in this study to obtain the average residence time and reactor dispersion numbers. These two parameters were needed in order to use the dispersion model with first-order kinetics to describe the inhibitory effects of zinc.

5.2.4 Toxicity Experiments

Three basic toxicity experiments were performed during this study. The experiments and the process conditions used are summarized in Table 5.2. The shock-load or slug fed toxicity experiments were completed from inhibition to bioreactor recovery. The continuous step function, because of a lack of time, was not completed to recovery, and ended at the stage where the inhibitory effects were caused by a continuous feeding of 1000 mg/L Zn.

5.3 Data Acquisition and Analysis

5.3.1 Average Residence Time and Reactor Dispersion Number

The RTD experiments were performed using the stimulus-response technique. This was done by injecting a slug containing 100 mL of 1000

Table 5.2. Summary of the Toxicity Experiments Performed and the Process Conditions Used.

Toxicity Experiment	Characteristics of Input Function	Bioreactor	Temperature °C	Flow Rate (ml/min)	Average Residence Time (h)	Synthetic Feed	Organic ¹ Loading kg ⁻¹ COD day ⁻¹ m ⁻³
1	Slug of 1000 mg/L Zn as ZnCl ₂ for 42 hours. Soluble zinc = 60 - 15%	T-1	20-25	10.5	43.8	1	1.64 (102.4)
2	Slug of 1000 mg/L Zn as ZnCl ₂ for 42 hours soluble Zn = 100%	T-1	25-35	10.5	43.8	2	1.64 (102.4)
3	100 mg/L Zn as ZnCl ₂ for 35 days	T-2	25-35	10.5	40.9	2	1.76
	300 mg/L Zn as ZnCl ₂ for 26 days						(109.7)
	1000 mg/L Zn as ZnCl ₂ for 13 days						
		Control	Same as T-1 and T-2	10.5	46.6	Same as T-1 and T-2	1.55 (96.4)

1. Number in parenthesis are the organic loadings in lb COD/day/1000ft³; based on the void volume.

2. Organic loading calculated as, $O.L. = \frac{C \cdot 0.24 \times \text{feed soluble COD}(=3000 \text{ mg/L})}{t \text{ (h)}}$; conversion factor to SI units = 62.3.

mg/L fluorescein dye at the bottom of the bioreactor and monitoring its concentration in the bioreactor effluent (see Figure 5.6). This dye was chosen because it was used before on the $19\text{-m}^3/\text{day}$ pilot plant, and the equipment necessary for its determination was readily available. The volume and the concentration of the injected pulse are experimental values obtained from a set of combinations of volumes and concentrations. The experiments were performed on a side bioreactor with the same design as the bioreactors used in this research, but it was not in operation. It was found that injecting 100 ml of 1000 mg per L of fluorescein dye (100 mg) produced a peak dye concentration in the bioreactor effluent of about 2 to 3 mg/L.

The dye in the effluent was determined by sampling the effluent on a continuous basis. At preset time intervals the effluent was pumped into individual test tubes in a fraction collector. The dye concentration in each test tube was determined using a fluorescence spectrophotometer (Perkin Elmer Model 203) and the experimental setup shown in Figure 5.7. Before pumping the effluent sample (about 30 ml) into the fluorescence spectrophotometer, the pH of the sample was raised to $\text{pH} > 8$ with a few drops of 50% w/w NaOH to enhance the fluorescence properties of fluorescein dye.⁴⁷ The pH dependence of fluorescein dye fluorescence for the conditions of the experiment is shown in Figure 5.8. The fluorescence spectrophotometer was calibrated (excitation wavelength = 490 nm, emission wavelength = 520 nm) with standards made of different concentrations of fluorescein dye. In general, the calibration curve has a straight portion from low % emissions to about 30% emission. This range expressed in mg/L depends on what dye concentration is chosen to be 100%

Figure 5.6. Experimental setup to continuously sample the bioreactor effluent during an RTD experiment.

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Figure 5.7. Experimental setup to analyze the effluent samples containing fluorescein dye.

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Figure 5.8. pH dependence of fluorescein dye fluorescence. Dye concentration = 2 mg/L.

emission. For the experimental conditions of this research (an expected peak dye concentration of less than 3.5 mg/L) 10 mg/L was chosen to represent 100% emission (see Figure 5.9).

The first preset time interval used in the sampling process was 10 min. In the tail portion of the RTD curve, where the concentration of the dye changed very slowly, this time was increased to 20 min. After the completion of the first RTD experiment, it was decided that 30-min sampling intervals were sufficient for the first portion of the RTD curve and 60-min intervals for the tail portion. The times and number of data points collected in each RTD experiment are summarized in Table 5.3.

Table 5.3. Summary of Residence Time Distribution Experiments

Bioreactor	First Time Interval Δt_1 (min)	Second Time Interval Δt_2 (min)	Number of Data Points with Δt_1	Number of Data Points with Δt_2	Total Number of Data Points
T-1	10	20	580	182	762
T-2	30	60	151	93	244
Control	30	60	145	95	240

The average residence time was calculated with a computer code developed based on a computational method for equations (3-18) and (3-19) as suggested by Levenspiel.²⁹ If the continuous distribution is measured only at a number of equidistant points, then equations (3-18) and (3-19) for the experimental conditions used, become:

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Figure 5.9. Percent emission as function of fluorescein dye concentration.

$$\bar{t} = \frac{\sum_{i=1}^{N_1} t_i C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} t_i C_i \Delta t_2}{\sum_{i=1}^{N_1} C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} C_i \Delta t_2} \quad (5-1)$$

and

$$\sigma_t^2 = \frac{\sum_{i=1}^{N_1} (t_i - \bar{t})^2 C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} (t_i - \bar{t})^2 C_i \Delta t_2}{\sum_{i=1}^{N_1} C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} C_i \Delta t_2} \quad (5-2)$$

which simplifies to

$$\sigma_t^2 = \frac{\sum_{i=1}^{N_1} t_i^2 C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} t_i^2 C_i \Delta t_2}{\sum_{i=1}^{N_1} C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} C_i \Delta t_2} - \bar{t}^2 \quad (5-3)$$

where: $t_i = \Delta t_1/2$, for $i = 0$

$t_i = t_{i-1} + \Delta t_1$, for $1 \leq i \leq N_1$

$t_i = t_{N_1} + (\frac{\Delta t_1 + \Delta t_2}{2})$, for $i = N_1 + 1$

$t_i = t_{i-1} + \Delta t_2$, for $N_1 + 2 \leq i \leq N_2$

N_1 = number of data points with Δt_1

N_2 = number of data points with Δt_2

The reactor dispersion number for each bioreactor was calculated using the variance method and the time of peak concentration method. The variance method was included in the same computer code used to calculate the average residence time.

The time of peak concentration was calculated on the assumption that the RTD was log-normally distributed. The equation that describes the

distribution of t when t is log-normally distributed is given as:

$$f(t) = \frac{1}{t \sigma_{1n} \sqrt{2\pi}} \exp \left[-1/2 \left[\frac{\ln(t/t_g)}{\sigma_{1n}} \right]^2 \right] \quad (5-4)$$

for $t \geq 0$,

where t_g is the geometric average time and σ_{1n} is the geometric standard deviation when t is in logarithmic units.

The relationships between the time at peak concentration (t_p) and the geometric average residence time (t_g) is obtained by setting the differential of $f(t)$ equal to zero and solving for t_p . The resulting equation,

$$t_p = t_g \exp (-\sigma_{1n}), \quad (5-5)$$

was used to calculate t_p and $\theta_p = t_p/\bar{t}$ and from equations (3-21) or (3-22) a reactor dispersion number is obtained.

A computer code was developed to calculate t_g and σ_{1n} from the RTD data. This computer code is similar to the one that calculates the arithmetic average residence time ($\bar{t}$) except that $\ln t_i$ is used instead of t_i . After substituting, equations (5-1) and (5-3) are expressed as:

$$\overline{\ln t} = \frac{\sum_{i=1}^{N_1} \ln t_i C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} \ln t_i C_i \Delta t_2}{\sum_{i=1}^{N_1} C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} C_i \Delta t_2} \quad (5-6)$$

$$t_g = \exp (\overline{\ln t}) \quad (5-7)$$

$$\sigma_{1n}^2 = \frac{\sum_{i=1}^{N_1} (\ln t_i)^2 C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} (\ln t_i)^2 C_i \Delta t_2}{\sum_{i=1}^{N_1} C_i \Delta t_1 + \sum_{i=N_1+1}^{N_2} C_i \Delta t_2} - (\overline{\ln t})^2 \quad (5-8)$$

The theoretical dispersion curve or RTD for a reactor with a given dispersion number (D/uL) was calculated using a computer code based on equation (3-12). It was found that 50 (and even less) roots were enough to characterize the theoretical RTD curve. This method represents an alternative to the variance and the time of peak concentration methods. The use of the method involves a trial and error operation. The reactor dispersion number is that dispersion number that describes the theoretical RTD curve that most closely fits the experimental residence time distribution curve.

5.3.2 Monitoring Bioreactor Response to Toxicity

Bioreactor response was monitored by determining soluble COD in the effluent, pH, alkalinity, gas production, gas composition, acetic and propionic acid profiles, and soluble COD profiles. The chemical analyses required to evaluate these parameters were performed following the basic procedures established in the Standard Methods for Water and Wastewater Analysis.⁴⁸ Reagent-grade materials were used in the chemical analyses. A brief description of each analysis is found in Sects. 5.3.2.1 to 5.3.2.5. The procedures for sample collection and treatment are covered in Sects. 5.3.2.6.

5.3.2.1 Chemical oxygen demand. The chemical oxygen demand (COD) analysis is a measure of the oxygen-equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong oxidant. The COD was determined using the EPA-approved Ampule COD Method,⁴⁹ COD range 25 to 900 mg/L. The organic matter is oxidized in a sealed 10-mL expendable ampule, which contains premixed reagents, at

$150 \pm 2^\circ\text{C}$ for 2 h (30 min in this research). The COD of the sample is determined using a Coleman Model 295 spectrophotometer at 600 nm by measuring the concentration of the Cr(III) ion. The path length of the ampules is 1.59 cm.

The use of this method depends on establishing a calibration curve that relates the COD of known samples with their measured absorbance. The recommended standard, potassium acid phthalate (KHP) has a COD content of 10,000 mg/L per every 8.5034 g or 1.176 g COD/g KHP. A typical COD standard curve is shown in Figure 5.10. This curve was checked weekly using 200-, 500-, and 800-mg/L COD standards. If the COD of the sample was suspected to be higher than 900 mg/L, sample dilutions were made in order to bring its concentration down to the operating range.

5.3.2.2 pH/Alkalinity. Sample pH was measured with a Microprocessor Ionalyzer Model 901 and a pH electrode.

The alkalinity content of the samples was determined by titrating 50 ml of the sample with 0.1N HCl to pH 4. The alkalinity in milligrams per liter as CaCO_3 was calculated from

$$\text{Alkalinity (mg/L as CaCO}_3\text{)} = 100 \times \text{volume of acid (mL)} \quad (5-9)$$

and in milliequivalents per L,

$$\text{Alkalinity (meg/L)} = 2 \times \text{volume of acid (mL)} \quad (5-10)$$

5.3.2.3 Gas production and composition. The cumulative gas production in each bioreactor was measured with a wet-test meter, located in the off-gas line. The gas production rate was calculated using the difference between two cumulative gas production readings and the time interval between them.

Figure 5.10. Calibration curve for the chemical oxygen demand analysis.

The composition of the major components in the off-gas, mainly methane, carbon dioxide, and air ($N_2 + O_2$) were determined using a Bendix Gas Chromatograph Model 80491 with a Porapak-Q packing. A calibration curve was obtained for each one of these gases by injecting a series of gas samples with volumes of 20, 40, 60, 80 and 100 μL with a pressure-locked syringe (see Figure 5.11). The response of the system was found to be linear and reproducible using the peak heights as the criteria. By injecting 100 μL of a gaseous mixture, the peak heights for each component will correspond to their respective volume percentages in the sample. It was found, however, that the system response changed from time to time but always maintained the linearity and proportionality for each gas. It was decided, then, to obtain a calibration and correct the gas percentages to 100%. The calibration curves obtained for methane, carbon dioxide, and air are shown in Figure 5.12.

Acetic and propionic acids, chosen to study the phenomena of toxicity in ANFLOW bioreactors, were analyzed using a Shimadzu Gas Chromatograph Model GC-Mini 1 with a Carbopack C/0.3% Carbowax 20 M/0.1% H_3PO_4 . Their concentration in mg/L were calculated by comparing their peak areas (using the paper-weight method) with samples containing a mixture of each acid in equal proportions. The standard mixtures used were 100, 250, 450 and 1000 mg/L of each acid. At least two replicates were obtained for each sample analyzed.

5.3.3 Bioreactor Performance for Soluble Zinc Removal

Effluent and bioreactor profile samples containing zinc analyzed using atomic absorption spectrophotometry (AAS) with an AAS Varian Techtron Model 1200, an acetylene-air mixture, a wavelength of 213.9 nm, and

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Figure 5.11. Experimental setup used to obtain the calibration curve shown on Fig. 5.12.

Figure 5.12. Calibration curves for the gas chromatograph used to determine the composition of the off-gas from the ANFLOW bioreactors.

a lamp current of 5 mA. The optimum working range under these conditions is 0.05 to 2.0 mg/L zinc.⁵⁰ A calibration curve was obtained in every analysis by combining the absorbance of a series of samples with known concentration before and after the analysis of the unknown sample. The standard curve was obtained using standards containing 0.2, 0.4, 0.8, 1.2, 1.6, and 1.8 mg/L zinc. To simplify the management of the data gathered, a linear regression equation of the form, $y = ax + b$ (where x = absorbance and y = concentration of zinc in mg/L), was obtained for every analysis and used to calculate the concentration of zinc in the samples. A typical regression coefficient of 0.9999 was not uncommon.

Soluble zinc is considered as that remaining in the supernatant after centrifuging the sample at 10,000 (16,300 $\times$ g) rpm for 15 min. Total zinc was determined after wet digestion (with sulfuric and nitric acids) of a representative portion of the sample.

5.3.4 Sample Collection and Treatment

The collection and treatment of the samples for the chemical analyses discussed in the previous sections are summarized in Table 5.4.

5.3.5 Sorption Factors and Distribution of Zinc

Solids (suspended and fixed-film) were obtained at different heights of the column using the vacuum technique shown in Figure 5.13. This technique consisted of inserting a sampling probe (60.96 cm long and 0.32 cm inside diameter) across the packed bed. By rotating and moving it, from one end to the other of the column, solids were vacuumed into a 500-mL flask. About 150 to 300 mL were collected during the operation.

Table 5.4. Summary of Collection and Treatment of Samples for the Chemical Analyses

Sample	Kind of Sample	Chemical Analysis	Treatment of Sample for Analysis	Treatment of Sample for Preservation
Effluent	Composite 1-1.5 h	Soluble COD	Centrifugation at 10,000 rpm for 15 min	Add concentrated H_2SO_4 to pH <2, and stored at a temperature = 4°C
		pH	None	Always analyzed immediately after collection
		Alkalinity	None	Same as above
		Soluble zinc	Centrifugation at 10,000 rpm for 15 min	Same as above
		Total zinc	Wet digestion with concentrated H_2SO_4 and HNO_3	Same as above
Off-gas, sample taken in gas line at top-end of bioreactor	Grab	Gas composition	None	None
Bioreactor profile	Grab	Soluble COD	Centrifugation at 10,000 rpm for 15 min	Add concentrated H_2SO_4 to pH <2, and stored at a temperature = 4°C
		Volatile acids	Same as above	Add concentrated formic acid to pH <2, and stored at a temperature = 4°C
		Soluble zinc	Same as above	Analyzed immediately after collection

Figure 5.13. Schematic diagram of the sampling method developed to obtain solids samples from the ANFLOW bioreactors. The collected sample will contain fixed-film as well as suspended solids.

The sampling probe was thin enough to follow a tortuous sampling path if necessary. Liquid was prevented from leaking out of the column by connecting tubing sections where the inside diameter of one section was equal to the outside diameter of the other (See section A-A on Figure 5.13). After collection, the solids were centrifuged at 10,000 rpm (16,300 x g) for 15 min and air-dried in a hood for 2 to 3 days. At the end of the drying period they were ground and stored in a desiccator for future analysis.

Weighed amounts of dry solids were wet-digested, and their zinc content was determined. This was done by suspending the solids in an acid solution made of 30 mL of deionized distilled water and 20 mL (10 mL each) of concentrated sulfuric and nitric acids. After adding a few glass beads, this mixture was boiled until the solution was clear and white fumes appeared. After cooling, this solution was filtered using a porcelain filtering funnel and diluted to 1000 mL or any other suitable volume. This final solution was analyzed for the zinc content.

The sorption factor as defined in this report is an indicator of the degree of association of a heavy metal with the dry solids. This factor was calculated using the following equation:

$$SF_T = \left(\frac{C \times V}{W} \right) \times \left(\frac{1000}{65.37} \right), \quad (5-11)$$

where: SF_T = sorption factor, moles zinc/kg dry solids

$(C \times V)$ = milligrams of zinc recovered from digestion of solids

C = concentration of zinc in final solution, mg/L

V = volume of final solution, L

W = milligrams of dry solids digested

65.37 g/mol = atomic weight of zinc

An extraction method was developed to characterize the heavy metal. A simplified version of the method used by Stover et al.⁵¹ was used to characterize zinc in two fractions: organically bound (associated with the biosolids) and precipitated (sulphides and carbonates). The method, shown in Figure 5.14 involves the extraction of zinc by suspending a weighed amount of dry solids in 100 mL of a 0.1 M tetrasodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) solution. This mixture was shaken at 200 rpm (1-in. stroke) for 24 h in a New Brunswick Scientific water-bath rotary shaker. At the end of shaking period this mixture was centrifuged at 10,000 rpm for 15 min and the supernatant was analyzed for zinc content. The amount of zinc in the supernatant was mainly that fraction of zinc organically bound in the solids. (These authors claimed that sodium pyrophosphate removes organically bound heavy metals and has a low extractive power for metals in a carbonate or sulfide form. Tetrasodium pyrophosphate is also widely used by workers in the Soil Sciences to extract metals from soil samples.⁵²⁻⁵⁴ That amount of metal removed with $\text{Na}_4\text{P}_2\text{O}_7$ is generally characterized as organically bound.) Wet digestion of the remaining solids provided the fraction of zinc that was in a precipitated form.

The sorption factors for each one of the fractions was calculated using equations (5-4) and (5-5). The organically bound zinc is calculated, in terms of percentages, as:

$$\% \text{ organically bound Zn} = \frac{\text{SF}_o}{\text{SF}_o + \text{SF}_p} \times 100; \quad (5-14)$$

Figure 5.14. Experimental method used to characterize zinc in ANFLOW solids as organically bound and precipitated (mainly carbonate and sulfide).

and the percent precipitated zinc, as:

$$\% \text{ precipitated Zn} = \frac{SF_p}{SF_o + SF_p} \times 100. \quad (5-15)$$

5.3.6 Determining Global and Regional First Order Rate Constants

The global and regional first-order rate constants were calculated by trial and error using a computer code to solve equation (3-28). The remaining soluble COD fraction for the overall model was simply the effluent soluble COD divided by 3000 (3000 mg/L was taken as the influent soluble COD for all computations). For the regional model the remaining soluble COD fractions were obtained from the soluble COD profiles performed during the toxicity experiments. This was done by considering that the effluent from the first region (sample port No. 1) was the influent for the second region, and the effluent from the second region (sample port No. 4) was the influent for the last region.

6. RESULTS AND DISCUSSION OF RESULTS

This section summarizes the results obtained after studying the phenomena of toxicity from zinc, as zinc chloride ($ZnCl_2$), in laboratory-scale ANFLOW bioreactors. It begins in Section 6.1 with the results obtained from the residence time distribution experiments, mainly the average hydraulic residence time and the reactor dispersion number. After determining these two characteristic parameters, the first-order rate constant was calculated from the plugflow with dispersion model. This kinetic parameter was used to characterize the phenomena of toxicity.

Section 6.2 describes the bioreactor response from toxicity as described by the first-order rate constant. Bioreactor response to toxicity as reflected by operational parameters such as effluent soluble COD, soluble COD profiles, gas production rate, gas composition, acetic and propionic acid profiles, and pH and alkalinity is covered in Section 6.3. Section 6.4 describes the bioreactor performance for soluble zinc removal, and Section 6.5 covers the sorption factors and distribution of zinc under step-feeding conditions. Possible association of these phenomena with the phenomena of toxicity is included. This chapter concludes with some discussions about the phenomena of recovering from inhibition or toxicity (see Section 6.6).

6.1 Average Residence Time and Reactor Dispersion Number

To test the assumption that the RTD curves were log-normally distributed, the concentration of fluorescein dye (as % emission) was plotted against the logarithm of t (t in minutes) as shown in Figures 6.1 and 6.3. As can be seen, they are reasonably normal, and equation (5.11) could be used to calculate the time of peak concentration (t_p).

Table 6.1 summarizes the results obtained from the RTD experiments. It can be seen that the variance (VAR) method gave lower reactor dispersion numbers as compared to the time of peak concentration (TPC) method. The dispersion numbers differ by a factor of 2 to 3.

To test which method gave better results the theoretical dispersion-curves described by the calculated dispersion numbers were obtained and compare with the experimental RTDs. As shown in Figures 6.4 to 6.9 the TPC method gave dispersion numbers that most closely described the experimental RTDs.

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Figure 6.1. Fluorescein dye concentration (percent emission) against the natural logarithm of t (in minutes) for bioreactor T-2.

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Figure 6.2. Fluorescein dye concentration (percent emission) against the natural logarithm of t (in minutes) for control bioreactor.

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Figure 6.3. Fluorescein dye concentration (percent emission) against the natural logarithm of t (in minutes) for bioreactor T-1.

Table 6.1. Summary of the Results Obtained from the
Residence Time Distribution Experiments

Bioreactor	$\bar{t}$ (h)	t_g (h)	σ_t^2 (h ²)	σ^2_{1n}	t_p (h)	θ_p	$(D/uL)_{VAR}^a$	$(D/uL)_{TPC}^b$
T-1	43.8	34.4	895.4	0.5177	16.8	0.38	0.33	0.64
T-2	40.9	33.2	890.3	0.7412	12.5	0.31	0.35	0.92
Control	46.6	36.8	979.1	0.5258	17.8	0.38	0.33	0.64

^aVariance method.

^bTime of peak concentration method.

Figure 6.4. Comparison between the experimental RTD for bioreactor T-2 and the theoretical RTD based on a reactor dispersion number of 0.92 obtained from the time of peak concentration method.

Figure 6.5. Comparison between the experimental RTD for bioreactor T-1 and the theoretical RTD based on a reactor dispersion number of 0.64 obtained from the time of peak concentration method.

Figure 6.6. Comparison between the experimental RTD for control bioreactor and the theoretical RTD based on a reactor dispersion number of 0.64 obtained from the time of peak concentration method.

Figure 6.7. Comparison between the experimental RTD for bioreactor T-1 and the theoretical RTD based on a reactor dispersion number of 0.33 obtained from the variance method.

Figure 6.8. Comparison between the experimental RTD for bioreactor T-2 and the theoretical RTD based on a reactor dispersion number of 0.35 obtained from the variance method.

Figure 6.9. Comparison between the experimental RTD for control bioreactor and the theoretical RTD based on a reactor dispersion number of 0.33 obtained from the variance method.

Even though the TPC method described the experimental RTD curves better than the VAR method some difference can be seen between the theoretical and experimental curves. Two factors that might explain the difference is the sorption/desorption phenomena between fluorescein dye and the packing-fixed-film system and the porosity of the packing. These two phenomena are not considered in the dispersion model. The porosity of the packing in a packed bed is part of the liquid holdup system and contributes to the tailing in the RTD curve.^{29,30,55} Packing with high porosities can be considered as the main cause of the tailing phenomena in the RTD for these systems. This tailing in the RTD curves can cause some inaccuracies when the dispersion number is calculated from the variance method.⁶⁰ Other models⁵⁶⁻⁵⁹ have been developed to account for these phenomena but they use three, four, or even more parameters to very closely describe experimental RTD curves.

An extensive study of hydraulic phenomena in ANFLOW bioreactors was beyond the scope of this work. Research in this area is suggested if the dispersion model is further developed to be incorporated as part of the design criteria for ANFLOW bioreactors. In this research, however, the main objective in performing the RTD experiment was to determine the reactor dispersion number and average residence time. As seen from the results obtained, the time of peak concentration method gave a better estimate of the reactor dispersion number for the experimental conditions used.

6.2 The Phenomena of Toxicity as Described by the Dispersion Model with First Order Kinetics

The response of an ANFLOW bioreactor to heavy metal-induced toxicity can be considered to depend, among other things, on the regional performance of the reactor under steady-state conditions (or free of inhibition), the toxic effects of the toxicant on the regional phenomena and the characteristics of the toxic input function. It seems to be a general trend for these bioreactors to have most of the biological activity located on the lower sections of the vessel. The volume of such region depends mainly on the organic loading rate, availability of nutrients, and environmental conditions.

Table 6.2 shows the characteristics parameters t and d for each bioreactor as needed to solve equation (3.28) for the global and regional first-order rate kinetic constants.

6.2.1 Global Phenomena

The response of the dosed ANFLOW bioreactors during the three toxicity experiments, as measured by the global first-order rate constant, are shown in Figures 6.10 to 6.12. By comparing the response curves of toxicity experiments No. 1 and No. 2 (under shock-load conditions) two major observations can be made: (1) the degree of inhibition as measured by ΔK was higher with a larger amount of soluble zinc, and (2) the lower the value of K during the days following the introduction of the shock load the longer it took to recover. These two observations were anticipated since the potential of a heavy metal to induce toxicity increases with increase in the soluble fraction.

Table 6.2. Summary of the Characteristic Parameters for
Global and Regional Kinetic Models

Bioreactor	Global			Region I			Region II			Region III		
	L^a	$\bar{t}^b$	d^c	L	$\bar{t}$	d	L	$\bar{t}$	d	L	$\bar{t}$	d
T-1	1.83	43.8	0.64	0.15	3.7	7.68	0.46	11.0	2.56	1.22	29.2	0.96
T-2	1.83	40.9	0.92	0.15	3.4	11.04	0.46	10.2	3.68	1.22	27.3	1.38
Control	1.83	46.6	0.64	0.15	3.9	7.68	0.46	11.7	2.56	1.22	31.1	0.96

a_L = length of bioreactor region (m).

$b_{\bar{t}}$ = calculated regional average residence time (h).

$c_d = (D/uL)$ = reactor dispersion number.

Figure 6.10. Global first-order rate constants for control and test (T-1) bioreactors during toxicity experiment No. 1.

Figure 6.11. Global first-order rate constants for control and test (T-1) bioreactors during toxicity experiment No. 2.

Figure 6.12. Global first-order rate constants for control and test (T-2) bioreactors during toxicity experiment No. 3.

For toxicity experiment No. 3 (step function) there is a gradual decrease in the global first-order rate constant during the first 20 days (see Figure 6.12). From 20 to 60 days there was not so much change in reactor performance even though the concentration of the heavy metal in the feed solution was increased by a factor of 3 to 300 mg/L. If Figure 6.12 were the only source of information it could be concluded that the system was developing some sort of acclimation to the inhibitory feed. Furthermore, when the concentration of zinc was increased to 1000 mg/L (a factor of about 3.3) there was not an immediate response as in shock-loading. The response pattern appears to follow the same trend as with 100 mg/L. All these observations are complemented when the phenomena of toxicity is described with the regional kinetic model and its kinetic lump parameter, the local or regional first-order constant.

The response pattern for shock-loads in terms of the global first-order rate constant can be generalized using Figure 6.13. Three parameters characterize the pattern: t_1 , t_2 , and K_{min} . The rate of inhibition and recovery can be expressed as:

$$\text{rate of inhibition} = -\frac{dK}{dt}, \text{ for } 0 \leq t \leq t_1 \quad (6-1)$$

$$\text{rate of recovery} = \frac{dK}{dt}, \text{ for } t_1 \leq t \leq t_2 \quad (6-2)$$

These parameters (t_1 , t_2 , K_{min}) depend among other things on the input function, regional phenomena, and flow characteristics.

An expression could be found to describe the variations of K with time after the introduction of the shock-load (slug). An equation of the form,

Figure 6.13. Reponse pattern of an ANFLOW bioreactor to a heavy metal injected as a shock load.

$$K(t) = \frac{K_{ss}}{I.F.}, \quad (6-3)$$

where K_{ss} is the global first-order rate constant at steady state, and I.F. is an inhibition function (its general form could be determined with further research), could be used to describe the response pattern. A similar approach for the study of inhibition from substrates on bacterial growth rates has been proposed by Andrews.⁶¹

With Equation 6-3 a methodology is developed for a kinetic approach to the study of the phenomena of toxicity in biological treatment processes. The study of the phenomena of toxicity will involve an initial evaluation of the reactor dispersion number, average residence time, and the steady-state first-order constant. After the induction of toxicity, it will involve the development of an inhibition function that could describe the variations of K . One property that the inhibition function should have is that it should reflect the conditions under which the induction of toxicity is made, either shock or step-test conditions. The process of developing such an inhibition function could provide useful information for a better understanding of the phenomena of toxicity.

6.2.2 Regional Phenomena

The steady-state performance of the laboratory-scale ANFLOW bioreactors could be considered as to consist of three regions with decreasing biological activity as measured by soluble COD removal. The first region, with a volume of about 8% of the total volume, is a very biologically active region and is responsible for the removal of more than 70% of the soluble COD removed by the entire bioreactor. The second region, responsible for the removal of the remaining COD not removed by the

first region, has a volume of about 25% of the total volume. The last region, with about 67% of the total volume, does not contribute to the overall soluble COD removal performance of the system. Instead, it may be considered as a region where biological decay or biosolids digestion are the predominant bioprocesses. This region, considered to be insignificant for the overall performance of the system under steady-state operation, might have some significance on the phenomena of toxicity.

As shown in Figures 6.14 to 6.17, the response of the vessel depends on the degree of inhibition to the active region. The inactive region will begin to become active from the resulting increase in organic loading reaching this region of the vessel. The upper regions considered inactive (small K 's) during steady-state operation now begin to see an increase in organic loading. With the increase in organic loading the biological activity of those regions begin to increase (K increase). Even though the lower regions become inactive, from a soluble COD removal point of view, they still remove the heavy metal protecting the upper regions by decreasing the inhibitory potential of the waste.

Since the regional first-order rate constants were calculated from soluble COD profiles they do not represent a major source of detailed information as the global constants obtained from the effluent COD. To the extent they were measured they are, however, indicative of the dynamic phenomena occurring within the vessel. A better procedure to obtain enough data to describe the regional constants to the same extent as the global constants would be to sample the effluent of every region (from sample ports) on a daily basis or even more often if necessary. Composite sampling instead of grab sampling is recommended.

Figure 6.14. Global and regional first-order rate constants for test (T-1) bioreactor during toxicity experiment No. 1. The constants were calculated from the soluble chemical oxygen demand profiles.

Figure 6.15. Global and regional first-order rate constants for test (T-2) bioreactor during toxicity experiment No. 2. The constants were calculated from the soluble chemical oxygen demand profiles.

Figure 6.16. Global and regional first-order rate constants for test (T-2) bioreactor during toxicity experiment No. 3. The constants were calculated from the soluble chemical oxygen demand profiles.

Figure 6.17. Global first-order rate constants for control and test (T-1) bioreactors during toxicity experiment No. 1. The constants were calculated from the soluble chemical oxygen demand profiles.

The global kinetic constants are shown in Figures 6.17, 6.18, and 6.19. These figures could be compared with Figures 6.10 to 6.12, respectively, to illustrate the difference in the amount of information provided by the sampling procedures. It can be seen that for the overall system both sampling procedures provided enough information. A closed look to Figures 6.14 to 6.16 indicates, however, that more data is required for a better description of the regional phenomena.

The information provided by studying the phenomena of toxicity regionally could be incorporated in the inhibition function defined by Equation (6-3). This could be done by the development of regional inhibition functions and combining them in a single expression which will result in the inhibition function for the entire vessel. As in steady-state operation, one particular region is anticipated to be the predominant contributor to the overall behavior.

The regional kinetic constants for the control bioreactor during all toxicity experiments are shown in Figures 6.20 to 6.22. One major observation from these figures is the relative magnitude of the kinetic constants for each region which indicates the extent of the biological activity and the relative contribution of each region to the overall performance of the bioreactor.

6.3 Bioreactor Response to Toxicity as Reflected in Operational Parameters

6.3.1 Chemical Oxygen Demand

The transients for the soluble COD in the effluent of control and dosed bioreactors are shown in Figures 6.23 to 6.25 for all toxicity

Figure 6.18. Global first-order rate constants for control and test (T-1) bioreactor during toxicity experiment No. 2. The constants were calculated from the soluble chemical oxygen demand profiles.

Figure 6.19. Global first-order rate constants for control and test (T-2) bioreactors during toxicity experiment No. 3. The constants were calculated from the soluble chemical oxygen demand profiles.

Figure 6.20. Global and regional first-order rate constants for control bioreactor during toxicity experiment No. 1. The constants were calculated from the soluble chemical oxygen demand profiles.

Figure 6.21. Global and regional first-order rate constants for control bioreactor during toxicity experiment No. 2. The constants were calculated from the soluble chemical oxygen demand profiles.

Figure 6.22. Global and regional first-order rate constants for control bioreactor during toxicity experiment No. 3. The constants were calculated from the soluble chemical oxygen demand profiles.

Figure 6.23. Soluble chemical oxygen demand in bioreactor effluent during toxicity experiment No. 1. (Influent COD = 3000 mg/L).

Figure 6.24. Soluble chemical oxygen demand in test (T-1) and control bioreactor effluents during toxicity experiment No. 2. (Influent COD = 3000 mg/L).

Figure 6.25. Soluble chemical oxygen demand in test (T-2) and control bioreactor effluents during toxicity experiment No. 3. (Influent COD = 3000 mg/L).

experiments. In general, there was a measurable gradual increase (in proportion to the concentration of soluble zinc) in the soluble COD, probably related with increase in biological activity in the upper regions and the dispersion characteristics of the vessel. If these two phenomena did not occur, particularly under shock conditions, then the increase in soluble COD in the effluent should be very sudden because the unremoved COD was moving upward as a slug. Dispersion is considered to be beneficial because it tends to reduce the toxic potential of a toxic waste.

The soluble COD profiles for the three experiments are shown in Figures 6.26 to 6.28. The general pattern is an upward shifting for inhibition and a downward shifting for recovery, and the rate of shifting could be considered as the rate of inhibition and the rate of recovery respectively. The profiles for the test bioreactor are compared with the average profile for the control bioreactor. These average profiles were obtained from the COD profiles for the control bioreactor during the time the particular toxicity experiment was being performed. The soluble COD profiles for the control bioreactor during the toxicity experiments are shown in Figures 6.29 to 6.31.

A comparison between Figures 6.26 and 6.27 (both under shock-load conditions) indicates that an increase in the soluble fraction of zinc resulted in a higher degree of inhibition in the upper regions and consequently more time would be needed for full recovery. The response pattern for shock and step-test conditions are somewhat similar in shape, but they differed in the rate of shifting or rate of inhibition.

Figure 6.26. Soluble chemical oxygen demand profiles for test bioreactor (T-1) during toxicity experiment No. 1. Also shown is the average profile for control bioreactor. (Influent COD = 3000 mg/L). ..

Figure 6.27. Soluble chemical oxygen demand profiles for test bioreactor (T-1) during toxicity experiment No. 2. Also shown is the average profile for control bioreactor. (Influent COD = 3000 mg/L).

Figure 6.28. Soluble chemical oxygen demand profiles for test bioreactor during toxicity experiment No. 3. Also shown are the concentrations of zinc in the feed, and the time when the profile was done relative to the time when the particular concentration was first introduced in the feed. (Influent COD = 3000 mg/L).

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Figure 6.29. Soluble chemical oxygen demand profiles for control bioreactor during toxicity experiment No. 1. (Influent COD = 3000 mg/L).

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Figure 6.30. Soluble chemical oxygen demand for control bioreactor during toxicity experiment No. 2. (Influent COD = 3000 mg/L).

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Figure 6.31. Soluble chemical oxygen demand profiles for control bioreactor during toxicity experiment No. 3. (Influent COD = 3000 mg/L).

Due to time constraints the toxicity experiment under step-test conditions was not completed to recovery. It is expected however that it might follow the same trend as the one shown in Figure 6.27. This observation is based on a comparison between curve B in Figure 6.27 and curve E in Figure 6.28.

6.3.2 Gas Production and Composition

As shown in Figures 6.32, 6.33, and 6.34 there is a decrease in the gas production rate as response from inhibition. This parameter by itself may not be good indicator of inhibitory conditions when there are large variations in the organic loading. The scattered nature of these figures is due mainly to the physical phenomena of bubble production and released in the packed bed. The gas production is not uniform but in random pulses. These phenomena added to variations in organic loading rates will probably cause wide variations in the gas production rate under field conditions (especially if the gas production rate is measured in L/h for example).

The methane and carbon dioxide percent patterns as response from inhibition are shown in Figures 6.35, 6.36, and 6.37. The effects from toxicity are only noticeable in toxicity experiment No. 3 where the heavy metal was fed continuously.

The relative percentages of carbon dioxide and methane (about 30 and 70% in this study respectively) depend on the rate of production of each gas and in their solubility in the liquid phase. The solubility of carbon dioxide is several orders of magnitude higher than the solubility of methane.⁶² Furthermore, under normal operating conditions, the ratio of

Figure 6.32. Gas production rate for control and test (T-1) bioreactors during toxicity experiment No. 1.

Figure 6.33. Methane and carbon dioxide percentages in the off-gas of control and test (T-1) bioreactors during toxicity experiment No. 1.

Figure 6.34. Gas production rate for control and test (T-1) bioreactors during toxicity experiment No. 2.

Figure 6.35. Methane and carbon dioxide percentages in the off-gas of control and test (T-1) bioreactors during toxicity experiment No. 2.

Figure 6.36. Gas production rate for control and test (T-2) bioreactors during toxicity experiment No. 3.

Figure 6.37. Methane and carbon dioxide percentages in the off-gas of control and test (T-2) bioreactors during toxicity experiment No. 3.

carbon dioxide to methane varies with the character of the substrate undergoing decomposition.⁶³

Under normal operations conditions some of the carbon dioxide produced dissolves in the water maintaining an alkalinity level high enough to prevent a decrease in pH when the volatile acids are being produced. The alkalinity of the system (whose major contributor is the carbonate system) under the existing near-neutral pH conditions is given primarily by the bicarbonate ion.⁶⁴ This alkalinity serves as potential buffer when unbalanced conditions appear and the concentration of volatile acids begin to increase. When the acids are neutralized, carbon dioxide is produced. This production of carbon dioxide coupled with a decrease in the production of methane contribute to an increase in its percentage in the off-gas.

The change in gas composition during toxicity experiments under shock conditions were not noticeable to a great extent as in continuous-feeding of the heavy metal. This phenomena probably happened because of the relatively short time involved during the toxicity induction phase and the dilution phenomena in the gas chamber on top of the packed section where the gas samples were taken. This response is also modified by the shifting of biological activity to upper regions resulting in a decrease in reactor response as determined from the effluent. When the heavy metal was fed continuously, the inhibitory phase was gradual and much longer times were involved. Shifting of biological activity to upper regions was observed, but it was expected that a point would eventually be reached where toxicity was going to be uniformly distributed along the entire vessel.

In summary, to observe a change in gas composition would require a continuous gas production with different composition for some time. For example, a sudden complete cessation in the gas production would result in no change in the composition of the gas in the gas chamber under the experimental conditions of this study.

6.3.3 Acetic and Propionic Acid Profiles

Acetic and propionic acids were found to be good indicators of inhibitor or toxicity to this system. As shown in Figures 6.38 to 6.43, the acids were produced in the lower or active regions; however, since they were not used to the same extent (as in steady-state operation) they kept moving upward and reached the upper or inactive regions. Since these regions do not have enough bacterial population (methane formers), these acids were not removed to a great extent. This state will exist for some time because of the long generation times for these microorganisms. Their capacity to use the acids and produce the necessary biomass will depend on the potential of the heavy metal to reach these regions. This potential depends on the metal removal capacity of the lower regions.

Based on these acid profiles and the soluble COD profiles shown before, it is concluded that the kinetic constant describes the biological activity of the methane formers. This observation could be anticipated since it is being considered that waste stabilization (soluble COD removal) occurs mainly with the production of methane (methanogenesis). In the operating mode the bioreactor is divided in two regions, one that produces the acids and the other that produces the methane. Since the

Figure 6.38. Acetic acid profiles for test bioreactor during toxicity experiment No. 1.

Figure 6.39. Propionic acid profiles for test bioreactors (T-1) during toxicity experiment No. 1.

Figure 6.40. Acetic acid profiles for test (T-1) bioreactor during toxicity experiment No. 2.

Figure 6.41. Propionic acid profiles for test bioreactor (T-1) during toxicity experiment No. 2.

Figure 6.42. Acetic acid profiles for test bioreactor during toxicity experiment No. 3. Also shown are the concentrations of zinc in the feed and the time when the profile was done relative to the time when the particular concentration was first introduced in the feed.

Figure 6.43. Propionic acid profiles for test bioreactor during toxicity experiment No. 2. Also shown are the concentrations of zinc in the feed and the time when the profile was done relative to the time when the particular concentration was first introduced in the feed.

acids increased in concentration, it is concluded that inhibition was induced mainly to the methane formers. The same degree of inhibition to the acid formers should result in a decrease in acid production (acetogenesis), resulting in lower acid concentrations.

The best way to monitor the performance of an ANFLOW bioreactor would be to determine and physically define the active region. The rate of increase in total volatile acids concentration in the active region should be one of the variables to monitor and the one indicator for the need for control action. Since the process is a series reaction, this increased rate can be caused by an increased rate of production of volatile acids by the acid-producing bacteria (response to an increase in organic loading) or a decreased rate of acid consumption by the methane bacteria (inhibition). As shown in Figures 6.44 to 6.52, the control bioreactor has its own volatile acid transients, especially in the active region, but they did not result in a decrease in bioreactor performance because this transient was damped by the upper regions. This observation suggests that probably the heavy metal was present when the acids reached the upper regions of the dosed bioreactor, and this factor reduced their potential to dampen the inhibition caused to the active regions. Experience in the operation of ANFLOW bioreactors under field conditions could also be useful in the assessment of the presence and cause of unbalanced conditions, mainly, increase in acetogenesis or decrease in methanogenesis.

6.3.4 pH and Alkalinity

The alkalinity in an ANFLOW bioreactor serves as a buffer preventing rapid changes in pH. Bioreactor stability is therefore enhanced by

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Figure 6.44. Propionic acid profiles for control bioreactor during toxicity experiment No. 1.

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Figure 6.45. Acetic acid profiles for control bioreactor during toxicity experiment No. 1.

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Figure 6.46. Average volatile acid profiles for control bioreactor during toxicity experiment No. 1.

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Figure 6.47. Acetic acid profiles for control bioreactor during toxicity experiment No. 2.

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Figure 6.48. Propionic acid profiles for control bioreactor during toxicity experiment No. 2.

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Figure 6.49. Average volatile acid profiles for control bioreactor during toxicity experiment No. 2.

Figure 6.50. Acetic acid profiles for control bioreactors during toxicity experiment No. 2.

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Figure 6.51. Propionic acid profiles for control bioreactor during toxicity experiment No. 3.

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Figure 6.52. Average volatile acid profiles for control bioreactor during toxicity experiment No. 3.

a high alkalinity concentration. Under normal operating conditions, the major buffering system is the carbon dioxide—bicarbonate system with the ammonium ion as the major cation. However, when the concentration of volatile acids is high, a substantial portion of the alkalinity as measured is caused by the volatile acids since the analysis for alkalinity is conducted by titrating with a strong acid to pH of 4.5 to 4.0. The pK of acetic and propionic acids is about 4.5-5,⁶⁵⁻⁶⁶ therefore a portion of the titrant is used to neutralize the volatile acid salts.

It is considered that bicarbonate alkalinity would be a better indicator of bioreactor conditions rather than the total alkalinity since bicarbonate alkalinity is more related to the biochemical changes associated with anaerobic waste treatment. The bicarbonate ion is the major chemical species of the carbonate system. Carbon dioxide, the source of bicarbonate, is one of the by-products of methanogenesis. A separate analysis as proposed by Banta and Pomeroy⁶⁷ could be useful. The alkalinity also depends on the kind of incoming waste. High protein wastes would have higher alkalinities because of the production of ammonia from proteins.

The pH is another indicator of possible problems within the reactor-vessel. It is influenced by other operational variables, and although it is easy to be measured, it is not a sensitive indicator since it is a logarithmic function. For a system with adequate buffering capacity, it does not decrease significantly until the system is seriously affected. In a system such as ANFLOW, the alkalinity and pH are functions that would depend on the location within the bioreactor.

Enough alkalinity was added during all toxicity experiments to help buffer the system. Based on pH and alkalinity measurements performed in the bioreactor effluents, the systems were well buffered (see Tables A.8, B.9, and C.8).

6.4 Bioreactor Performance for Soluble Zinc Removal

The removal of zinc was investigated during toxicity experiment No. 3 when the heavy metal was being fed continuously. As shown in Figure 6.53, the removal efficiencies for soluble zinc were more than 95% when the influent concentration was 100 mg/L, about 90% for 300 mg/L, and about 85% for 1000 mg/L. This trend indicates that the sorption capacity of the system was decreasing. Some analyses of total zinc concentration in the effluent were performed. It was found that the fraction of suspended zinc increased from about 10 to 20% at a zinc concentration in the feed of 100 and 300 mg/L to about 30 to 40% at 1000 mg/L. These phenomena could be explained by an increase in biosolids sloughing from the fixed-films or by an increase in precipitated (carbonate or sulfide) zinc associated with a decrease in the sorption capacity of the column or both. Unfortunately, no data are available to support these or any other phenomena.

The soluble zinc concentrations in the bioreactor effluent during shock-load conditions are indicated in Figure 6.54. As can be seen, the curve begins with a gradual increase in concentration from background levels. This concentration level is believed to be the result of dispersion, as well as sorption of the heavy metal by the biomasses. The concentration of zinc in the effluent kept increasing until a maximum

Figure 6.53. Soluble zinc concentration in test (T-2) and control bioreactor effluents during toxicity experiment No. 3.

Figure 6.54. Soluble zinc in test (T-1) bioreactor effluent during toxicity experiment No. 2.

was reached. After the shock load was stopped and feeding without zinc was resumed, wash out of the metal began to occur. Thereafter, the concentration of zinc began to decrease. It was not determined if this wash out was from the voids only or if it also included desorption from the fixed-films or biosolids.

6.5 Sorption Factors and Distribution of Zinc Under Step-Feeding Conditions

It was found that depending on the influent concentration and sorption factors the efficiency for soluble zinc removal in the active region (Region I) decreased from about 80 to 85% to about 20 to 30%. As shown in Figure 6.55 and supported by Figure 6.56, the upward shifting in the soluble zinc profiles, mainly in the lower regions of the bio-reactor, could be caused by a decrease of the sorption capacity or the number of binding sites available in these regions. After these regions began to saturate, the upper regions received the increase in metal concentration and the sorption factors began to increase too.

As shown in Figures 6.55 and 6.56, soluble zinc profiles and sorption factor profiles follow the same pattern as the soluble COD profiles. It seems reasonable to conclude that inhibition seems to be associated with the amount of heavy metal sorbed by the biosolids.

An attempt was made to determine if there was any relation between the sorption factor and the soluble zinc concentration at that location. Because of its flexibility, the Freundlich equation is the most widely used equation for complex systems that involve sorption phenomena. The equation as applied to this study can be expressed as

Figure 6.55. Soluble zinc profiles for test (T-2) bioreactor during toxicity experiment No. 3. Times given refer to the time when the profile was done relative to the time when the particular concentration was first introduced in the feed.

Figure 6.56. Sorption factor profiles for test (T-2) bioreactor during toxicity experiment No. 3. Times given refer to the time when the profile was done relative to the time when the particular concentration was first introduced.

$$SF_T = K_F C^{1/n}, \quad (6-4)$$

where K_F and $1/n$ are constants, SF_T is the sorption factor in moles of zinc per kg of dry solids, and C is the concentration of soluble zinc in moles per m^3 . This equation can be linearized to become

$$\ln SF_T = 1/n \ln C + \ln K_F. \quad (6-5)$$

The parameter $\ln K_F$ could be considered as an indicator of sorption capacity and $1/n$ of sorption intensity. K_F and $1/n$ are obtained from the intercept and slope of the straight line that results when the logarithm of the sorption factor is plotted against the logarithm of the soluble zinc concentration (see Figure 6.57).

From a least-squares fit of the sorption factors data, the following equation was obtained:

$$\ln SF_T = 0.5090 \ln C - 0.0383 \quad (6-6)$$

with a regression coefficient (r) of 0.8955. In its exponential form the equation is

$$SF_T = 0.9624 C^{0.5090}. \quad (6-7)$$

These two equations are illustrated in Figures 6.57 and 6.58.

As shown in Table 6.3, it was found that over 90% of the heavy metal was organically bound in the solids sampled from the ANFLOW bioreactor. This finding supports other observed phenomena associated with inhibition or toxicity. The analyses were performed when the zinc concentration in the influent was 100 and 300 mg/L. Not enough solids were collected, probably because of previous sampling, when zinc was being fed at a concentration of 1000 mg/L. Previous sampling was performed on every other sample port to avoid not having enough solids to perform the

Figure 6.57. Freundlich-type relationship between the sorption factor and the soluble zinc concentration when plotted in log-log paper.

Figure 6.58. Freundlich equation describing the relationship between the soluble zinc concentration and the amount of zinc sorbed by the solids in an ANFLOW bioreactor. The solids would include fixed-films as well as suspended masses.

Table 6.3. Distribution of Zinc in ANFLOW Bioreactor Solids During Toxicity Experiment No. 3

Influent Zn (mg/L)	Soluble Zinc in Sample (mg/L)	Sorption Factors			(SF _O + SF _P)	% Recovery ^a
		SF _T	SF _O	SF _P		
300	199	98.0	92	6.4	98.4	101.4
300	262	89	82	5	87.0	97.8
300	147.3	154.7	152	3.2	155.2	100.3
100	21.8	19.2	22.1	1.3	23.4	121.9
100	33.2	36.3	36	4.3	40.3	111.0
100	49.9	54.2	43.1	2.5	45.6	84.1
100	34.3	43.5	40.7	1.2	41.9	95.9
100	29	36.2	28.3	0.8	29.1	80.4
100	17	19.6	23.4	0.8	24.2	123.5
100	18.7	38.3	37.7	0.5	38.2	99.7
100	7.5	25.0	27.7	.8	28.5	114.0
100	4.9	18.2	21.4	0.5	21.9	120.3

$$^a\% \text{ recovery} = \frac{\text{SF}_O + \text{SF}_P}{\text{SF}_T} \times 100$$

characterization of the heavy metal. Most of the solids were located on the lower regions of the bioreactor, mainly the first four sample ports. It is suspected that solids sampling probably enhanced the inhibitory state of the bioreactor by decreasing the amount of biosolids in the system. This would result in a decrease of its sorption capacity for heavy-metal removal in addition to decrease bioreactor performance for the removal of soluble COD.

6.6 Overview of the Recovery Phenomena

Recovery or resumption of normal operating conditions, global and regionally, is a very important aspect of the overall phenomena of toxicity. The basic components of the phenomena of toxicity from heavy metals in ANFLOW bioreactors and the benefits that could result by investigating them are summarized in Table 6.4.

The aspect of inducing toxicity has been studied to some extent and the basic benefit from it is that the toxicity potential of a heavy metal is reduced by precipitation from solution. This control measure reduces the interaction between the heavy metal and biomasses. The response from heavy-metal-induced toxicity was the basic objective of this work. The recovery aspect will be surveyed briefly, and research in this area as well as in acclimation is suggested. Research in the area of acclimation would be somewhat similar to toxicity experiment No. 3, where the concentration of the heavy metal is increased gradually, but it would involve much lower concentrations (6-10 mg/L) and smaller increments in heavy-metal concentration.

Table 6.4. Basic Aspects of the Phenomena of Toxicity and
the Engineering Significance of Their Study

Basic Aspect of Toxicity Phenomena	Engineering Significance of Studying Basic Aspect
Induction of Toxicity	Development of control measures, for example, precipitation of heavy metals.
Response	Proper identification of the presence of toxic conditions during operation and the need for immediate control actions.
Recovery	Development of routine procedures to minimize the recovery time and establishment of normal operating conditions.
Acclimation	Development of procedures to enhance system resistance and have broader applications.

The discussion that will follow is only speculative in nature and is not based on actual data as evidence of its existence. Instead, it is mainly a rationalization that could serve as one explanation to the response data presented in previous sections.

During normal operation, bioreactor performance in the treatment of organic wastes probably depends on fixed-films and to some degree on suspended solids. It is not known with certainty the contribution of each phase nor whether this contribution depends on location. Under field conditions these suspended solids may come in the waste or are generated within the vessel by sloughing mechanisms. These mechanisms are probably caused by factors such as film growth, flow of liquid, bubble growth and generation, and upward movement of the produced bubbles. Depending on factors such as particle size, fluid velocity, and the tortuous path they need to follow within the packed bed they either settle down and are trapped in the voids, are removed by impact or any other mechanisms, or are washed out of the system by the flowing liquid or by the gas bubbles as in flotation. For the most part it is considered in this work that ANFLOW bioreactors depend on fixed-films.

One of the advantages of this system, of particular significance under inhibitory conditions, is that since its performance depends on fixed-films, the biosolids remain in the system probably resulting in a decrease in the recovery time. One disadvantage, caused by the reactor type (i.e., packed vs mixed vessel), is the dilution phenomena during shock-loads. A packed bed probably has some degree of dispersion or

dilution potential, but it is smaller than in mixed reactors. The safety factor involved in such situations depends on the size of the shock-load relative to the volume of the vessel and the degree of development or sophistication of the control actions in each system. Once inhibition is induced in a mixed reactor, the suspended solids are washed out further decreasing the cell residence time or number of viable cells. This situation is reduced by full recycle. In any event, recovery times may be long, and seeding may be required.

The treatment process in the anaerobic fixed-films of an ANFLOW bio-reactor could be described in a very simple manner using the simple bio-film model shown in Figure 6.59. This model is an extension of the bio-film model proposed by Atkinson⁶⁸⁻⁷⁰ as applied to an anaerobic fixed-film. It is assumed that methanogenesis or fermentation of the volatile acids is the rate-limiting step in the removal of soluble COD.⁷¹ The performance per unit volume of fixed film is considered to depend on the number of viable methane formers per cubic centimeter (viable cell density) and the growth rate associated with them. This cell density may not be uniform throughout the film, but for simplicity it is assumed to be uniform. An increasing degree of inhibition, as reflected by the performance of the system to remove soluble COD, is the result of decreasing the number of viable cells in the film. The concentration of volatile acids within the film is the balance between production, utilization, and net diffusion rates. During inhibitory conditions, the rate of utilization decreases more than the rate of production, and buildup of acids occurs. These acids diffuse out of the film and enter the bulk solution. In the upper regions of the system where the biological

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Figure 6.59. Simple model for a fixed-film in an ANFLOW bioreactor. The film is formed by a heterogeneous mixture of acid and methane formers.

activity is much lower, these acids diffuse into the film providing enough food for increasing the biological activities. The rate of increase is controlled by the presence of the heavy metal.

System recovery by a regeneration phenomena could be explained by supposing that a fixed-film that has been inhibited to a certain extent is allowed to recover. The regeneration mechanism proposed from this study is that the cell layers closer to the structural support are inhibited to a lesser extent than the upper layers of the film. These layers can grow and repopulate, increasing the thickness of the fixed film. As in normal operation, sloughing will occur but in this case inhibition would probably enhance the sloughing process by weakening the upper layer of the film. Cell death, lysis, and digestion by other bacteria (acid formers for example) are considered as the most probable sloughing-enhancing factors.

The time required to regenerate a fixed-film to the viable cell densities present during normal operation depends on the generation time and the initial density of the microorganisms involved. The generation time can be modified by environmental factors such as nutrients, pH, temperature, substrate, and absence of toxic materials. For example, an increase in volatile acids to very high concentrations may induce some inhibition on the methane formers and even on the acid formers.⁶¹ Relatively long recovery times are expected to be required in anaerobic treatment simply because of the long generation times of the methane bacteria.

7. SUMMARY AND CONCLUSIONS

Oak Ridge National Laboratory has developed an energy-conserving wastewater treatment system based on an anaerobic, upflow (ANFLOW) bioreactor. Since many applications of the ANFLOW process could involve the treatment of wastewaters containing heavy metals, the potentially toxic effects of these metals on the biological processes occurring in ANFLOW columns (primarily acetogenesis and methanogenesis) were investigated.

An experimental program was developed where zinc (as zinc chloride) in pulse inputs of 1000 mg/L and step inputs ranging from 100 to 1000 mg/L were added to synthetic wastewaters being treated in ANFLOW columns with 0.057-m^3 volumes. The global and regional kinetic aspects of the toxicity phenomena were described with a mathematical model based on plugflow with axial dispersion and first-order kinetics for soluble COD removal. Bioreactor response to toxicity was determined by monitoring operational parameters such as gas production and composition, acetic and propionic acid concentration profiles, soluble COD profiles, effluent soluble COD, pH, and alkalinity. Metal sorption, removal and distribution were investigated under step-input conditions.

Several conclusions related with the phenomena of toxicity from heavy metals in ANFLOW bioreactors can be drawn from this research project:

1. The inhibitory effects of Zn on soluble substrate removal were found to be associated with the sorption of the heavy metal by the

biosolids. Sorption (and consequently high metal-removal efficiencies) initially occurred in the lower sections of the column moving upward as the sorption capacity of these regions was beginning to decrease. This saturation trend resulted in a decrease in heavy-metal removal efficiencies. Consequently, a much larger reactor volume was needed to obtain the removal efficiencies previously obtained in the lower regions. It was found that the sorption phenomena could be described by a Freundlich equation. This equation relates the amount of heavy metal (in moles of zinc per kilogram of dry solids) with the concentration of soluble metal (in moles of zinc per cubic meter) surrounding the solids. It was also found that most of the heavy metal present in the solids sampled from the ANFLOW bioreactor was organically bound or associated with biomasses. It is expected that under field conditions this distribution might be different.

Precipitation of heavy metals by formation of sulfide is considered to play a major role when the metals are introduced under stepwise conditions. At pH values of 8 and above, most of the reduced sulfur exist in solution as HS^- and $\text{S}^{=}$ ions. At pH levels below 8, the equilibrium shifts toward the formation of un-ionized H_2S and is about 80 percent complete at pH 7.⁶⁶ When the concentration of a heavy metal is increased gradually, the precipitation effectiveness of the hydrogen sulfide system is enhanced. This happens because the H_2S can supply sulfide ions continuously. Under shock conditions, only the sulfide ion will be able to protect the system.

2. The methane formers were the main group of bacteria inhibited by zinc. This finding was anticipated since the methane-forming bacteria

are generally considered to be more susceptible to heavy metals than the acid-forming bacteria. An increase in volatile acid concentrations (acetic and propionic acids) was the local response after inhibiting these bacteria. Although acetic and propionic acids were used to determine the inhibitory effects of heavy metals on methanogenesis, a determination of total volatile acid concentration by steam distillation or chromatographic separation could be used as a criteria in the control of ANFLOW bioreactors. Inhibition, on a global scale, was reflected as an upward shifting in the concentration profiles for these two acids. It is believed that the shifting in the soluble COD profiles was primarily caused by the increase in volatile acid concentrations. Inhibition to the acid-forming bacteria is expected to result in a shifting of the maxima or peak of the volatile acids profile to upper regions in the reactor in addition to the upward/downward shifting in volatile acids concentration profiles. By the time the toxicant starts causing inhibition to the acid-forming bacteria the system has received extensive damage.

Gas production and composition were other variables used to monitor methanogenesis. In general, it was found that inhibition from zinc was reflected as a decrease in gas production rate and a decrease in methane percent. The general characteristics of the response (magnitude and pattern) was found to be associated with the way inhibition was induced, either shock or stepwise, and the degree of inhibition. Since methane production rates are representative of methanogenesis it is proposed that a more sensitive parameter to monitor this stage will be the methane production rate (i.e. gas production rate x methane percent).

Based on the previous research findings it is concluded that a sound approach for operating and controlling an ANFLOW bioreactor will consist of determining the active region and monitoring methanogenesis with three basic parameters:

- a. rate of methane production from whole vessel,
- b. rate of change in volatile acids concentrations in active region, and
- c. bicarbonate alkalinity in active region.

The first two parameters combined will be used as indicators of process stability and to establish the need for early control actions. The bicarbonate alkalinity shall be kept at a high enough level to help buffer any increase in volatile acid concentration resulting from inhibition to the methane bacteria. This technique will help prevent any synergistic effects between the toxicity of heavy metals and a decrease in pH.

3. The plugflow with axial dispersion model with first-order kinetics was found to be useful in characterizing toxicity data. The kinetic parameter, a lump constant, reflected the inhibitory effects of heavy metals on the global and regional kinetics as determined from soluble COD removal data.

It was found that the biological activity progressively moved upward to upper regions of the reactor. It is believed that their relative rates of increase in biological activity depended on the concentration of the heavy metal. Its potential to induce inhibition being dependent on the sorption capacity of lower regions.

It was observed that there is a characteristic response pattern as a result of inhibition in this system under shock-load and continuous-feed

conditions. Each response pattern could be described by a function of the form:

$$K(t) = K_{SS}/I.F.,$$

where the inhibition function (I.F.) characterizes the response and the conditions under which toxicity was induced.

4. System recovery was observed when feeding of wastewaters without zinc was resumed. It is postulated that this phenomena could be based on a regeneration process that involved sloughing of layers of biofilms, or other biosolids involved in zinc sorption, followed by continued growth of lower layers of biofilms not involved in heavy-metal sorption. Recovery times are considered to depend, among other things, on the long generation times of the methane-forming bacteria.

8. RECOMMENDATIONS FOR FUTURE STUDY

The research program of this project was designed to emphasize the development of methods, models, and definitions of mechanisms rather than to conduct a survey of varying effects among several metals. Future studies on ANFLOW bioreactors should be oriented toward the research areas of toxicity and development of design principles which may incorporate the phenomena of toxicity. Several projects recommended for future studies on these research areas are the following:

1. In the Area of Toxicity

- a. study the toxicity phenomena under field conditions;
- b. develop a technique for continuously monitoring the production of methane;

- c. explore ways of minimizing the recovery time of an ANFLOW system that has been toxified;
- d. determine the general form of the inhibition function and the parameters that define it;
- e. use the mixed-tanks-in-series model as an approach to the regional aspects of the toxicity phenomena;
- f. study the toxic effects of other insulting agents.

2. In the Area of Design

Study the feasibility of using the plug flow with dispersion model with first-order kinetics, or any other rate expression, as part of the design criteria for ANFLOW bioreactors. A similar approach has been proposed by Thirumurthi⁷² in the design of waste stabilization ponds.

The following research projects would provide significant information:

- a. application of the kinetic model to describe total COD or BOD removal in large-scale processes;
- b. determination of the effects of temperature, loading rates, kind of waste and other factors on the global rate constant(s) (a regional approach may be very useful);
- c. development of simpler techniques for determining accurately the reactor dispersion number and average residence time (these techniques should simplify the gathering and management of data);
- d. determination of the effects of scaling-up on the reactor dispersion number and average residence time.

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9. APPENDIXES

APPENDIX A: DATA FOR TOXICITY EXPERIMENT NO. 1

Toxicity experiment No. 1 was a shock-load (slug) of 1000 mg/L zinc as ZnCl_2 for 42 h; variable soluble fraction. The shockload began with 60% soluble zinc and ended with 15% soluble zinc. Time zero is taken as the time when the experiment began.

Table A.1. Effluent Soluble Chemical Oxygen Demand for Control and Test Bioreactors During Toxicity Experiment No. 1

Time (days)	Soluble COD (mg/L)	
	Control Bioreactor	Test Bioreactor
-33	213	210
-30	204	213
-28	175	175
-26	167	166
-22	182	200
-20	186	192
-15	183	183
-13	231	227
-7	183	185
-2	192	192
-1	200	200
0	172	180
0.771	198	193
1.0	192	187
1.729	195	285
2.815	187	410
3.875	182	288
4.56	182	273
5.0	185	278
5.75	174	278
6.0	175	279
7.0	179	295
8.729	183	285
9.875	189	268
11.75	194	279
12.771	209	283
13.771	175	298
14.75	194	294
15.77	194	276
18.75	213	298
19.75	219	291
20.75	211	300
21.75	211	281
26.75	173	182
27.75	175	187
28.625	162	173
32.75	159	170
33.75	155	168
40	132	152

Table A.2. Soluble Chemical Oxygen Demand Profiles for Control and Test Bioreactors
During Toxicity Experiment No. 1^a

Time (days)	Bioreactor	Bioreactor Length (cm x 3.281 x 10 ⁻²)											
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
-1	Control	600	355	262	213	213	192	190	185	177	170	168	172
	Test	528	348	287	268	236	220	212	202	196	187	183	176
20	Control	403	290	281	232	222	223	225	227	219	211	194	190
	Test	2352	1097	900	616	505	435	417	380	345	340	320	267
29	Control	447	308	260	178	182	178	170	170	162	162	162	162
	Test	1203	528	457	340	314	254	232	236	184	186	178	173
40	Control	752	429	244	178	165	162	153	140	136	141	138	137
	Test	985	675	492	492	372	305	267	236	219	175	162	152
Control average		551	346	262	200	196	189	185	181	174	171	166	165
S.D.		159	62	15	27	27	26	31	36	35	29	23	22

^aCOD concentrations in mg/L.

Table A.3. Acetic Acid Profiles for Control and Test Bioreactors During Toxicity Experiment No. 1^a

Time (days)	Bioreactor	Bioreactor Length (cm x 3.281 x 10 ⁻²)											
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
-1	Control	220	142	114	103	96	91	38	30	25	25	23	16
	Test	114	108	90	70	58	54	51	51	37	30	26	15
20	Control	35	26	47	26	33	25	20	25	24	19	31	34
	Test	1000	1066	920	720	740	540	580	595	490	501	389	394
29	Control	423	178	63	57	32	41	18	20	6	17	16	37
	Test	537	249	207	186	85	961	80	77	45	50	396	50
40	Control	450	450	125	30	50	20	20	20	10	5	5	5
	Test	450	450	100	50	50	20	20	20	10	10	10	10
Control Average		282	199	87	54	53	44	24	24	16	17	19	23
S. D.		194	179	38	35	30	32	9	5	10	8	11	15

^aAcetic Acid concentrations are in mg/L.

Table A.4. Propionic Acid Profiles for Control and Test Bioreactors During
During Toxicity Experiment No. 1^a

Time (days)	Bioreactor	Bioreactor length (cm x 3.281 x 10 ⁻²)											
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
-1	Control	75	45	20	7	10	5	0	0	0	0	0	0
	Test	31	16	14	11	9	8	6	4	4	4	0	0
20	Control	11	4	14	2	4	7	0	0	0	0	0	0
	Test	238	180	103	123	131	79	92	87	103	110	68	53
29	Control	108	60	16	4	1	1	0	0	0	0	0	0
	Test	313	122	100	85	30	42	34	26	6	7	6	7
40	Control	200	120	25	10	5	0	0	0	0	0	0	0
	Test	200	200	40	20	20	10	10	10	5	5	0	0
Control Average		99	57	19	6	5	3	0	0	0	0	0	0
S. D.		79	48	5	4	4	3	0	0	0	0	0	0

^aPropionic acid concentrations are in mg/L, a value of zero is used to indicate a nondetectable concentration.

Table A.5. Cumulative Gas Production, in Liters, for Control and Test Bioreactors During Toxicity Experiment No. 1

Time (days)	Bioreactor	
	Control	Test
-33.042	0	0
-30.042	55.85	54.72
-29.042	71.81	69.85
-28.042	95.01	91.44
-27.042	116.00	113.27
-26.042	137.64	134.93
-23.042	195.96	189.23
-22.042	215.48	209.53
-21.042	237.50	230.77
-20.042	259.04	251.2
-19.042	282.75	273.80
-16.230	343.25	330.61
-15.042	366.56	352.83
-14.125	386.34	372.09
-13.042	410.34	395.55
-12.084	432.41	417.08
-7.082	541.52	525.43
-6.042	565.31	550.19
-5.125	585.57	571.44
-2.042	642.76	629.04
-1.208	659.66	648.11
-1.111	661.81	650.52
-0.974	665.15	653.50
-0.253	682.32	672.89
-0.163	684.55	675.25
0.045	689.33	679.35
0.722	704.13	690.25
0.821	706.24	691.68
0.911	708.27	693.05
0.996	710.15	694.39
1.038	711.09	695.11
1.716	725.08	708.55
1.823	727.23	710.48
1.942	729.69	712.55
2.007	731.18	713.68
2.038	731.91	714.22
2.792	750.60	729.24
2.99	755.5	733.94
3.913	779.24	752.7
5.028	805.68	773.99
6.052	832.75	795.6
7.042	858.43	815.37
8.048	885.61	836.56

Table A.5. (Continued)

Time (days)	Bioreactor	
	Control	Test
9.052	912.33	857.24
12.035	987.42	917.80
13.052	1022.85	935.01
14.050	1048.16	957.16
15.039	1073.26	978.70
16.047	1098.27	1000.11
19.050	1167.57	1063.89
20.030	1190.09	1084.71
21.028	1213.26	1106.87
22.035	1236.9	1129.07
23.038	1260.60	1151.37
26.042	1319.93	1210.38
27.042	1344.22	1231.74
28.051	1365.82	1256.41
29.045	1387.98	1278.54
33.042	1492.13	1373.23

Table A.6. Gas Production Rate, in Liters per Day, for Control and Test Bioreactors During Toxicity Experiment No. 1^a

Δt (days)	$t_{1/2}$ (days)	Bioreactor	
		Control	Test
3	-31.542	18.62	18.24
1	-29.542	15.96	15.13
1	-28.542	23.2	21.59
1	-27.542	20.99	21.83
1	-26.542	21.64	21.66
3	-22.542	19.44	18.10
1	-21.542	19.52	20.3
1	-20.542	22.02	21.24
1	-19.542	21.54	20.43
1	-18.542	23.71	22.60
2.812	-17.636	21.51	20.20
1.188	-15.636	19.62	18.70
0.917	-14.584	21.57	21.00
1.083	-13.584	22.16	21.66
.958	-12.563	23.04	22.47
5.002	-9.583	21.81	21.66
1.040	-6.562	22.88	23.81
.917	-5.584	22.09	23.17
3.083	-3.584	18.55	18.68
0.834	-1.625	20.26	22.98
0.084	-1.250	23.21	24.64
0.097	-1.160	22.16	24.85
0.137	-1.043	24.38	21.75
0.781	-0.614	23.81	26.89
0.090	-0.208	24.78	26.22
0.208	-0.059	22.98	19.71
0.677	0.384	21.86	16.10
0.099	0.772	21.31	14.44
0.090	0.866	22.22	15.22
0.085	0.954	22.12	15.76
0.042	1.017	22.38	17.14
0.678	1.377	20.63	19.82
0.107	1.770	20.09	18.04
0.119	1.883	20.67	17.39
0.065	1.975	22.92	17.38
0.031	2.023	23.55	17.42
0.754	2.415	24.79	19.92
0.2	2.892	24.5	23.5
.923	3.452	25.75	20.33
1.115	4.471	23.71	19.09
1.024	5.540	26.44	21.10
0.990	6.547	25.94	19.97
1.006	7.545	27.02	21.06

Table A.6. (Continued)

Δt (days)	$t_{1/2}$ (days)	Bioreactor	
		Control	Test
1.004	8.550	26.61	20.60
2.983	10.554	25.17	20.30
1.017	12.544	34.84	16.92
0.998	13.551	25.36	22.19
0.989	14.545	25.38	21.23
1.000	15.543	25.01	21.54
3.003	17.549	23.08	21.24
0.980	19.540	22.98	21.24
0.998	20.529	23.22	22.20
1.007	21.532	23.48	22.05
1.003	23.035	23.63	22.23
3.004	24.540	19.75	19.64
1.000	26.542	24.29	21.36
1.009	27.547	21.41	24.45
0.994	28.548	22.29	22.26
3.997	31.044	26.06	23.69

$a_{t_{1/2}} = \frac{(t_2 + t_1)}{2}$, $\Delta t = t_2 - t_1$, and t_1 and t_2 were obtained from Table

A.5.

Table A.7. Gas Composition for Control and Test Bioreactors
During Toxicity Experiment No. 1

Δt (days)	Control Bioreactor			Test Bioreactor		
	% air	% CH ₄	% CO ₂	% air	% CH ₄	% CO ₂
-29.125	2.0	74.3	23.7	2.4	74.2	23.4
-28.135	2.2	71.3	26.5	2.2	70.1	27.7
-26.125	1.6	71.1	27.2	1.6	71.4	26.9
-22.125	2.5	70.7	26.8	3.4	69.7	26.9
-20.146	3.8	68.9	27.3	3.4	69.4	27.2
-19.125	3.1	68.5	28.4	3.0	68.9	28.1
-15.104	3.1	70.7	26.2	3.2	70.6	26.2
-14.021	1.9	68.6	29.5	2.3	69.4	28.3
-13.094	2.6	68.6	28.8	1.4	69.6	29.0
-12.115	1.6	69.3	29.1	1.9	69.2	29.9
-8.063	2.3	71.3	26.4	2.5	71.0	26.5
-7.104	2.3	70.9	26.8	2.0	70.9	27.1
-6.042	2.0	69.5	28.5	2.1	69.4	28.5
-5.021	1.8	71.3	26.9	2.0	71.0	27.0
-2.0	3.4	75.0	21.6	2.5	75.2	22.3
-1.0	1.7	68.4	29.8	1.7	68.6	29.7
0	1.9	69.5	28.6	2.4	69.2	28.4
0.833	2.2	69.6	28.2	3.0	65.0	32
1.750	2.3	70.9	26.8	2.5	66.3	31.3
1.917	2.3	71.2	26.5	2.8	66.6	30.6
2.979	2.3	69.2	28.5	2.2	67.2	30.6
3.792	3.2	69.4	27.4	2.9	67.6	29.6
4.792	2.9	68.6	28.5	2.5	67.0	30.5
5.0	2.4	67.5	30.1	3.3	65.7	31.0
5.729	2.1	68.7	29.2	2.5	67.5	30.0
6.75	2.6	69.5	27.9	2.2	68.5	29.3
7.0	1.6	70.9	27.5	2.6	68.0	29.4
7.75	2.1	69.0	29.9	2.5	68.1	29.4
8.0	1.8	69.7	28.5	1.8	69.1	29.1
8.75	2.6	69.8	27.6	3.2	68.5	28.3
9.0	2.9	69.0	28.1	1.8	69.1	29.1
11.895	2.4	70.3	27.3	2.4	69.5	28.1
12.813	2.3	75.3	22.4	2.4	72.1	25.5
13.0	1.7	75.2	23.1	2.6	70.8	26.6
13.771	2.1	71.6	26.4	3.1	68.8	28.1
14.75	2.0	71.0	27.0	2.0	69.7	28.3
15.75	3.2	69.5	27.3	2.2	69.5	28.4
16.0	3.0	69.2	27.8	1.9	69.8	28.3
18.792	2.4	72.4	25.2	2.2	72.4	25.4
19.75	2.1	70.8	27.1	2.1	69.7	28.2
20.75	3.0	70.2	26.8	3.5	69.4	27.1
21.792	4.8	68.4	26.8	2.7	69.4	27.9
22.75	3.1	69.2	27.7	3.0	68.8	28.1

Table A.7. (Continued)

Δt (days)	Control Bioreactor			Test Bioreactor		
	% air	% CH ₄	% CO ₂	% air	% CH ₄	% CO ₂
25.792	2.6	77.4	20.0	2.6	76.2	21.2
26.000	2.8	76.5	20.7	2.8	76.1	21.1
26.813	1.5	74.3	24.2	1.7	74.5	23.8
27.792	2.1	70.5	27.4	2.3	70.4	27.3
28.333	1.9	70.9	27.2	1.6	71.1	27.3

Table A.8. Effluent pH and Alkalinity for Control and Test Bioreactors During Toxicity Experiment No. 1

Time (days)	Control				Test	
	Control pH	Test pH	Alkalinity		Alkalinity	
			(mg/L as) CaCO ₃	(meq/L)	(mg/L as) CaCO ₃	(meq/L)
-30.031	7.13	7.12	-	-	-	-
-26.250	7.11	7.15	-	-	-	-
-22.042	7.31	7.28	-	-	-	-
-21.042	7.32	7.26	-	-	-	-
-20.083	7.27	7.27	-	-	-	-
-14.000	7.45	7.35	-	-	-	-
-13.042	7.37	7.37	-	-	-	-
-12.052	7.26	7.34	-	-	-	-
-7.042	7.19	-	-	-	-	-
-6.271	7.33	-	-	-	-	-
-5.083	7.35	-	-	-	-	-
-1.167	7.09	7.27	3018	60.4	2982	59.6
-0.188	7.34	7.27	-	-	-	-
0.979	7.21	7.12	2941	58.8	2851	57.0
1.938	7.27	7.08	2940	58.8	2420	48.4
2.792	7.20	7.17	2965	59.3	2500	50.0
3.781	7.17	7.09	2940	58.8	2520	50.4
4.729	7.31	7.2	2920	58.4	2580	51.6
5.75	7.32	7.33	2950	59.0	2650	53.0
6.74	7.29	7.13	2940	58.8	2750	55.0
7.76	7.39	7.34	2920	58.4	2740	54.8
8.729	7.38	7.36	3033	60.7	2805	56.1
11.75	7.25	7.22	2850	57.0	2750	55.0
12.833	7.50	7.22	3122	62.4	2841	56.8
13.781	7.37	7.39	3123	62.5	2920	58.4
14.760	7.33	7.33	3150	63.0	2900	58.0
16.037	7.46	7.56	3080	61.6	3050	61.0
18.740	7.37	7.36	3070	61.4	3000	60.0
19.75	7.48	7.32	3011	60.2	2906	58.1
20.823	7.26	7.32	3036	60.7	2970	59.4
21.923	7.34	7.44	3030	60.6	3000	60.0
22.778	7.47	7.44	2970	59.4	2970	59.4
25.948	7.67	7.62	2950	59.0	2944	58.9
27.865	7.72	7.39	3020	60.4	2970	59.4
28.990	7.34	7.34	3006	60.1	2995	59.9

Table A.9. Global First-Order Rate Constants^a for Control and Test Bioreactor During Toxicity Experiment No. 1

Time (days)	Control Bioreactor ^b			Test Bioreactor ^c		
	f_o	$K_o \bar{t}_o$	K_o	f_o	$K_o \bar{t}_o$	K_o
-33	0.071	5.39	0.12	0.070	5.43	0.12
-30	0.068	5.53	0.12	0.071	5.39	0.12
-28	0.058	6.06	0.14	0.058	6.06	0.14
-26	0.056	6.18	0.13	0.055	6.24	0.14
-22	0.061	5.88	0.13	0.067	5.57	0.13
-20	0.062	5.83	0.13	0.064	5.72	0.13
-15	0.061	5.88	0.13	0.061	5.88	0.13
-13	0.077	5.13	0.11	0.076	5.17	0.12
-7	0.061	5.88	0.13	0.062	5.83	0.13
-2	0.064	5.72	0.12	0.064	5.72	0.13
-1	0.067	5.57	0.12	0.067	5.57	0.13
0	0.057	6.11	0.13	0.060	5.94	0.14
-0.771	0.066	5.62	0.12	0.064	5.72	0.13
1.0	0.064	5.72	0.12	0.062	5.83	0.13
1.729	0.065	5.67	0.12	0.095	4.49	0.10
2.815	0.062	5.83	0.13	0.137	3.48	0.08
3.875	0.061	5.88	0.13	0.096	4.46	0.10
4.56	0.061	5.88	0.13	0.091	4.62	0.11
5.0	0.062	5.83	0.13	0.093	4.55	0.10
5.75	0.058	6.06	0.14	0.093	4.55	0.10
6.0	0.058	6.06	0.14	0.093	4.55	0.10
7.0	0.060	5.94	0.13	0.098	4.40	0.10
8.729	0.061	5.88	0.13	0.095	4.49	0.10
9.875	0.063	5.78	0.12	0.089	4.68	0.11
11.75	0.065	5.67	0.12	0.093	4.55	0.10
12.771	0.070	5.43	0.12	0.094	4.52	0.10
13.771	0.058	6.06	0.13	0.099	4.37	0.10
14.75	0.065	5.67	0.12	0.098	4.40	0.10
15.77	0.065	5.67	0.12	0.092	4.59	0.10
18.75	0.071	5.39	0.12	0.099	4.37	0.10
19.75	0.073	5.30	0.11	0.097	4.43	0.10
20.75	0.070	5.43	0.12	0.100	4.34	0.10
21.75	0.070	5.43	0.12	0.094	4.52	0.10
26.75	0.058	6.06	0.14	0.061	5.88	0.13
27.75	0.058	6.06	0.14	0.062	5.83	0.13
28.625	0.054	6.30	0.14	0.058	6.06	0.14
32.75	0.053	6.37	0.14	0.057	6.11	0.14
33.75	0.052	6.43	0.14	0.056	6.18	0.14
40	0.044	7.03	0.15	0.051	6.50	0.15

^aCalculated from effluent soluble chemical oxygen demand (see Table A.1).

^b $\bar{t}_o$ for control bioreactors = 46.6 h.

^c $\bar{t}_o$ for test bioreactors = 43.8 h.

Table A.10 Global and Local First-Order Rate Constants_a for Control and Test Bioreactors During Toxicity Experiment No. 1.

Time (days)	Bioreactor	f_0	$K_0 \bar{t}_0$	$K_0(h^{-1})$	f_1	$K_1 \bar{t}_1$	$K_1(h^{-1})$	f_2	$K_2 \bar{t}_2$	$K_2(h^{-1})$	f_3	$\bar{f}_3 t_3$	$K_3(h^{-1})$
-1	Control	0.057	6.11	0.13	0.200	3.70	0.95	0.355	1.65	0.14	0.808	0.23	0.0074
	Test	0.059	6.00	0.14	0.176	4.29	1.16	0.507	0.92	0.084	0.657	0.50	0.017
20	Control	0.063	5.78	0.12	0.134	5.74	1.47	0.576	0.71	0.061	0.819	0.21	0.0067
	Test	0.089	4.68	0.11	0.748	0.27	0.073	0.262	2.45	0.22	0.433	1.20	0.041
29	Control	0.054	6.30	0.14	0.149	5.14	1.32	0.398	1.39	0.12	0.910	0.10	0.0032
	Test	0.058	6.06	0.14	0.401	1.45	0.39	0.283	2.23	0.20	0.509	0.90	0.031
40	Control	0.046	6.87	0.15	0.251	2.81	0.72	0.237	2.75	0.24	0.770	0.29	0.0093
	Test	0.051	6.50	0.15	0.328	1.97	0.53	0.499	0.95	0.086	0.309	1.94	0.066

^aCalculated from soluble chemical oxygen demand profiles (see Table A.2).

APPENDIX B: DATA FOR TOXICITY EXPERIMENT NO. 2

Toxicity experiment No. 2 was a shock-load (slug) of 1000 mg/L zinc as ZnCl_2 for 42 h; 100% soluble zinc. Time zero is taken as the time when the experiment began.

Table B.1. Effluent Soluble Chemical Oxygen Demand^a for
Control and Test Bioreactors During Toxicity
Experiment No. 2

Time (days)	Bioreactor	
	Control	Test
-14.25	173	182
-13.25	175	187
-12.25	162	173
- 8.25	159	170
- 7.25	155	168
- 6.25	189	205
- 5.25	138	146
- 4.25	147	140
- 0.083	139	125
0.792	153	175
1.771	126	141
2.813	121	855
3.563	130	1382
3.823	123	931
4.823	132	916
5.781	132	858
7.01	150	1200
8.906	150	1050
9.740	175	949
13.719	180	1240
14.740	176	1111
16.792	170	840
19.740	210	835
20.740	165	860
21.740	155	805
22.740	165	675
23.760	146	615
27.740	160	727
28.740	194	653
29.740	-	727
30.740	-	720
33.740	160	-
34.740	175	740
35.927	198	-
36.760	186	-
37.760	195	730
38.802	135	780
39.844	140	770
41.719	151	610
42.719	150	500
43.719	150	530
44.802	148	499

Table B.1. (Continued)

Time (days)	Bioreactor	
	Control	Test
45.875	148	481
47.740	152	382
48.740	150	353
49.771	150	353
50.750	142	338
51.771	138	303
52.750	137	299
55.823	161	265
56.792	157	311
57.792	129	351
58.792	130	306
61.771	143	243
62.792	138	204
63.792	136	280
64.792	134	277
65.792	141	253
68.854	147	179
69.792	169	208
70.792	172	240
71.792	163	198
72.792	150	184
73.792	165	213
74.771	161	185
75.771	154	164
76.750	176	180
78.917	159	169
80.792	152	163
81.979	144	144
82.750	162	144
83.750	167	151
84.750	152	157
87.771	154	175
88.750	149	170
89.771	113	134
90.750	117	109
91.771	133	123

^aValues given in mg/L.

Table B.2 Soluble Chemical Oxygen Demand Profiles for Control and Test Bioreactors During Toxicity Experiment No. 2^a

Time (days)		Bioreactor Length (cm x 3.281 x 10 ⁻²)											
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
-1	Control	752	429	244	178	165	162	153	140	136	141	138	137
	Test	985	675	492	372	305	267	236	219	175	162	155	152
6	Control	620	280	225	195	192	192	185	170	175	178	174	170
	Test	2650	2140	1980	1772	1740	1464	1428	1460	1380	1420	1420	1340
24	Control	746	258	198	175	167	178	159	152	160	160	166	167
	Test	1658	1555	1048	960	907	845	826	744	773	747	764	615
50	Control	1009	361	173	160	156	153	155	149	148	138	145	142
	Test	1730	856	752	620	658	505	556	476	374	414	370	338
88	Control	705	217	133	143	135	133	113	113	107	107	102	105
	Test	463	334	318	237	207	218	170	148	170	157	185	151
Control Ave.		766	309	195	169	163	164	153	145	145	145	145	144
S. D.		146	35	44	19	21	23	26	21	26	27	28	26

^aCOD concentrations in mg/L.

Table B.3 Cumulative Gas Production, in Liters, for Control
and Test Bioreactors During Toxicity Experiment No. 2

Time (days)	Bioreactor	
	Control	Test
-14.000	0	0
-12.976	22.00	25.02
-12.000	43.69	46.15
-8.000	128.22	127.00
-6.958	148.83	142.47
-5.962	180.34	167.33
-4.953	208.29	192.4
-3.949	236.73	218.38
-1.983	314.60	288.67
-0.292	332.29	304.09
0.0	339.75	310.64
0.708	357.50	323.09
1.042	366.28	327.98
1.708	382.28	336.58
2.038	389.99	342.35
2.714	405.84	352.53
3.038	414.38	357.28
3.809	434.70	369.29
3.978	439.37	372.10
4.813	462.80	387.21
4.951	466.77	389.63
5.698	485.84	402.30
6.015	493.48	407.03
6.717	511.45	418.47
7.045	520.51	424.17
7.713	537.88	435.46
8.016	546.48	440.89
8.688	564.59	453.11
9.037	574.12	459.09
9.713	591.65	471.02
10.043	599.63	475.57
12.703	668.59	518.85
13.047	677.45	524.14
13.712	693.25	534.72
14.042	700.81	539.69
14.713	715.55	550.12
15.042	722.50	554.96
16.715	754.31	576.69
17.050	759.66	580.95
20.00	808.61	614.24
21.039	827.60	627.02
22.049	844.32	639.98
23.051	859.18	651.10

Table B.3 (continued)

Time (days)	Bioreactor	
	Control	Test
24.035	875.83	661.34
25.222	897.56	675.12
26.708	917.87	689.58
27.042	934.70	701.71
28.049	955.27	718.28
29.044	973.70	732.97
30.038	991.03	745.97
31.052	1012.06	761.22
34.046	1077.00	811.87
35.042	1103.50	831.67
36.042	1124.03	852.49
37.051	1151.40	874.51
38.042	1176.81	895.32
39.003	1200.34	914.16
40.104	1227.54	936.66
42.052	1271.16	972.51
43.048	1296.02	991.96
44.038	1319.66	1012.12
45.045	1343.77	1031.80
45.868	1364.73	1050.17
47.045	1413.72	1093.57
48.050	1437.71	1114.41
49.042	1461.58	1136.44
50.042	1483.90	1156.61
50.717	1498.77	1176.45
54.042	1544.21	1239.90
55.046	1552.93	1256.97
56.038	1573.64	1275.62
57.046	1596.53	1295.63
58.044	1620.74	1318.11
61.047	1675.13	1373.57
62.047	1700.20	1394.39
63.042	1725.22	1417.61
64.038	1749.56	1440.07
65.042	1765.28	1463.80
68.042	1831.98	1526.05
69.046	1852.69	1546.35
70.044	1874.34	1566.62
71.047	1895.21	1587.12
72.042	1917.22	1608.48
75.049	1986.63	1679.27
76.044	2007.86	1700.51
77.052	2030.96	1724.13
78.042	2053.14	1747.05

Table B.3 (continued)

Time (days)	Bioreactor	
	Control	Test
79.856	2094.88	1790.06
82.053	2146.97	1844.03
83.045	2169.38	1866.81
84.045	2194.21	1893.58
85.047	2218.45	1919.48
86.046	2238.85	1944.07
86.814	2257.09	1962.83
89.042	2312.22	2000.52
90.049	2336.79	2025.96
91.045	2356.9	2045.36
92.042	2382.41	2068.25
93.049	2395.62	2091.45
93.942	2414.77	2109.75

Table B.4. Gas Production Rate (Liters Per Day) for the Control and Test Bioreactors During Toxicity Experiment No. 2

Δt (days) ^a	$t_{1/2}$ (days) ^b	Bioreactor	
		Control	Test
1.024	-13.488	21.48	24.43
.976	-12.488	22.22	21.65
4.000	-10.000	21.13	20.21
1.042	-7.479	19.78	14.85
1.009	-5.458	27.70	25.07
1.004	-4.451	28.33	25.88
2.966	-2.466	26.25	23.71
.691	-.638	25.60	22.32
.292	-.146	25.55	22.43
.708	.354	25.07	17.58
.334	.875	26.29	14.64
.666	1.375	24.02	12.91
.330	1.873	23.36	17.48
.676	2.376	23.45	15.06
.324	2.876	26.36	14.66
.771	3.424	26.36	15.58
.169	3.894	27.63	16.63
.835	4.396	28.06	18.10
.138	4.882	28.77	17.54
.747	5.325	25.53	16.96
.317	5.857	24.10	14.92
.702	6.366	25.56	16.30
.328	6.881	27.62	17.38
.668	7.379	26.00	16.90
.303	7.865	28.38	17.92
.672	8.352	26.95	18.18
.349	8.863	27.31	17.13
.676	9.375	25.93	17.65
.330	9.878	24.18	13.79
2.660	11.373	25.92	16.27
.344	12.875	25.76	15.38
0.665	13.380	23.76	15.91
0.330	13.877	22.91	15.06
0.671	14.378	21.97	15.54
0.329	14.878	21.12	14.71
1.673	15.879	19.01	12.99
0.335	16.883	15.97	12.72
2.950	18.525	16.59	11.28
1.039	20.520	18.28	12.30
1.010	21.544	16.55	12.83
1.002	22.550	14.83	11.10
0.984	23.543	16.92	10.41
1.187	24.629	18.31	11.61

Table B.4. (Continued)

Δt (days)	$t_{1/2}$ (days)	Bioreactor	
		Control	Test
0.994	29.541	17.43	13.08
1.014	30.545	20.74	15.04
2.994	32.549	21.69	16.92
0.996	34.544	26.61	19.88
1.000	35.342	20.53	20.82
1.009	36.547	27.13	21.82
0.991	37.547	25.64	20.99
0.961	38.523	24.48	19.90
1.010	39.554	24.70	20.44
1.948	41.078	22.39	18.40
0.996	42.550	24.96	19.53
0.990	43.543	23.88	20.36
1.007	44.542	23.94	19.54
0.823	45.457	25.47	22.32
1.177	46.457	41.62	36.87
1.005	47.548	23.87	20.74
0.992	48.546	24.06	22.21
1.000	49.542	22.32	20.17
1.000	50.380	24.910	19.84
3.325	52.379	13.67	19.08
1.004	54.544	8.68	17.00
0.992	55.542	20.87	18.80
1.008	56.542	22.61	19.85
0.998	57.545	24.26	22.52
3.003	59.546	18.11	18.47
1.000	61.547	25.07	20.82
0.995	62.545	25.15	23.34
0.996	63.540	24.44	22.55
1.004	64.540	15.65	23.63
3.000	66.542	22.23	20.75
1.004	68.544	20.63	20.22
0.998	69.545	21.69	20.31
1.003	70.546	20.81	20.44
0.995	71.545	22.12	21.47
3.007	73.546	23.08	23.54
0.995	75.547	21.34	21.35
1.008	76.548	22.92	23.43
0.990	77.547	22.40	23.23
1.814	78.949	23.01	23.71
2.197	80.955	23.71	24.56
0.992	82.549	22.59	22.96
1.000	83.545	24.83	26.77
1.002	84.546	24.19	25.85
0.999	85.547	20.42	24.61

Table B.4. (Continued)

Δt (days)	$t_{1/2}$ (days)	Bioreactor	
		Control	Test
0.768	86.430	23.75	24.43
2.228	87.928	24.74	16.92
1.007	89.546	24.40	25.26
0.996	90.547	20.19	19.48
0.997	91.544	25.59	22.96
1.007	92.546	13.12	23.04
0.893	93.496	21.44	20.49

^a $\Delta t = t_2 - t_1$; t_1 and t_2 were obtained from Table B.3.

^b $t_{1/2} = (t_2 + t_1)/2$.

Table B.5. Gas Composition for Control and Test Bioreactors
During Toxicity Experiment No. 2

Time (days)	Control Bioreactor			Test Bioreactor		
	% Air	% CH ₄	% CO ₂	% Air	% CH ₄	% CO ₂
-14.177	1.5	74.3	24.2	1.7	74.5	23.8
-13.198	2.1	70.5	27.4	2.3	70.4	27.3
-12.167	1.9	70.7	27.4	1.6	71.1	27.3
-8.108	2.4	72.9	24.7	2.8	72.2	25.0
-7.972	2.4	71.8	25.8	2.5	72.0	25.5
-7.260	3.1	70.5	26.4	2.9	70.7	26.4
-6.260	2.3	69.7	28.1	2.2	70.7	27.1
-5.257	3.1	70.6	26.3	2.6	70.6	26.8
-4.219	2.9	70.4	26.7	2.4	70.3	27.3
-1.250	2.0	73.2	24.8	2.4	72.7	24.9
-0.243	1.7	74.3	24.0	1.7	73.8	23.0
0.75	1.7	74.7	23.6	2.5	71.8	25.7
1.729	1.8	74.9	23.3	3.0	69.2	27.8
2.740	3.0	75.4	21.6	3.0	67.9	29.1
3.010	2.5	74.1	23.4	3.4	65.0	31.6
3.844	2.0	74.2	23.8	3.4	65.4	31.2
4.840	2.6	73.5	23.9	2.4	66.3	31.3
5.750	2.1	75.3	22.6	2.7	69.2	28.1
6.736	1.9	74.3	23.8	2.8	69.1	28.1
7.740	2.0	73.4	24.6	2.4	68.5	29.1
8.733	3.7	71.9	24.4	3.0	68.8	28.3
9.760	1.9	75.5	22.6	2.8	70.9	26.3
12.813	1.8	75.4	22.8	2.6	71.1	26.3
13.740	2.8	74.3	22.9	2.5	70.8	26.7
14.771	2.8	74.9	22.3	3.3	70.9	25.8
16.750	3.0	76.9	20.1	4.5	72.9	22.6
19.927	3.1	78.2	18.7	3.2	76.2	20.6
20.750	3.0	77.0	20.0	3.6	74.7	19.1
21.740	3.2	75.4	21.3	2.9	74.9	22.5
22.760	3.6	75.7	20.7	3.1	74.5	22.4
23.771	2.9	78.3	18.8	2.1	77.4	20.5
26.730	2.5	74.8	22.7	4.0	71.8	24.2
27.760	2.8	73.5	23.7	6.0	68.8	25.2
28.771	2.8	74.8	22.4	3.2	72.4	24.5
29.729	4.2	75.9	20.0	3.9	78.7	17.4
30.740	3.6	74.0	22.4	3.5	72.7	23.8
33.740	2.6	74.2	23.3	2.1	72.4	25.5
34.826	2.1	73.3	24.6	1.8	71.6	26.6
35.948	2.5	71.6	25.8	2.5	69.5	28.0
36.760	5.0	71.0	24.0	2.8	69.4	27.8
37.760	3.4	71.0	25.7	2.7	68.0	29.3
38.736	1.9	74.6	23.5	3.3	70.6	26.2
39.740	2.7	72.2	25.0	2.7	69.9	27.4

Table B.5. (Continued)

Time (days)	Control Bioreactor			Test Bioreactor		
	% Air	% CH ₄	% CO ₂	% Air	% CH ₄	% CO ₂
40.740	1.6	73.5	24.9	2.2	71.1	26.7
41.781	2.7	74.3	23.0	3.2	72.0	24.9
43.750	3.8	74.0	22.2	3.9	72.4	23.7
44.781	2.0	74.1	23.9	1.1	73.9	25.0
45.771	4.0	71.0	25.0	4.8	68.9	26.3
46.792	2.4	74.0	23.6	2.9	73.3	23.8
47.813	3.5	73.8	22.7	2.9	73.3	23.8
50.969	3.8	75.9	20.2	3.3	75.8	20.9
51.804	3.8	76.7	19.5	2.7	78.0	19.3
52.802	2.5	78.0	19.5	2.5	77.1	20.4
53.896	2.7	77.3	19.9	2.1	76.7	21.2
54.813	2.5	74.9	22.5	2.9	74.1	23.0
57.804	1.4	70.6	28.0	1.4	70.6	28.0
58.750	2.3	72.8	24.9	1.2	72.6	26.2
59.823	1.7	73.1	25.2	1.7	73.2	25.1
60.760	2.6	75.4	22.0	2.5	74.2	23.3
61.781	3.1	72.8	24.1	2.0	73.9	24.1
63.896	-	-	-	2.3	73.6	24.1
64.781	2.8	73.5	23.7	2.4	72.3	25.3
65.750	1.7	75.5	22.8	1.6	74.7	23.7
66.802	2.3	75.8	22.0	2.7	74.9	22.4
67.792	1.8	75.0	23.2	1.7	75.1	23.3
70.792	2.8	75.0	22.3	3.2	74.6	22.1
71.771	2.2	76.6	21.1	2.2	76.7	21.1
72.802	1.8	75.6	22.7	2.0	75.1	22.9
73.792	1.8	72.9	25.3	1.8	72.7	25.5
75.896	1.9	72.7	25.4	1.8	72.3	25.8
77.792	3.2	72.7	24.1	1.5	74.1	24.4
78.885	2.4	73.0	24.7	1.4	73.3	25.3
79.781	0.9	71.8	27.2	1.8	71.0	27.1
80.771	1.0	71.9	27.1	2.8	70.0	27.2
81.927	2.1	71.2	26.7	3.2	70.9	25.9
84.823	1.6	72.2	26.2	2.8	69.7	27.5
85.875	1.3	73.0	25.6	1.6	72.2	26.2
86.804	1.8	72.8	25.4	1.4	72.6	26.0
87.804	1.6	73.6	24.8	2.0	72.4	25.6
88.833	2.5	73.2	24.3	2.0	74.3	23.6
91.792	1.5	74.5	24.0	1.5	74.0	24.5

Table B.6. Acetic Acid Profiles for Control and Test Bioreactors During Toxicity Experiment No. 2^a

Time (days)	Bioreactor	Bioreactor Length (cm x 3.281 x 10 ⁻²)											
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
-1	Control	450	450	125	30	50	20	20	20	10	5	5	5
	Test	450	450	100	50	50	20	20	20	10	10	10	10
6	Control	*	*	*	*	*	*	*	*	*	*	*	*
	Test	1202	1223	1092	1058	1058	989	982	824	762	646	639	769
24	Control	107	37	22	7	10	11	2	0	0	0	0	0
	Test	1000	1066	920	720	740	540	580	595	490	501	389	394
50	Control	712	33	31	31	31	31	30	22	22	22	38	15
	Test	1211	517	442	529	478	337	393	288	198	235	243	206
88	Control	468	380	37	40	16	0	0	0	0	0	0	0
	Test	215	162	147	91	58	52	32	25	18	14	14	0
Control Ave.		434	225	66	27	27	16	13	11	8	7	11	5
S. D.		249	221	49	14	18	13	14	12	10	10	18	7

^aAcetic acid concentration in mg/L; a value of zero is used to denote a nondetectable concentration.

Table B.7. Propionic Acid Profiles for Control and Test Bioreactors
During Toxicity Experiment No. 2^a

Time (days)	Bioreactor	Bioreactor Length (cm x 3.281 x 10 ⁻²)											
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
-1	Control	200	120	25	10	5	0	0	0	0	0	0	0
	Test	200	200	40	20	20	10	10	10	5	5	0	0
6	Control	*	*	*	*	*	*	*	*	*	*	*	*
	Test	316	329	289	267	280	236	254	200	178	169	169	191
24	Control	36	10	0	0	0	0	0	0	0	0	0	0
	Test	238	180	103	123	131	79	92	87	103	110	68	53
50	Control	107	0	0	0	0	0	0	0	0	0	0	4
	Test	248	94	85	109	86	60	63	56	27	31	25	27
88	Control	64	43	0	0	0	0	0	0	0	0	0	0
	Test	32	24	21	12	0	0	0	0	0	0	0	0
Control Ave.		102	43	6	3	1	0	0	0	0	0	0	1
S.D.		72	54	13	5	3	0	0	0	0	0	0	2

^aPropionic acid concentrations in mg/L; a value of zero is used to denote nondetectable concentrations.

Table B.8. Effluent pH and Alkalinity for Control and Test Bioreactors During Toxicity Experiment No. 2

Time (days)	pH		Control Bioreactor		Test Bioreactor	
	Control	Test	Alkalinity	Alkalinity	Alkalinity	Alkalinity
			(mg/L as CaCO ₃)	(meq/L)	(mg/L as CaCO ₃)	(meq/L)
-13.135	7.72	7.39	3120	62.40	2970	59.40
-12.01	7.34	7.35	3006	60.12	2995	59.90
-16.073	7.35	7.40	3430	68.60	3353	67.06
-4.198	7.47	7.47	3970	79.40	3870	77.40
.802	7.61	7.59	4191	83.82	4085	81.70
2.01	7.45	7.40	-	-	-	-
2.813	7.67	7.15	4220	84.40	3640	72.80
3.823	7.18	7.43	-	-	-	-
4.823	7.42	7.24	-	-	-	-
6.740	7.54	7.50	-	-	-	-
9.760	7.48	7.25	4200	84.00	4015	80.30
13.802	7.78	7.21	-	-	-	-
20.740	-	-	3140	62.80	3160	63.20
21.740	7.59	7.32	-	-	-	-
22.740	7.52	7.21	-	-	-	-
23.729	7.4	7.27	-	-	-	-
26.740	7.47	7.16	-	-	-	-
27.740	7.44	7.21	3180	63.60	3090	61.80
28.740	7.39	7.19	-	-	-	-
29.740	7.36	7.22	-	-	-	-
30.740	7.67	7.32	-	-	-	-
33.740	7.42	7.20	-	-	-	-
34.760	7.63	7.34	-	-	-	-
35.927	7.54	7.43	-	-	-	-
36.760	7.50	7.50	-	-	-	-
37.719	7.54	7.47	-	-	-	-
38.823	7.63	7.52	-	-	-	-
39.823	7.47	7.24	-	-	-	-
41.719	7.46	7.30	-	-	-	-
42.719	7.56	7.51	-	-	-	-
43.719	7.49	7.50	-	-	-	-
44.740	7.48	7.49	-	-	-	-
45.740	7.50	7.51	-	-	-	-
48.740	7.67	7.50	-	-	-	-
51.750	7.53	7.61	-	-	-	-
54.813	7.67	7.53	-	-	-	-
55.8 3	7.78	7.57	-	-	-	-
73.000	7.87	7.80	4070	81.40	4050	81.00
80.771	7.62	7.55	-	-	-	-
81.771	7.62	7.56	4100	82.00	4120	82.40
84.750	7.56	7.55	4050	81.00	4075	81.50
85.750	7.57	7.64	-	-	-	-

Table B.9. Global First-Order Rate Constants^a for Control and Test Bioreactors During Toxicity Experiment No. 2

Time (days)	Control Bioreactor			Test Bioreactor		
	f_o	$K_o \bar{t}_o$	K_o (h ⁻¹)	f_o	$K_o \bar{t}_o$	K_o (h ⁻¹)
-14.25	0.058	6.06	0.13	0.061	5.88	0.13
-13.25	0.058	6.06	0.13	0.062	5.83	0.13
-12.25	0.054	6.30	0.14	0.058	6.06	0.14
-8.25	0.053	6.37	0.14	0.057	6.11	0.14
-7.25	0.052	6.43	0.14	0.056	6.18	0.14
-6.25	0.063	5.78	0.12	0.068	5.53	0.13
-5.25	0.046	6.87	0.15	0.049	6.64	0.15
-4.25	0.049	6.64	0.14	0.047	6.79	0.16
-0.083	0.046	6.87	0.15	0.042	7.21	0.16
0.792	0.051	6.50	0.14	0.058	6.06	0.14
1.771	0.042	7.21	0.15	0.047	7.69	0.16
3.563	0.043	7.12	0.15	0.461	0.98	0.022
3.823	0.041	7.30	0.16	0.310	1.66	0.038
4.823	0.044	7.03	0.15	0.305	1.69	0.039
5.781	0.044	7.03	0.15	0.286	1.81	0.041
7.01	0.050	6.57	0.14	0.400	1.21	0.028
8.906	0.050	6.57	0.14	0.350	1.44	0.033
9.740	0.058	6.06	0.13	0.316	1.62	0.037
13.719	0.060	5.94	0.13	0.413	1.16	0.026
14.740	0.059	6.00	0.13	0.370	1.34	0.031
16.792	0.057	6.11	0.13	0.280	1.85	0.042
19.740	0.070	5.43	0.12	0.278	1.87	0.043
20.740	0.055	6.24	0.13	0.287	1.81	0.041
21.740	0.052	6.43	0.14	0.268	1.94	0.044
22.740	0.055	6.24	0.13	0.225	2.30	0.053
23.760	0.049	6.64	0.14	0.205	2.51	0.057
27.740	0.053	6.37	0.14	0.242	2.15	0.049
28.740	0.065	5.67	0.12	0.218	2.37	0.054
29.740	-	-	-	0.242	2.15	0.049
30.740	-	-	-	0.240	2.17	0.050
33.740	0.053	6.37	0.14	-	-	-
34.740	0.058	6.06	0.13	0.247	2.11	0.048
35.927	0.066	5.62	0.12	-	-	-
36.760	0.062	5.83	0.13	-	-	-
37.760	0.065	5.67	0.12	0.243	2.14	0.049
38.802	0.045	6.95	0.15	0.260	2.00	0.046
39.844	0.047	6.79	0.15	0.257	2.03	0.046
41.719	0.050	6.57	0.14	0.203	2.53	0.058
42.719	0.050	6.57	0.14	0.167	2.98	0.068
43.719	0.050	6.57	0.14	0.177	2.84	0.065
44.802	0.049	6.64	0.14	0.166	3.00	0.068
45.875	0.049	6.64	0.14	0.150	3.25	0.074

Table B.9. (Continued)

Time (days)	Control Bioreactor			Test Bioreactor		
	f_o	$K_o \bar{t}_o$	K_o (h^{-1})	f_o	$K_o \bar{t}_o$	K_o (h^{-1})
47.740	0.051	6.50	0.14	0.127	3.68	0.084
48.740	0.050	6.57	0.14	0.118	3.88	0.089
49.771	0.050	6.57	0.14	0.118	3.88	0.089
50.750	0.047	6.79	0.15	0.113	4.00	0.091
51.771	0.046	6.87	0.15	0.101	4.31	0.10
52.750	0.046	6.87	0.15	0.100	4.34	0.10
55.823	0.054	6.30	0.14	0.088	4.72	0.10
56.792	0.052	6.43	0.14	0.104	4.23	0.10
57.792	0.043	7.12	0.15	0.117	3.90	0.09
58.792	0.043	7.12	0.15	0.102	4.28	0.10
61.771	0.048	6.72	0.14	0.081	4.97	0.11
62.792	0.046	6.87	0.15	0.068	5.53	0.13
63.792	0.045	6.95	0.15	0.093	4.55	0.10
64.792	0.045	6.95	0.15	0.092	4.59	0.10
65.792	0.047	6.79	0.15	0.084	4.86	0.11
68.854	0.049	6.64	0.14	0.060	5.94	0.14
69.792	0.056	6.18	0.13	0.069	5.48	0.13
70.792	0.057	6.11	0.13	0.080	5.01	0.11
71.792	0.054	6.30	0.14	0.066	5.62	0.13
72.792	0.050	6.57	0.14	0.061	5.88	0.13
73.792	0.055	6.24	0.13	0.071	5.39	0.12
74.771	0.054	6.30	0.14	0.062	5.83	0.13
75.771	0.051	6.50	0.14	0.055	6.24	0.14
76.750	0.059	6.00	0.13	0.060	5.94	0.14
78.917	0.053	6.37	0.14	0.056	6.18	0.14
80.792	0.051	6.50	0.14	0.054	6.30	0.14
81.979	0.048	6.72	0.14	0.048	6.72	0.15
82.750	0.054	6.30	0.14	0.048	6.72	0.15
83.750	0.056	6.18	0.13	0.050	6.57	0.15
84.750	0.051	6.50	0.14	0.052	6.43	0.15
87.771	0.051	6.50	0.14	0.058	6.06	0.14
88.750	0.050	6.57	0.14	0.057	6.11	0.14
89.771	0.038	7.58	0.16	0.045	6.95	0.16
90.750	0.039	7.49	0.16	0.036	7.79	0.18
91.771	0.044	7.03	0.15	0.041	7.30	0.17

^aCalculated from effluent soluble chemical oxygen demand (see Table B.1).

^b $\bar{t}_o$ for control bioreactor = 46.6 hours.

^c $\bar{t}_o$ for test bioreactor = 43.8 hours.

Table B.10. Effluent Dissolved Zinc Concentrations^a for Control and Test Bioreactors During Toxicity Experiment No. 2

Time (days)	Bioreactor	
	Control	Test
0.802	<0.05	1.38
1.771	<0.05	2.5
2.813	<0.05	20.82
3.823	<0.05	5.26
4.823	<0.05	4.57
5.781	<0.05	2.30
6.740	<0.05	1.8
8.740	<0.05	1.34
9.760	0.06	1.48
12.719	<0.05	1.5
16.740	<0.05	1.3
19.740	0.14	1.3
20.740	<0.05	1.5
22.740	<0.05	1.3
34.760	0.08	0.64
37.990	0.45	-
45.875	0.5	0.75
47.740	0.2	0.5
48.740	0.16	0.38
49.750	0.1	0.3
50.771	0.09	-
54.823	0.14	0.2
55.792	0.1	0.2
56.750	0.2	0.3
57.750	<0.05	0.1
58.750	0.1	0.1
61.771	<0.05	0.23
62.792	<0.05	<0.05
63.792	0.1	0.1
64.792	0.2	0.5
65.792	0.1	0.2
68.854	<0.05	0.27
69.792	<0.05	0.09
70.792	<0.05	0.08
71.792	<0.05	0.11
72.792	0.07	0.13
75.792	<0.05	0.1
76.771	<0.05	0.09
77.771	<0.05	0.12
78.750	<0.05	0.13
80.917	<0.05	0.17
82.792	<0.05	0.12
83.958	<0.05	0.28

Table B.10. (Continued)

Time (days)	Bioreactor	
	Control	Test
84.750	<0.05	0.17
85.750	<0.05	0.15
86.750	0.06	0.14
89.771	0.05	0.11
90.750	<0.05	0.14
91.771	0.21	0.20
92.750	0.23	0.30

^aZinc concentrations in mg/L.

Table B.11. Overall and Regional First-Order Rate Constants^a for Control and Test Bioreactors During Toxicity Experiment No. 2

Time (days)	Bioreactor	f_0	$K_0 \bar{t}_0$	K_0	f_1	$K_1 \bar{t}_1$	K_1	f_2	$K_2 \bar{t}_2$	K_2	f_3	$K_3 \bar{t}_3$	K_3
-1	Control	0.046	6.87	0.15	0.251	2.81	0.72	0.237	2.75	0.24	0.770	0.29	0.0093
	Test	0.051	6.50	1.15	0.328	1.97	0.53	0.378	1.51	0.14	0.409	1.23	0.042
6	Control	0.057	6.11	0.13	0.207	3.56	0.91	0.315	1.94	0.17	0.872	0.15	0.0048
	Test	0.447	1.03	0.024	0.883	0.13	0.035	0.669	0.48	0.044	0.756	0.31	0.011
24	Control	0.056	6.18	0.13	0.249	2.86	0.73	0.224	2.93	0.25	0.954	0.05	0.0016
	Test	0.205	2.51	0.057	0.553	0.79	0.21	0.579	0.70	0.064	0.641	0.52	0.0018
50	Control	0.47	6.79	0.15	0.336	1.90	0.49	0.159	4.17	0.36	0.888	0.12	0.0039
	Test	0.113	4.00	0.091	0.577	0.72	0.19	0.358	1.63	0.15	0.545	0.75	0.026
88	Control	0.035	7.90	0.17	0.235	3.06	0.78	0.203	3.26	0.28	0.734	0.35	0.011
	Test	0.050	6.57	0.15	0.154	4.96	1.34	0.512	0.90	0.082	0.637	0.53	0.018

^aCalculated from the soluble chemical oxygen demand profiles (see Table B.2).

APPENDIX C: DATA FOR TOXICITY EXPERIMENT NO. 3

Toxicity experiment No. 3 was a step increase in feed zinc concentration as follows:

100 mg/L zinc, 100% soluble for 35 d

300 mg/L zinc, 100% soluble for 26 d

1000 mg/L zinc, 100% soluble for 13 d

Time zero is taken as the time when the experiment began with an influent zinc concentration of 100 mg/L.

Table C.1. Effluent Soluble Chemical Oxygen Demand for Control and Test Bioreactors During Toxicity Experiment No. 3

Time (d)	Control Bioreactor COD (mg/L)	Test Bioreactor COD (mg/L)
-20.125	172	172
-19.125	170	175
-16.177	210	190
-15.177	165	160
-14.177	165	150
-12.156	146	146
-9.177	160	155
-7.177	194	194
-2.177	160	160
-1.177	175	170
0.010	198	198
0.844	186	186
1.844	195	243
2.885	135	223
3.927	140	290
5.802	151	331
6.802	150	315
7.802	150	315
8.885	148	292
9.958	148	340
11.802	152	332
12.802	150	335
13.833	142	463
14.854	138	379
15.833	137	436
18.906	161	526
19.875	157	561
20.833	145	854
21.833	129	879
22.833	130	958
25.854	143	767
26.875	138	773
27.875	136	848
28.875	134	803
29.875	141	922
32.938	147	793
33.875	169	810
34.875	172	850
35.875	163	802
36.875	150	785
39.875	165	776
40.854	161	818
41.854	154	879

Table C.1. (Continued)

Time (d)	Control Bioreactor COD (mg/L)	Test Bioreactor COD (mg/L)
42.833	176	868
45.000	159	855
46.985	152	843
48.063	144	881.
48.833	162	815
49.833	167	840
50.833	152	893
53.854	154	884.
54.833	149	820
55.854	113	887
56.833	117	744
57.854	133	835
58.958	105	893
60.844	117	912
61.844	95	880
62.854	100	920
63.833	92	930
64.833	100	1060
65.958	114	1078
67.792	103	1094
68.833	111	1132
69.844	100	1229
70.854	91	960
71.833	102	983
72.917	107	1139
73.958	132	1233

Table C.2. Soluble Chemical Oxygen Demand,^a Profiles for Control and Test Bioreactor During Toxicity Experiment No. 3

Test (days)	Bioreactor	Bioreactor Length (cm x 3.281 x 10 ⁻²)											
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
-12	Control	746	258	198	167	167	178	159	152	160	160	166	167
-2	Test	725	600	480	340	260	225	190	180	175	170	180	180
14	Control	1009	361	173	160	156	153	155	149	148	138	145	142
	Test	1075	843	839	673	528	577	496	499	490	468	477	463
28	Test	2022	1674	1305	1389	1274	1047	1050	953	891	887	850	848
52	Control	705	217	133	143	135	133	113	113	107	107	102	105
	Test	2192	1886	1550	1680	1313	1389	1214	1190	1076	1062	929	893
74	Test	2596	2488	2388	2202	1969	1866	1469	1443	1257	1269	1221	1233
Control Ave.		820	279	168	157	153	155	142	138	138	135	138	138
S.D.		165	74	33	12	16	23	25	22	28	27	33	32

^aCOD concentrations are in mg/L.

Table C.3. Cumulative Gas Production (Liters) for Control and Test Bioreactors During Toxicity Experiment No. 3

Time (days)	Test Bioreactor	Control Bioreactor
-18.972	0	0
-15.970	55.11	50.09
-15.050	73.75	67.05
-13.967	93.38	82.23
-12.985	108.62	99.49
-12.008	126.40	116.21
-11.194	151.85	140.01
-10.208	173.40	160.32
-8.981	188.51	174.77
-7.978	209.80	195.38
-6.945	229.61	214.81
-5.878	249.57	233.48
-4.983	268.38	251.21
-1.928	340.24	317.97
-0.875	370.94	345.95
0.042	397.93	364.15
0.826	420.05	385.10
1.134	429.08	393.85
1.792	446.36	410.62
2.125	455.02	419.26
2.896	473.15	437.90
3.087	478.42	442.79
3.917	498.74	463.52
4.188	505.18	469.99
5.795	538.30	505.80
6.135	544.78	513.61
6.795	558.71	529.99
7.131	566.24	538.47
7.797	581.30	554.50
8.121	588.00	562.11
8.837	602.34	578.29
9.128	608.95	586.22
9.951	627.92	607.18
11.810	666.24	649.37
12.128	671.94	656.17
12.802	685.56	672.00
13.135	692.51	680.16
13.808	707.41	696.48
14.125	713.60	704.03
14.796	727.01	719.03
15.129	733.14	726.35
15.801	746.18	741.22
16.128	752.26	751.26
18.794	806.67	781.05

Table C.3. (Continued)

Time (days)	Test Bioreactor	Control Bioreactor
19.125	810.33	786.66
19.801	819.99	792.11
20.129	824.52	795.38
20.795	835.38	809.17
21.122	840.13	816.09
21.794	851.37	830.95
22.129	856.84	838.98
22.792	869.87	855.07
23.128	876.07	863.19
25.795	933.43	909.55
26.130	940.43	917.58
26.795	953.40	934.45
27.130	959.53	942.65
27.797	972.89	959.66
28.125	978.97	967.67
28.795	991.09	983.89
29.122	996.65	992.01
29.796	1009.77	1002.16
30.125	1015.57	1007.73
32.796	1060.99	1070.89
33.125	1066.19	1074.43
33.795	1077.37	1087.94
34.129	1082.66	1095.14
34.795	1093.28	1109.65
35.128	1098.38	1116.79
35.796	1108.87	1130.93
36.130	1114.03	1137.66
36.794	1125.47	1152.41
37.125	1131.09	1159.67
39.794	1178.52	1222.53
40.133	1183.65	1229.08
40.792	1193.93	1243.38
41.128	1198.89	1250.31
41.797	1210.61	1265.65
42.135	1216.40	1273.41
42.792	1228.69	1288.81
43.125	1234.15	1295.59
44.939	1264.77	1337.33
46.801	1296.27	1381.71
47.137	1301.27	1389.42
47.795	1311.81	1404.37
48.128	1317.07	1411.83
48.795	1329.31	1428.61
49.128	1335.17	1436.66
49.797	1347.66	1452.56

Table C.3. (Continued)

Time (days)	Test Bioreactor	Control Bioreactor
50.130	1353.63	1460.90
50.798	1365.12	1474.49
51.129	1370.70	1481.30
51.897	1383.70	1499.54
53.797	1415.68	1546.68
54.125	1420.76	1554.67
54.840	1432.29	1572.63
55.133	1436.96	1579.24
55.795	1447.06	1592.23
56.170	1451.49	1599.35
56.792	1461.61	1616.56
57.125	1466.58	1624.86
57.796	1477.13	1631.72
58.133	1481.92	1638.07
58.984	1493.80	1657.22
60.795	1515.46	1706.31
61.128	1517.69	1713.58
61.795	1523.32	1729.21
62.125	1526.75	1736.68
62.797	1534.87	1747.85
63.125	1539.08	1753.71
63.796	1548.94	1768.67
64.127	1553.84	1776.13
64.793	1564.86	1791.99
65.125	1570.07	1800.05
65.926	1583.59	1819.58
66.152	1587.24	1824.00
67.793	1611.22	1856.18
68.128	1615.75	1862.67
68.801	1625.58	1872.20
69.135	1630.17	1876.99
69.795	1639.87	1891.26
70.126	1644.36	1898.65
70.800	1654.46	1914.53
71.128	1658.41	1921.92
71.792	1667.53	1938.11
72.130	1672.02	1945.99
72.896	1681.31	1957.14
73.137	1684.51	1961.15
73.927	1696.00	1979.71
74.797	1707.65	1998.94

Table C.4. Total Gas Production Rates (Liters per Day) for Control and Test Bioreactors During Toxicity Experiment No. 3

Δt (days) ^a	$t_{1/2}$ (days) ^b	Control Bioreactor	Test Bioreactor
3.0020	-17.4710	16.69	18.36
0.9200	-15.5100	18.43	20.26
1.0830	-47.5087	14.02	18.13
0.9820	-13.4785	17.58	15.52
0.9770	-12.4965	17.11	18.20
0.8140	-11.6010	29.24	31.27
0.9860	-10.7010	20.60	21.86
1.2270	-9.5945	11.78	12.31
1.0030	-8.4795	20.55	21.23
1.0330	-7.4615	18.81	19.18
1.0670	-6.4115	17.50	18.71
0.8950	-5.4305	19.81	21.02
3.0550	-3.4555	21.85	23.52
1.0530	-1.4015	26.57	29.15
0.9170	-0.4165	19.85	29.43
0.7840	0.4340	26.72	28.21
0.3080	0.9802	28.41	29.32
0.6580	1.4630	25.49	26.26
0.3330	1.9612	27.03	26.01
0.7710	2.5105	24.18	23.51
0.1910	2.9915	25.60	27.59
0.8300	3.5020	24.98	24.48
0.2710	4.0525	23.87	23.76
1.6070	4.9915	22.28	20.61
0.3400	5.9650	22.97	19.06
0.6600	6.4680	24.82	21.11
0.3360	6.9630	25.24	22.41
0.6660	7.4640	24.07	22.61
0.3240	7.9590	23.49	20.68
0.7160	8.4790	22.60	20.03
0.2910	8.9825	27.25	22.71
0.8230	9.5395	25.47	23.05
1.8590	10.8805	22.70	20.61
0.3180	11.9640	21.38	17.92
0.6740	12.4650	23.49	20.21
0.3330	12.9685	24.50	20.87
0.6730	13.4715	24.25	22.14
0.3170	13.9930	23.82	19.53
0.6710	14.4605	22.35	19.99
0.3330	14.9625	21.98	18.41
0.6720	15.4650	22.13	19.40
0.3270	15.9645	30.70	18.59
2.6660	17.4610	11.17	20.41
0.3310	18.9595	16.95	11.06

Table C.4. (Continued)

Δt (days)	$t_{1/2}$ (days)	Control Bioreactor	Test Bioreactor
0.6760	19.4630	8.06	14.29
0.3280	19.9650	9.97	13.81
0.6660	20.4623	20.71	16.31
0.3270	20.9585	21.16	14.53
0.6720	21.4580	22.11	16.73
0.3350	21.9615	23.97	16.33
0.6630	22.4605	24.27	19.65
0.3360	22.9600	24.17	18.45
2.6670	24.4615	17.38	21.51
0.3350	25.9625	23.97	20.90
0.6650	26.4625	25.37	19.53
0.3350	26.9625	24.48	18.30
0.6670	27.4635	25.50	20.03
0.3280	27.9610	24.42	18.54
0.6700	28.4600	24.21	18.09
0.3270	28.9585	24.83	17.00
0.6740	29.4590	15.06	19.47
0.3290	29.9605	16.93	17.63
2.6710	31.4605	23.65	17.00
0.3290	32.9605	10.76	15.81
0.6700	33.4600	20.16	16.69
0.3340	33.9620	21.56	15.84
0.6660	34.4620	21.79	15.95
0.3330	34.9615	21.44	15.32
0.6680	35.4620	21.17	15.70
0.3340	35.9630	20.15	15.45
0.6640	36.4620	22.21	17.23
0.3310	36.9595	21.93	16.98
2.6690	38.4595	23.55	17.77
0.3390	39.9635	19.32	15.13
0.6590	40.4625	21.70	15.60
0.3360	40.9600	20.63	14.70
0.6690	41.4625	22.93	17.52
0.3380	41.9660	22.96	17.13
0.6570	42.4635	23.44	18.71
0.3330	42.9585	20.36	16.40
1.8140	44.0320	23.01	16.88
1.8620	45.8700	23.83	16.92
0.3360	46.9690	22.95	14.88
0.6580	47.4660	22.72	16.02
0.3330	47.9615	22.40	15.80
0.6670	48.4615	25.16	18.35
0.3330	48.9615	24.17	17.60
0.6690	49.4625	23.77	18.67
0.3330	49.9635	25.05	17.93

Table C.4. (Continued)

Δt (days)	$t_{1/2}$ (days)	Control Bioreactor	Test Bioreactor
0.6680	50.4640	20.34	17.20
0.3310	50.9635	20.57	16.86
0.7680	51.5130	23.75	16.93
1.9000	52.8470	24.81	16.83
0.3280	53.9610	24.36	15.49
0.7150	54.4825	25.12	16.13
0.2930	54.9865	22.56	15.94
0.6620	55.4640	19.62	15.26
0.3750	55.9825	18.99	11.81
0.6220	56.4810	27.67	16.27
0.3330	56.9585	24.92	14.92
0.6710	57.4605	10.22	15.72
0.3370	57.9645	18.84	14.21
0.8510	58.5585	22.50	13.96
1.8110	59.8895	27.11	11.96
0.3330	60.9615	21.83	6.70
0.6670	61.4615	23.43	8.44
0.3300	61.9600	22.64	10.39
0.6720	62.4610	16.62	12.08
0.3280	62.9610	17.87	12.84
0.6710	63.4605	22.30	14.69
0.3310	63.9615	22.54	14.80
0.6660	64.4600	23.81	16.55
0.3320	64.9590	24.28	18.70
0.8010	65.5255	24.38	16.88
0.2260	66.0390	19.56	16.15
1.6410	67.3415	19.61	14.61
0.3350	67.9605	19.37	13.52
0.6730	68.4645	14.61	14.61
0.3340	68.9680	14.34	13.74
0.6600	69.4650	21.62	14.70
0.3310	69.9605	22.33	13.56
0.6740	70.4630	23.56	14.99
0.3280	70.9640	22.53	12.04
0.6640	71.4600	24.38	13.73
0.3380	71.9610	23.31	13.28
0.7660	72.5130	14.56	12.13
0.2410	73.0165	16.64	13.28
0.7900	73.5320	23.49	14.54
0.8700	74.3620	22.10	13.39

^a $\Delta t = t_2 - t_1$; t_1 and t_2 were obtained from Table C.3.

^b $t_{1/2} = (t_2 + t_1)/2$.

Table C.5. Gas Percentages in Off-Gas for Control and Test Bioreactors During Toxicity Experiment No. 3

Time (days)	Control Bioreactor			Test Bioreactor		
	% Air	% CH ₄	% CO ₂	% Air	% CH ₄	% CO ₂
-19.146	3.0	76.9	20.1	3.1	75.2	21.7
-15.979	3.1	78.2	18.7	1.4	71.0	27.6
-15.146	3.0	77.0	20.0	2.8	75.9	21.3
-14.167	3.2	75.4	21.3	2.6	74.9	22.4
-13.135	3.6	75.7	20.7	3.1	75.1	21.8
-12.042	2.9	78.3	18.8	2.7	77.2	20.1
-9.167	2.5	74.8	22.7	3.7	73.4	22.9
-8.146	2.8	73.5	23.7	2.9	73.1	24.0
-7.125	2.8	74.8	22.7	3.7	73.4	22.9
-6.146	4.2	75.9	20.0	2.7	76.1	21.2
-5.156	3.6	74.0	22.4	2.4	74.6	23.0
-2.156	2.6	74.2	23.3	4.4	72.8	22.8
-1.063	2.1	73.3	24.6	2.4	72.3	25.3
0.042	2.5	71.6	25.8	3.9	69.9	26.2
0.885	5.0	71.0	24.0	5.4	69.9	24.7
1.875	3.4	71.0	25.7	3.0	70.5	26.5
5.844	1.9	74.6	23.5	2.4	71.3	26.3
6.844	2.7	72.2	25.0	1.0	71.2	27.8
7.854	1.6	73.5	24.9	1.6	71.0	27.4
8.885	2.7	74.3	23.0	4.1	70.9	25.0
11.854	3.8	74.0	22.0	3.7	71.9	24.0
12.882	2.0	74.1	23.9	2.3	70.4	27.3
13.879	4.0	71.0	25	3.1	69.6	27.3
14.897	2.4	74.0	23.6	3.1	70.1	26.7
15.948	3.5	73.8	22.7	2.6	70.6	26.8
19.080	3.8	76.0	20.2	6.1	73.1	20.8
19.923	3.8	76.7	19.5	2.8	75.7	21.5
21.027	2.5	78.0	19.5	2.5	72.9	24.6
21.987	2.7	77.4	19.9	3.1	71.5	25.4
22.014	2.5	74.9	22.5	1.9	68.9	29.2
25.909	1.4	70.6	28.0	2.1	65.0	32.9
26.914	2.3	72.8	24.9	1.6	72.4	26.0
27.978	1.7	73.1	25.2	3.7	67.8	28.5
28.876	2.6	75.4	22.0	2.2	71.0	26.8
29.919	3.1	72.8	24.1	2.4	70.0	27.6
32.958	2.0	74.0	24.0	1.3	71.0	27.6
33.899	2.8	73.5	23.7	1.4	70.2	28.4
34.862	1.7	75.5	22.8	1.7	70.0	28.3
35.907	2.3	75.7	22.0	1.8	71.1	27.1
36.875	1.8	75.0	23.2	1.8	68.2	30.0
39.902	2.8	75.0	22.2	1.4	69.5	29.1
40.877	2.2	76.7	21.1	1.6	70.0	28.4
41.936	1.8	75.5	22.7	1.4	67.4	31.2

Table C.5. (Continued)

Time (days)	Control Bioreactor			Test Bioreactor		
	% Air	% CH ₄	% CO ₂	% Air	% CH ₄	% CO ₂
42.894	1.8	72.9	25.3	1.1	65.5	33.4
45.000	1.9	72.7	25.4	2.1	64.4	33.5
46.969	3.2	72.7	24.1	2.8	65.6	31.6
47.991	2.4	73.0	24.7	1.0	66.4	32.6
48.882	0.9	71.9	27.2	1.2	63.5	35.3
49.881	1.0	71.9	27.1	1.2	62.8	36.0
51.004	2.1	71.3	26.7	3.7	62.2	34.1
53.882	1.6	72.2	26.2	1.9	63.9	34.2
54.967	1.3	73.1	25.6	1.3	64.5	34.2
55.911	1.8	72.8	25.4	2.0	63.2	34.8
56.908	1.6	73.6	24.8	2.1	63.5	34.4
57.936	2.5	73.2	24.3	1.5	66.0	32.5
60.898	1.5	74.5	24.0	0	68.6	31.4
61.904	1.5	73.9	24.6	2.4	65.7	31.9
62.880	2.1	75.0	22.9	2.2	62.8	35.0
63.867	1.6	74.9	23.5	1.6	61.6	36.8
64.864	2.0	73.2	24.8	2.4	58.2	39.4
65.983	2.2	72.7	25.1	1.1	57.3	41.6
67.889	2.5	75.0	22.5	2.4	57.5	40.1
68.853	3.0	73.3	23.7	1.4	57.2	41.4
69.869	2.4	73.8	23.8	2.6	55.9	41.6
70.874	3.4	71.6	25.0	0.8	55.9	43.3
71.861	2.5	71.0	26.5	2.5	53.1	44.4
72.938	0.9	73.8	25.3	2.0	55.1	42.9
73.982	1.8	72.1	26.1	2.0	54.8	43.2

Table C.6. Acetic Acid Profiles^a for Control and Test Bioreactors
During Toxicity Experiment No. 3

Time (days)	Bioreactor	Bioreactor Length (cm x 3.281 x 10 ⁻²)											
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
-12	Control	107	37	22	7	10	11	2	0	0	0	0	0
-2	Test	232	201	115	104	85	40	20	0	0	0	0	0
14	Control	712	33	31	31	31	31	30	22	22	22	38	15
	Test	764	573	407	276	296	289	291	228	369	263	293	362
28	Test	1201	759	938	839	787	602	538	458	445	422	469	417
52	Control	468	380	87	40	16	0	0	0	0	0	0	0
	Test	1315	1083	826	981	748	678	681	574	686	661	631	601
74	Test	1073	1070	1050	1022	943	786	667	616	657	559	619	646
Control Ave.		429	150	47	26	19	14	11	7	7	7	13	5
S.D.		304	199	35	17	11	16	17	13	13	13	22	9

^aAcetic acid concentrations in mg/L; a value of zero is used to denote a nondetectable concentration.

Table C.7. Propionic Acid Profiles^a for Control and Test Bioreactors During Toxicity Experiment No. 3

Time (days)	Bioreactor	Bioreactor Length (cm x 3.281 x 10 ⁻²)											
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
-12	Control	36	10	1	0	0	0	0	0	0	0	0	0
-2	Test	39	24	19	0	0	0	0	0	0	0	0	0
14	Control	107	0	0	0	0	0	0	0	0	0	0	0
	Test	94	112	72	54	51	73	57	75	60	53	53	68
28	Test	184	153	163	168	158	137	124	115	105	104	104	102
52	Control	64	43	0	0	0	0	0	0	0	0	0	0
	Test	240	193	232	163	125	110	116	96	107	109	109	106
74	Test	318	328	281	284	251	214	175	159	176	156	150	167
Control Ave.		69	15	0.3	0	0	0	0	0	0	0	0	0
S. D.		36	23	0.6	0	0	0	0	0	0	0	0	0

^aPropionic acid concentrations in mg/L; a value of zero is used to denote a nondetectable concentration.

Table C.8. Effluent pH and Alkalinity for Control and Test Bioreactors During Toxicity Experiment No. 3

Time (days)	Control Bioreactor			Test Bioreactor		
	pH	Alkalinity (meq/L)	Alkalinity (mg/L as) CaCO ₃	pH	Alkalinity (meq/L)	Alkalinity (mg/L as) CaCO ₃
-15.177	-	62.8	3140	-	61.0	3050
-14.177	7.59	-	-	7.26	-	-
-13.177	7.52	-	-	7.25	-	-
-12.177	7.40	-	-	7.20	-	-
-9.177	7.47	-	-	7.18	-	-
-8.177	7.44	63.6	3180	4.34	62.0	3100
-7.177	7.39	-	-	7.23	-	-
-6.177	7.36	-	-	7.22	-	-
-5.177	7.67	-	-	7.30	-	-
-2.177	7.42	-	-	7.34	-	-
-1.177	7.63	-	-	7.40	-	-
0.010	7.54	-	-	7.39	-	-
0.844	7.50	-	-	7.36	-	-
1.802	7.54	-	-	7.42	-	-
2.885	7.63	-	-	7.43	-	-
3.875	7.47	-	-	7.26	-	-
5.802	7.46	-	-	7.26	-	-
6.802	7.56	-	-	7.35	-	-
7.802	7.49	-	-	7.40	-	-
8.823	7.48	-	-	7.45	-	-
9.823	7.50	-	-	7.36	-	-
12.823	7.67	-	-	7.45	-	-
18.917	7.67	-	-	7.50	-	-
19.885	7.78	-	-	7.53	-	-
33.875	7.87	81.4	4070	7.53	75.0	3750
40.854	7.87	82.4	4120	7.36	72.2	3610
41.854	7.62	82.0	4100	7.46	72.3	3610
42.833	7.62	81	-	7.44	-	-
48.833	7.65	-	-	7.32	69.8	3490
49.833	7.56	80	4000	7.33	-	-
53.854	7.64	-	-	7.17	67.2	3360
55.854	7.64	-	-	-	-	-
56.833	-	-	-	-	64.8	3240
60.844	7.61	-	-	7.33	-	-
61.833	7.60	-	-	7.40	65.6	3280
62.854	7.63	78	3900	7.62	67.8	3390
63.833	7.58	79	3950	7.23	65.2	3260
64.833	7.56	80.2	4010	7.10	62.4	3120
65.958	7.7	80	4000	7.074	59	2950
67.813	7.69	80	4000	7.11	58	2900

Table C.8. (Continued)

Time (days)	Control Bioreactor			Test Bioreactor		
	pH	Alkalinity (meq/L)	Alkalinity (mg/L as) CaCO ₃	pH	Alkalinity (meq/L)	Alkalinity (mg/L as) CaCO ₃
68.833	7.64	76	3800	7.05	57.6	2880
69.884	7.60	76	3800	7.00	57	2850
70.854	-	-	-	7.01	57.2	2860
71.833	-	-	-	7.05	56	2800
72.917	-	-	-	7.00	-	-

Table C.9. Soluble Zinc Profiles for Test Bioreactor During Toxicity
Experiment No. 3^a

Influent Zn (mg/L)	Time (days)	Conc. Units	Bioreactor Length (cm x 3.281 x 10 ⁻²)											
			0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
100	14	mg/L	21.8	7.3	11.1	4.4	3.0	4.6	3.0	2.5	2.5	1.9	2.0	1.9
		%	21.8	7.3	11.1	4.4	3.0	4.6	3.0	2.5	2.5	1.9	2.0	1.9
	28	mg/L	34.3	29.0	17.0	18.7	18.7	10.8	10.3	7.5	5.9	4.9	5.5	5.1
		%	34.3	29.0	17.0	18.7	18.7	10.8	10.3	7.5	5.9	4.9	5.5	5.1
300	17	mg/L	147.3	101.9	75.4	94.4	60.8	49.3	43.8	39.9	34.8	35.9	32.6	33.3
		%	49.1	34.0	25.1	31.5	20.3	16.4	14.6	13.3	11.6	12.0	10.9	11.1
1000	13	mg/L	736.1	596.4	413.0	424.5	387.6	262.9	246.3	200.0	158.2	160.0	172.0	141.0
		%	73.6	59.6	41.3	42.5	38.8	26.3	24.6	20.0	15.8	16.0	17.2	14.1

^aTime = 0, at the beginning of the feeding of the particular zinc concentration (i.e., 100, 300, and 1000 mg/L); concentration in % relative to zinc concentration in feed.

Table C.10. Effluent Dissolved Zinc for Control and Test Bioreactors During Toxicity Experiment No. 3^a

Time (days)	Control Bioreactor	Test Bioreactor
-19.177	<0.05	<0.05
-16.177	0.1	<0.05
-15.177	<0.05	<0.05
-13.177	<0.05	<0.05
-0.156	0.1	<0.05
2.073	0.5	5.2
2.906	0.1	9.9
3.927	<0.05	2.9
5.896	0.2	6.5
6.896	<0.05	2.5
7.833	0.1	2.4
8.885	0.2	2.3
9.958	0.5	2.8
11.177	0.2	1.6
12.177	0.2	1.6 (2.4)
13.833	0.1	1.9
14.854	0.1	1.8
18.906	0.1	2.4
19.875	0.1	2.2
20.833	0.2	3.7
21.833	<0.05	4.1
22.833	0.1	2.4 (3.0)
25.854	<0.05	4.0 (5.1)
26.875	<0.05	4.2
27.875	0.1	5.1
28.875	0.2	7.0
29.875	0.1	5.8
32.958	<0.05	5.9
33.875	<0.05	6.4
34.875	<0.05	6.8
35.875	<0.05	9.0
36.875	0.1	13.4
39.875	<0.05	15.3
40.854	<0.05	20.2
41.854	<0.05	24.0
42.833	<0.05	21.3
45.000	<0.05	26.5
46.875	<0.05	24.8
48.063	<0.05	26.5
48.833	<0.05	28.3
49.833	<0.05	29.5 (33.2)
50.833	0.1	27.4
53.979	0.1	31.2
54.833	<0.05	28.8

Table C.10. (Continued)

Time (days)	Control Bioreactor	Test Bioreactor
55.854	0.2	41.5
56.833	0.2	31.8
57.854	<0.05	28.6
60.844	<0.05	24.9
61.844	0.2	25.0
62.854	0.3	58.2
63.833	<0.05	100.6
64.833	0.2	113.5
65.958	0.1	107.9 (168.6)
67.813	0.1	122 (189.4)
68.833	0.6	137 (209.6)
69.844	<0.05	147.4 (249)
70.854	<0.05	138 (198.8)
71.833	<0.05	143 (168)
72.917	<0.05	138.8 (203.2)
73.958	<0.05	141

^aNumbers in parenthesis represent the total zinc concentration for that sample; concentrations are in mg/L.

Table C.11. First Order Rate Constants^a for Control and Test Bioreactors During Toxicity Experiment No. 3

Time (days)	Control Reactor ^b			Test Bioreactor ^c		
	f_o	$K_o \bar{t}_o$	K_o (h ⁻¹)	f_o	$K_o \bar{t}_o$	K_o (h ⁻¹)
-20.125	0.057	6.11	0.13	0.057	6.95	0.17
-19.125	0.057	6.11	0.13	0.058	6.88	0.17
-16.177	0.070	5.43	0.12	0.063	6.54	0.16
-15.177	0.055	6.24	0.13	0.053	7.25	0.18
-14.177	0.055	6.24	0.13	0.050	7.50	0.18
-12.156	0.049	6.64	0.14	0.049	7.59	0.19
-9.177	0.053	6.37	0.14	0.052	7.34	0.18
-7.177	0.065	5.67	0.12	0.065	6.42	0.16
-2.177	0.053	6.37	0.14	0.053	7.25	0.18
-1.177	0.058	6.06	0.13	0.057	6.95	0.17
0.010	0.066	5.62	0.12	0.066	6.36	0.16
0.844	0.062	5.83	0.13	0.062	6.61	0.16
1.844	0.065	5.67	0.12	0.081	5.57	0.14
2.885	0.045	6.95	0.15	0.074	5.91	0.14
3.927	0.047	6.79	0.15	0.097	4.93	0.12
5.802	0.050	6.57	0.14	0.110	4.51	0.11
6.802	0.050	6.57	0.14	0.105	4.66	0.11
7.802	0.050	6.57	0.14	0.105	4.66	0.11
8.885	0.049	6.64	0.14	0.097	4.93	0.12
9.958	0.049	6.64	0.14	0.113	4.42	0.11
11.802	0.051	6.50	0.14	0.111	4.48	0.11
12.802	0.050	6.57	0.14	0.112	4.45	0.11
13.833	0.047	6.79	0.15	0.154	3.47	0.085
14.854	0.046	6.87	0.15	0.126	4.07	0.10
15.833	0.046	6.87	0.15	0.145	3.65	0.089
18.906	0.054	6.30	0.14	0.175	3.12	0.076
19.875	0.052	6.43	0.14	0.187	2.94	0.072
20.833	0.048	6.72	0.14	0.285	1.93	0.047
21.833	0.043	7.12	0.15	0.293	1.87	0.046
22.833	0.043	7.12	0.15	0.319	1.69	0.041
25.854	0.048	6.72	0.14	0.256	2.17	0.053
26.875	0.046	6.87	0.15	0.258	2.15	0.053
27.875	0.045	6.95	0.15	0.283	1.95	0.048
28.875	0.045	6.95	0.15	0.268	2.07	0.051
29.875	0.047	6.79	0.15	0.307	1.77	0.043
32.938	0.049	6.64	0.14	0.264	2.10	0.051
33.875	0.056	6.18	0.13	0.270	2.05	0.050
34.875	0.057	6.11	0.13	0.283	1.95	0.048
35.875	0.054	6.30	0.14	0.267	2.07	0.051
36.875	0.050	6.57	0.14	0.262	2.12	0.052
39.875	0.055	6.24	0.13	0.259	2.14	0.052
40.854	0.054	6.30	0.14	0.273	2.02	0.049

Table C.11. (Continued)

Time (days)	Control Bioreactor			Test Bioreactor		
	f_o	$K_o \bar{t}_o$	K_o (h^{-1})	f_o	$K_o \bar{t}_o$	K_o (h^{-1})
41.854	0.051	6.50	0.14	0.293	1.87	0.046
42.833	0.059	6.00	0.13	0.289	1.90	0.046
45.000	0.053	6.37	0.14	0.285	1.93	0.047
46.875	0.051	6.50	0.14	0.281	1.96	0.048
48.063	0.048	6.72	0.14	0.294	1.86	0.045
48.833	0.054	6.30	0.14	0.272	2.03	0.050
49.833	0.056	6.18	0.13	0.280	1.97	0.048
50.833	0.051	6.50	0.14	0.298	1.84	0.045
53.854	0.051	6.50	0.14	0.295	1.86	0.045
54.833	0.050	6.57	0.14	0.273	2.02	0.049
55.854	0.038	7.58	0.16	0.296	1.85	0.045
56.833	0.039	7.49	0.16	0.248	2.24	0.055
57.854	0.044	7.03	0.15	0.278	1.98	0.048
58.958	0.035	7.90	0.17	0.298	1.84	0.045
60.844	0.039	7.49	0.16	0.304	1.79	0.044
61.844	0.032	8.26	0.18	0.293	1.87	0.046
62.854	0.033	8.13	0.17	0.307	1.77	0.043
63.833	0.031	8.38	0.18	0.310	1.75	0.043
64.833	0.033	8.13	0.17	0.353	1.49	0.036
65.958	0.038	7.58	0.16	0.359	1.46	0.036
67.792	0.034	8.02	0.17	0.365	1.43	0.035
68.833	0.037	7.69	0.17	0.377	1.37	0.033
69.844	0.033	8.13	0.17	0.410	1.22	0.030
70.854	0.030	8.52	0.18	0.320	1.69	0.041
71.833	0.034	8.02	0.17	0.328	1.64	0.040
72.917	0.036	7.79	0.17	0.380	1.34	0.033
73.958	0.044	7.03	0.15	0.411	1.21	0.030

^aCalculated from effluent soluble COD (see Table C.1).

^b $\bar{t}_o$ for control bioreactor = 46.6 h.

^c $\bar{t}_o$ for test bioreactor = 40.9 h.

Table C.12. Global and Local First Order Rate Constants^a for Control and Test Bioreactors During Toxicity Experiment No. 3

Time (days)	Bioreactor	f_0	$K_0 \bar{t}_0$	K_0^b (h ⁻¹)	f_1	$K_1 \bar{t}_1$	K_1^b (h ⁻¹)	f_2	$K_2 \bar{t}_2$	K_2^b (h ⁻¹)	f_3	$K_3 \bar{t}_3$	K_3^b (h ⁻¹)
-37	Control	0.046	6.87	0.15	0.251	2.81	0.72	0.237	2.75	0.24	0.770	0.29	0.0093
-2	Test	0.060	6.74	0.17	0.242	3.00	0.88	0.469	1.08	0.11	0.529	0.82	0.030
-30	Control	0.57	6.11	0.13	0.207	3.56	0.91	0.315	1.94	0.17	0.872	0.15	0.0048
14	Test	0.154	3.47	0.085	0.358	1.75	0.51	0.626	0.58	0.057	0.688	0.43	0.016
-12	Control	0.056	6.18	0.13	0.249	2.86	0.73	0.224	2.93	0.25	0.954	0.05	0.0016
28	Test	0.283	1.95	0.048	0.674	0.48	0.14	0.687	0.45	0.044	0.611	0.60	0.022
14	Control	0.047	6.79	0.15	0.336	1.90	0.49	0.159	4.17	0.36	0.888	0.12	0.0039
52	Test	0.298	1.84	0.045	0.731	0.37	0.11	0.766	0.30	0.029	0.532	0.81	0.030
52	Control	0.035	7.90	0.17	0.235	3.06	0.78	0.203	3.26	0.28	0.734	0.35	0.011
74	Test	0.411	1.21	0.030	0.865	0.16	0.047	0.848	0.18	0.018	0.560	0.73	0.027

^aCalculated from the soluble chemical oxygen demand profiles.

^bValues for K_0 , K_1 , K_2 , and K_3 are expressed in h⁻¹.

Table C.13. Sorption Factors Profile for Test Bioreactor During Toxicity
Experiment No. 3 Using a Feed Zinc Concentration of 100 mg/L

Bioreactor Length (cm x 3.281 x 10 ⁻²)	Dry Solids (mg)	mg Zn Recovered	SF _T (mg/g Dry Solids)	Soluble Zn (mg/L)	Wet Digestion	
					mg Zn Recovered	SF _p
0.5	94.6/141.3	4.2/6.1	43.8	34.3		
1.0	44.2	1.6	36.2	29.0		
1.5	30.6	0.6	19.6	17.0		
2.0	26.1	1.0	38.3	18.7		
3.0	24.8	0.5	20.2	10.8		
4.0	20.0	0.5	25.0	7.5		
5.0	27.5	0.5	18.2	4.9		
Extraction with 0.1 M Na ₄ P ₂ O ₇			SF _O			
0.5	90.7/74.4	3.6/3.1	40.7	34.3	0.2/0.2	1.2
1.0	35.4	1.0	28	29.0	0.03	0.8
1.5	25.6	0.6	23.4	17.0	0.02	0.8
2.0	21.2	0.8	37.7	18.7	0.01	0.5
4.0	25.3	0.7	28	7.5	0.02	0.8
5.0	18.7	0.4	21	4.9	0.01	0.5

Table C.14. Sorption Factors Profile for Test Bioreactor During Toxicity
Experiment No. 3 Using a Feed Zinc Concentration of 300 mg/L

Bioreactor Length (cm x 3.281×10^{-2})	Dry Solids (mg)	mg Zn Recovered	SF _T (mg/g Dry Solids)	Soluble Zn (mg/L)	Wet Digestion	
					mg Zn Recovered	SF _p
0.5	148.0	22.9	154.7	147.3		
1.0	18.1	2.6	143.6	101.9		
1.5	6.2	0.80	129	75.4		
2.5	50.7	3.7	73	60.8		
3.5	67.7	2.6	38	43.8		
4.5	63.2	1.6	25	34.8		
5.5	15.3	0.5	33	32.6		
Extraction with Na ₄ P ₂ O ₇ 0.1 M			SF _O			
0.5	124.8	19.0	152	147.3	0.4	3.2

Table C.15. Sorption Factors Profile for Test Bioreactor
 During Toxicity Experiment No. 3 Using a
 Feed Zinc Concentration of 1000 mg/L

Bioreactor Length (cm x 3.281×10^{-2})	Dry Solids (mg)	mg Zn Recovered	SF (mg Zn/g Dry Solids)	Soluble zinc (mg/L)
0.5	142.1	31.0	218.2	736.4
1.0	28.7	5.5	191.6	596.4
2.0	10	1.6	160	424.5
4.0	10	1.1	110	200.0
5.0	13.0	1.4	108	160.0

APPENDIX D: COMPUTER CODES

```

C      THIS PROGRAM CALCULATES THE GEOMETRIC AVERAGE RESIDENCE TIME
C      AND THE STANDARD DEVIATION OF RESIDENCE TIME DISTRIBUTION
C      DATA FROM AN ANFLOW COLUMN ASSUMING A LOG-NORMAL DISTRIBUTION.
C      THE RESULTS MAY BE USEFUL TO CALCULATE THE REACTOR DISPERSION
C      NUMBER USING THE TIME OF PEAK CONCENTRATION TECHNIQUE.
C
C      DIMENSION C(800),T(800),CE(800),SUM1(800),TT(800),SLOT(800)
C
C      THIS SECTION OF THE PROGRAM OBTAINS THE DATA
C      FROM A DATA FILE. IT ASKS FOR THE NUMBER OF
C      DATA POINTS, HOW MANY DATA POINTS WITH DELTA T1
C      (THE PROGRAM WAS MADE FOR ONE OR TWO DELTA T'S),
C      THE FIRST TIME INTERVAL, AND THE SECOND TIME
C      INTERVAL (MINUTES).
C
C      LOGICAL*1 L1(11),L2(6)
C      EQUIVALENCE (L2(1),L1(2))
C      DATA L1/'R','O','9','0','5','7','9','.',',','D','A','T'/
C      CALL ASSIGN(1,'TT7:')
C      CALL INITT(120)
C      TYPE 800
800    FORMAT('$  DATE OF DESIRED RUN?')
C      ACCEPT 900,L2
900    FORMAT(6A1)
C      CALL ASSIGN(2,L1,11)
C      TYPE 1
1      FORMAT(' NUMBER OF DATA POINTS')
C      ACCEPT 2,N
2      FORMAT(I3)
C      TYPE 3
3      FORMAT(' NUMBER OF DATA POINTS WITH DELTA T1')
C      ACCEPT 4,M
4      FORMAT(I3)
C      TYPE 5
5      FORMAT(' FIRST TIME INTERVAL')
C      ACCEPT 6,T1
6      FORMAT(F2.0)
C      TYPE 7
7      FORMAT(' SECOND TIME INTERVAL')
C      ACCEPT 8,T2
8      FORMAT(F2.0)
C      READ(2,*)(T(I),CE(I),I=1,N)
C      TYPE 200
200    FORMAT(1X,'DATA PRINT OUT?/FUNCTION E=C PRINT OUT?, 0=NO,1=YES')
C      ACCEPT 201,IND1,IND2
201    FORMAT (2I2)
C      IF(IND1)700,700,600
600    TYPE 300,(T(I),CE(I),I=1,N)
300    FORMAT(' ',2E15.3)
C
C      THE FOLLOWING STATEMENT CALLS THE SUBROUTINE CI WHICH
C      CONVERTS THE DATA FROM% EMISSION TO MG/L.
C
700    CALL CI(CE,C,N)
C
C      THE FOLLOWING STATEMENT CALLS THE SUBROUTINE SUMCI
C      WHICH CALCULATES THE SUM OF THE PRODUCT:
C      CONCENTRATION (MG/L) * DELTA T (MINUTES).
C
C      CALL SUMCI(C,CUM1,SUM1,N,M,T1,T2)
C

```

```

C      THE FOLLOWING STATEMENT CALLS THE SUBROUTINE SUMCT
C      WHICH CALCULATES THE SUM OF THE PRODUCT:
C      THE CONCENTRATION * DELTA T * LOG T AND * (LOG T)**2
C      CALL SUMCT(CUM2,CUM3,SUM1,N,T,TT,SLOT)
C
C      THE FOLLOWING STATEMENT CALCULATES THE AVERAGE
C      RESIDENCE TIME (AT).
C
C      R=CUM2/CUM1
C      AT=EXP(R)
C
C      THE FOLLOWING STATEMENT CALCULATES THE VARIANCE
C      AS A FUNCTION OF TIME (T).
C
C      SIGMAT=CUM3/CUM1-R**2
C
C      PRINT THE RESULTS
C
C      9      FORMAT(' THE SUM OF C*DELTA T=',2X,F20.5,/)
C           TYPE 10,CUM2
C      10     FORMAT(' THE SUM OF C*DELTA T*LOG T=',2X,F20.5,/)
C           TYPE 11,CUM3
C      11     FORMAT(' THE SUM OF C*DELTA T*(LOG T)**2=',2X,F20.5,/)
C           TYPE 12,AT
C      12     FORMAT(' THE AVERAGE RESIDENCE TIME =',2X,F20.5,5X,'HRS.',/)
C           TYPE 13,SIGMAT
C      13     FORMAT(' THE VARIANCE = ',F20.5)
C
C      THIS SUBROUTINE PLOTS % EMISSION VS. LOG T (MIN.)
C
C      LX=0
C      LY=0
C      CALL GRID3(LX,LY,20,20,1)
C      CALL SWINDO(0,LX,0,LY)
C      CALL IWINDO(0,10,0,40.)
C      DO 69 I=1,N
C      69     CALL POINTA(SLOT(I),CF(I))
C           CALL HD COPY
C           STOP
C           END
C
C      THE SUBROUTINES:
C
C      SUBROUTINE CI(CE,C,N)
C      DIMENSION C(800),CE(800)
C      DO 10 I=1,N
C      C(I)=.0788*CE(I)-.0581
C      IF(I-N)10,11,11
C      10     CONTINUE
C      11     RETURN
C      END
C
C      SUBROUTINE SUMCI(C,CUM1,SUM1,N,M,T1,T2)
C      DIMENSION C(800),SUM1(800)
C      SUM=0.0
C      DO 10 I=1,N
C      IF(I-M)1,1,2
C      1      DT=T1
C      GO TO 3
C      2      DT=T2
C      3      SUM1(I)=C(I)*DT

```

```

      CUM1=SUM1(I)+SUM
      SUM=CUM1
      IF(I-N)10,11,11
10     CONTINUE
11     RETURN
      END

C     SUBROUTINE SUMCT(CUM2,CUM3,SUM1,N,T,TT,SLOT)
      DIMENSION SUM1(800),T(800),TT(800),SLOT(800)
      SCUM2=0.0
      SCUM3=0.0
      DO 10 I=1,N
      TT(I)=T(I)/60.0
      SLOT(I)=ALOG(TT(I))
      S2=SUM1(I)*SLOT(I)
      S3=S2*SLOT(I)
      CUM2=S2+SCUM2
      SCUM2=CUM2
      CUM3=S3+SCUM3
      SCUM3=CUM3
      IF(I-N)10,11,11
10     CONTINUE
11     RETURN
      END

```

```

C      THIS PROGRAM PERFORMS A DETAILED ANALYSIS OF RESIDENCE TIME
C      DISTRIBUTION DATA OF AN ANFLOW COLUMN. IT CALCULATES THE REACTOR
C      DISPERSION NUMBER OF THE 'PLUG FLOW W/ DISPERSION MODEL'
C      USING THE VARIANCE METHOD.
C
C      DIMENSION C(800),E(800),TETA(800),T(800),CE(800),SUM1(800)
C
C      THIS SECTION OF THE PROGRAM OBTAINS THE DATA
C      FROM A DATA FILE. IT ASKS FOR THE NUMBER OF
C      DATA POINTS, HOW MANY DATA POINTS WITH DELTA T1
C      (THE PROGRAM WAS MADE FOR ONE OR TWO DELTA T'S),
C      THE FIRST TIME INTERVAL, AND THE SECOND TIME
C      INTERVAL (MINUTES).
C
C      LOGICAL*1 L1(11),L2(6)
C      EQUIVALENCE (L2(1),L1(2))
C      DATA L1/'R','0','9','0','5','7','9',':','D','A','T'/
C      CALL ASSIGN(1,'TT7:')
C      CALL INITT(120)
C      TYPE 8000
8000   FORMAT(////,50X,' INPUT DATA',////)
C      TYPE 800
800    FORMAT('$  DATE OF DESIRED RUN?')
C      ACCEPT 900,L2
900    FORMAT(6A1)
C      CALL ASSIGN(2,L1,11)
C      TYPE 1
1      FORMAT(' NUMBER OF DATA POINTS')
C      ACCEPT 2,N
2      FORMAT(I3)
C      TYPE 3
3      FORMAT(' NUMBER OF DATA POINTS WITH DELTA T1')
C      ACCEPT 4,M
4      FORMAT(I3)
C      TYPE 5
5      FORMAT(' FIRST TIME INTERVAL')
C      ACCEPT 6,T1
6      FORMAT(F2.0)
C      TYPE 7
7      FORMAT(' SECOND TIME INTERVAL')
C      ACCEPT 8,T2
8      FORMAT(F2.0)
C      READ(2,*)(T(I),CE(I),I=1,N)
C      TYPE 200
200    FORMAT(1X,'DATA PRINT OUT?/FUNCTION E=C PRINT OUT?, 0=NO,1=YES')
C      ACCEPT 201,IND1,IND2
201    FORMAT (2I2)
C      IF(IND1)700,700,600
600    TYPE 300,(T(I),CE(I),I=1,N)
300    FORMAT(' ',2E15.3)
C
C      THE FOLLOWING STATEMENT CALLS THE SUBROUTINE CI WHICH
C      CONVERTS THE DATA FROM% EMISSION TO MG/L.
C
700    CALL CI(CE,C,N)

```

```

C
C THE FOLLOWING STATEMENT CALLS THE SUBROUTINE SUMCI
C WHICH CALCULATES THE SUM OF THE PRODUCT:
C CONCENTRATION (MG/L) * DELTA T (MINUTES).
C
C CALL SUMCI(C,CUM1,SUM1,N,M,T1,T2)
C
C THE FOLLOWING STATEMENT CALLS THE SUBROUTINE SUMCT
C WHICH CALCULATES THE SUM OF THE PRODUCT:
C THE CONCENTRATION * DELTA T * T AND * T**2.
C
C CALL SUMCT(CUM2,CUM3,SUM1,N,T)
C
C THE FOLLOWING STATEMENT CALCULATES THE AVERAGE
C RESIDENCE TIME (AT).
C
C AT=CUM2/CUM1
C
C THE FOLLOWING STATEMENT CALCULATES THE VARIANCE
C AS A FUNCTION OF TIME (T).
C
C SIGMAT=CUM3/CUM1-AT**2
C
C THE FOLLOWING STATEMENT CALCULATES THE VARIANCE
C AS A FUNCTION OF THETA, THE REDUCED TIME.
C
C SIGMA=SIGMAT/AT**2
C S=SIGMA
C
C THE FOLLOWING STATEMENT CALLS THE SUBROUTINE EC
C WHICH CALCULATES THE E=C FUNCTION AND THETA
C THE REDUCED TIME.
C
C CALL EC(C,N,CUM1,T,E,TETA,AT)
C
C THE FOLLOWING STATEMENT CALLS THE SUBROUTINE
C DFF WHICH CALCULATES THE DISPERSION COEFFICIENT
C OF THE 'PLUG FLOW WITH DISPERSION MODEL'.
C
C CALL DFF(DF,S)
C
C
C AT=AT/60.0
C
C PRINT THE RESULTS
C
C TYPE 9000
9000 FORMAT(////,50X,' RESULTS',////)
C TYPE 9,CUM1
9 FORMAT(' THE SUM OF C*DELTA T=',2X,F20.5,/)
C TYPE 10,CUM2
10 FORMAT(' THE SUM OF C*DELTA T*T=',2X,F20.5,/)
C TYPE 11,CUM3
11 FORMAT(' THE SUM OF C*DELTA T*T**2=',2X,F20.5,/)
C TYPE 12,AT
12 FORMAT(' THE AVERAGE RESIDENCE TIME =',2X,F20.5,5X,'HRS',/)
C TYPE 13,SIGMAT,SIGMA
13 FORMAT(' THE VARIANCE FOR E(T)=' ,2X,F20.5, 'AND FOR E=C =' ,2X,
1F20.5,/)

```

```

      TYPE 14,IF
14      FORMAT(' THE DISPERSION COEFFICIENT =',2X,F20.5, '// )
      IF(IND2)68,68,202
202     TYPE 15
15     FORMAT(' THE FUNCTION E=C FOR THIS PROBLEM IS',2X, '//)
      TYPE 16
16     FORMAT(25X,'TETA',15X,'E=C', '//)
      DO 100 I=1,N
100    TYPE 17, TETA(I),E(I)
17    FORMAT(15X,F20.5,10X,F20.5)
      C
      C      THE FOLLOWING 8 STATEMENTS PLOT THE FUNCTION E=C
      C      VERSUS THETA USING THE CRT FOR OUTPUT.
      C
68     LX=0
      LY=0
      CALL GRID3(LX,LY,20,20,1)
      CALL SWINDO(0,LX,0,LY)
      CALL IWINDO(0,4.0,0,1.)
      DO 69 I=1,N
69     CALL POINTA(TETA(I),E(I))
      CALL HDICOPY
      STOP
      END

      C
      C      THE SUBROUTINES:
      C
      SUBROUTINE CI(CE,C,N)
      DIMENSION C(800),CE(800)
      DO 10 I=1,N
      C(I)=.0788*CE(I)-.0581
      IF(I-N)10,11,11
10     CONTINUE
11     RETURN
      END

      C
      SUBROUTINE SUMCT(C,CUM1,SUM1,N,M,T1,T2)
      DIMENSION C(800),SUM1(800)
      SUM=0.0
      DO 10 I=1,N
      IF(I-M)1,1,2
1     DT=T1
      GO TO 3
2     DT=T2
3     SUM1(I)=C(I)*DT
      CUM1=SUM1(I)+SUM
      SUM=CUM1
      IF(I-N)10,11,11
10    CONTINUE
11    RETURN
      END

```

```

C      SUBROUTINE SUMCT(CUM2,CUM3,SUM1,N,T)
        DIMENSION SUM1(800),T(800)
        SCUM2=0.0
        SCUM3=0.0
        DO 10 I=1,N
            S2=SUM1(I)*T(I)
            S3=S2*T(I)
            CUM2=S2+SCUM2
            SCUM2=CUM2
            CUM3=S3+SCUM3
            SCUM3=CUM3
            IF(I-N)10,11,11
10         CONTINUE
11         RETURN
        END

C      SUBROUTINE EC(C,N,CUM1,T,E,TETA,AT)
        DIMENSION C(800),T(800),TETA(800),E(800)
        DO 10 I=1,N
            ET=C(I)/CUM1
            E(I)=AT*ET
            TETA(I)=T(I)/AT
            IF(I-N)10,11,11
10         CONTINUE
11         RETURN
        END

C      SUBROUTINE DFF(DF,S)
        IMPLICIT DOUBLE PRECISION (A-H,O-Z)
        DF=S/2
3         DF=DF+0.001
            B=DEXP(-1./DF)
            FR=S-2*(DF-(DF**2)*(1-B))
            IF(FR.GE.0.0)GO TO 3
            IF(FR.LT.0.0)GO TO 4
4         DF=DF-1.0E-5
            B=DEXP(-1.0/DF)
            FR=S-2.0*(DF-(DF**2)*(1-B))
            IF(FR.LE.0.0) GO TO 4
            IF(FR.GT.0.0)GO TO 5
5         DF=DF+1.0E-7
            B=DEXP(-1.0/DF)
            FR=S-2.0*(DF-(DF**2)*(1-B))
            IF(FR.GT.0.0)GO TO 5
            IF(FR.LE.0.0)GO TO 6
6         RETURN
        END

```

```

C      THIS PROGRAM DETERMINES THE THEORETICAL DISPERSION CURVE FOR
C      A PULSE INPUT TO AN ANFLOW BIOREACTOR.
C
      DIMENSION ROT(100),E(500),THETA(500)
      IMPLICIT DOUBLE PRECISION (A-H,O-Z)
      LOGICAL*1 L1(11),L2(6)
      EQUIVALENCE (L2(1),L1(2))
      CALL ASSIGN(1,'TT7:')
      CALL INITT(120)
      TYPE 1000
1000    FORMAT(////,50X,' INPUT DATA',////)
      TYPE 100
100     FORMAT(' DISPERSION FACTOR')
      ACCEPT 200,D
200     FORMAT(F10.6)
      TYPE 300
300     FORMAT(' NUMBER OF ROOTS')
      ACCEPT 400,N
400     FORMAT(I2)
      TYPE 1002
1002    FORMAT(' INCREMENT FOR THETA(100X)')
      ACCEPT 1003,L
1003    FORMAT(I2)
      TYPE 1004
1004    FORMAT(' WANT TYPE THETA AND E/O=NO,1=YES')
      ACCEPT 1005,NO
1005    FORMAT(I1)
      TYPE 1001
1001    FORMAT(////,50X,' RESULTS',////)
      TYPE 700,D
700     FORMAT(' DISPERSION FACTOR =',4X,F10.6,/)
      TYPE 800
800     FORMAT(//,18X,'ROOTS',30X,'FR(I)',/)
      CALL SROOT1(D,ROT)
      CALL SROOT3(D,N,ROT)
      TETA=0.0
      M=400/L
      B=0.5/D
      TI=L/100.0
      DO 12 IJ=1,M
      THETA(IJ)=TETA+TI
      SUM1=0.0
      SSUM=0.0
      DO 13 II=1,N
      X=ROT(II)*(B*DSIN(ROT(II))+ROT(II)*DCOS(ROT(II)))
      Y=B**2+2.0*B+ROT(II)**2
      F=(ROT(II)**2+B**2)/(2.0*B)
      F=F*THETA(IJ)
      H=B-F
      W=DEXP(H)
      SUM=(X/Y)*W
      CUM=SSUM+SUM
      IF(ABS(SUM+SUM1).LE.1.0E-9)GO TO 15
      SUM1=SUM
      SSUM=CUM
13     CONTINUE

```



```

15      E(IJ)=2.0*CUM
      TETA=THETA(IJ)
12      CONTINUE
      IF(NO.LE.0)GO TO 11
      TYPE 902
902     FORMAT(///,18X,'THETA(I)',30X,'E(I)',/)
      TYPE 901,(THETA(I),E(I), I=1,M)
901     FORMAT(15X,F3.1,29X,F20.15)
C
C      PLOT THE RESULTS
C
11      LX=0
      LY=0
      CALL GRID3(LX,LY,20,20,1)
      CALL SWINDO(0,LX,0,LY)
      CALL DWINDO(0,2.0,0,2.0)
      DO 69 I=1,M
69      CALL POINTA(THETA(I),E(I))
      CALL HDCOPY
      STOP
      END

C
C      THE SUBROUTINES
C
C      THIS SUBROUTINE CALCULATES AN APPROXIMATION FOR THE FIRST ROOT.
C
      SUBROUTINE SROOT1(D,ROT)
      DIMENSION ROT(100)
      IMPLICIT DOUBLE PRECISION (A-H,O-Z)
      INDEX=0
      ICOUNT=0
      B=0.5/D
      AINC=0.1
      A1=AINC
      FRR=A1**2-2.0*A1*B*(DCOS(A1)/DSIN(A1))-B**2
      IF(FRR)10,70,10
20      FRR=FR
10      A2=A1+AINC
      FR=A2**2-2.0*A2*B*(DCOS(A2)/DSIN(A2))-B**2
      IF(FR)40,70,40
40      IF(FRR)50,60,50
50      RATIO=FR/FRR
      IF(RATIO)70,70,60
60      A1=A2
      GO TO 20
70      ICOUNT=ICOUNT+1
      IF(ICOUNT/2*2-ICOUNT)71,80,71
71      INDEX=INDEX+1
      ROT(INDEX)=A2
      IF(INDEX-1)80,90,90
80      A1=A2
      GO TO 20
90      RETURN
      END

```

```

C
C      THIS SUBROUTINE CALCULATES ALL THE ROOTS(OPTIMUN NUMBER=50).
C
      SUBROUTINE SROOT3(D,N,ROT)
      DIMENSION ROT(100)
      IMPLICIT DOUBLE PRECISION (A-H,O-Z)
      J=1
      R=0.5/D
810    ROT(J)=ROT(J)-0.001
      A=ROT(J)
      FR=A**2-2.0*A*B*(ICOS(A)/ISIN(A))-B**2
      IF(FR.GE.0.0)GO TO 810
      IF(FR.LT.0.0)GO TO 820
820    ROT(J)=ROT(J)+0.00001
      A=ROT(J)
      FR=A**2-2.0*A*B*(ICOS(A)/ISIN(A))-B**2
      IF(FR.LE.0.0)GO TO 820
      IF(FR.GT.0.0)GO TO 830
830    ROT(J)=ROT(J)-1.0E-7
      A=ROT(J)
      FR=A**2-2.0*A*B*(ICOS(A)/ISIN(A))-B**2
      IF(FR.LE.0.0)GO TO 840
      IF(FR.GT.0.0)GO TO 830
840    ROT(J)=ROT(J)+1.0E-9
      A=ROT(J)
      FR=A**2-2.0*A*B*(ICOS(A)/ISIN(A))-B**2
      IF(FR.LT.0.0)GO TO 840
      IF(FR.GE.0.0)GO TO 850
850    TYPE 4001,ROT(J),FR,J
4001    FORMAT(10X,F21.15,14X,F20.15,5X,I2)
      J=J+1
      ROT(J)=ROT(J-1)+3.1417
      IF(J-N)810,810,890
890    RETURN
      END

```

```

C      THIS PROGRAM CALCULATES THE FIRST ORDER RATE CONSTANT
C      FOR THE PLUG FLOW WITH DISPERSION AND FIRST ORDER RATE
C      KINETICS MODEL.
C

```

```

      DIMENSION AKTO(200),FO(200),CODE(200),RK(200)
      TYPE 1000
1000   FORMAT(////,' INPUT DATA:',////)
      TYPE 10
10     FORMAT(' DISPERSION NUMBER')
      ACCEPT 60,D
60     FORMAT(F7.4)
      TYPE 70
70     FORMAT(' NUMBER OF K VALUES,01-99')
      ACCEPT 90,N
90     FORMAT(I2)
      TYPE 30
30     FORMAT(' AVERAGE RESIDENCE TIME IN HOURS')
      ACCEPT 31,TO
31     FORMAT(F5.1)
      TYPE 34
34     FORMAT(' OVERALL MODEL(0)/REGIONAL MODEL(1)')
      ACCEPT 35,MODEL
35     FORMAT(I1)
      IF(MODEL.EQ.0)GO TO 888
      TYPE 38
38     FORMAT(' SOLUBLE COD REMAINING FRACTION(FO)')
      DO 400 KK=1,N
      ACCEPT 36,FO(KK)
36     FORMAT(F5.3)
400    CONTINUE
      GO TO 999
888    TYPE 32
32     FORMAT(' EFFLUENT COD IN MG/L(INFLUENT=3000)')
      DO 300 K=1,N
      ACCEPT 33,CODE(K)
33     FORMAT(F7.0)
300    CONTINUE
999    DO 100 J=1,N
      IF(MODEL.EQ.1)GO TO 777
      FO(J)=CODE(J)/3000.0
777    AKTO(1)=0.01
      AKT=AKTO(1)
      DO 200 I=1,200
      CALL CALCF(AKT,F,D)
      IF(F.LE.FO(J))GO TO 75
      AKTO(I+1)=AKTO(I)+0.5
      AKT=AKTO(I+1)
      GO TO 200

```

```

75     AKT=AKT0(I-1)
      GO TO 1
200    CONTINUE
1      AKT=AKT+1.0E-3
      CALL RATE(AKT,D,F)
      DIF=FO(J)-F
      IF(DIF.LE.0.0)GO TO 1
      IF(DIF.GT.0.0)GO TO 2
2      AKT=AKT-1.0E-5
      CALL RATE(AKT,D,F)
      DIF=FO(J)-F
      IF(DIF.GT.0.0)GO TO 2
      IF(DIF.LE.0.0)GO TO 3
3      RK(J)=AKT/T0
100    CONTINUE
      TYPE 20
20     FORMAT(////,' RESULTS:',////)
      TYPE 21
21     FORMAT(13X,'DN',12X,'COD/COD0',10X,'K(1/HR)',/)
      DO 500 IJ=1,N
      TYPE 80,I,FO(IJ),RK(IJ)
80     FORMAT(10X,F7.4,11X,F5.3,12X,F5.2)
500    CONTINUE
      TYPE 600
600    FORMAT(/////////)
      END
C
C
      SUBROUTINE CALCF(AKT,F,D)
      A=SQRT(1.0+4.0*AKT*D)
      B=4.0*A*EXP(1.0/(2.0*D))
      C=(1+A)**2*EXP(A/(2.0*D))
      E=(1-A)**2*EXP(-A/(2.0*D))
      H=C-E
      F=B/H
      RETURN
      END
C
      SUBROUTINE RATE(AKT,D,F)
      A=SQRT(1.0+4.0*AKT*D)
      B=4.0*A*EXP(1.0/(2.0*D))
      C=(1+A)**2*EXP(A/(2.0*D))
      E=(1-A)**2*EXP(-A/(2.0*D))
      H=C-E
      F=B/H
      RETURN
      END

```

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