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Protocol for early detection and evaluation of potentially toxic wastewaters using aerobic respirometric and anaerobic batch techniques

by

Sock-Hoon Koh

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Civil Engineering (Environmental Engineering)

Program of Study Committee:
Timothy G. Ellis (Major Professor)
ShihWu Sung
SayKee Ong
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Iowa State University

Ames, Iowa

2002

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Graduate College
Iowa State University

This is to certify that the master's thesis of

Sock-Hoon Koh

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

To my teachers, who taught me how to make a life.

To my friends, who made my journey joyous.

To my panda, who always "rolled" with me.

And

To my family, who made me strong!

BEGIN AND YOU ARE ½ WAY THERE.

~ *Alfred A. Montapert*

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CHAPTER 1. INTRODUCTION

1.1 Background

Nothing ever exists at steady state, but is subject to incessant disturbances. This applies to every wastewater treatment plant where flow rate, influent pH, wastewater composition and concentrations are constantly changing. Currently, municipal wastewater treatment plants are faced with the task of treating significant volumes of complex industrial wastewaters in addition to meeting the demands of the municipality. Many of the industrial effluents may contain appreciable amounts of toxic or inhibitory constituents that are detrimental to the proper operation of the biological treatment systems in the plants. This could adversely affect the effluent quality and result in permit violations and higher operation costs. Therefore, there is need to develop an early warning system that could rapidly identify toxic or inhibitory constituents in the incoming wastewaters.

An upset early warning system is defined as "an instrument that is capable of indicating the presence of biodegradable or non-biodegradable toxic compounds in the influent, or sudden changes in loads of nontoxic, biodegradable substrates" (Love and Bott, 2000). It is important that the system is capable of detecting inhibition effects within a short period of time in order to protect the plant. An early warning system should also possess the necessary sensitivity to function over a broad spectrum of conditions (aerobic, anoxic, and anaerobic).

This research focused on the use of aerobic respirometers as a potential early warning device. Several different types of microorganisms (heterotrophs, autotrophs, and anaerobes) commonly found in the biological treatment system were subjected to toxic compounds and wastewaters from various sources to maximize the sensitivity of the protocol developed. In addition to the aerobic respirometers, an anaerobic inhibition/toxicity bioassay was also studied as part of the protocol development. The original plan for the research was to use both aerobic and anaerobic respirometers. However, due to the inadequacy of the anaerobic respirometers' data acquisition system used in the research (ANR-100, Challenge Environmental Systems, Inc.) and the difficulty to obtain consistent results, the anaerobic

respirometric part was abandoned. An anaerobic toxicity assay (ATA) using serum bottles was adopted as an alternative to the anaerobic respirometric system. Testing procedures and analysis methods for both the aerobic respirometers and anaerobic toxicity assay were developed based on the samples tested.

1.2 Hypothesis

Two main hypotheses were tested in this research.

1. There are inhibitory compounds present in the Genencor wastewaters received at the Cedar Rapids WPCF that have the potential to upset the biological processes at the plant.

2. Different groups of microorganisms in a biological wastewater treatment plant respond to the inhibiting nature of the wastewaters with varying degrees of sensitivity.

1.3 Objectives

The objective of this research project was to develop a protocol for rapid detection and evaluation of the inhibitory/toxicity characteristics of wastewaters from industrial sources. Wastewater from Genencor International, a biotechnology company, discharged at the Cedar Rapids Water Pollution Control Facility (WPCF), was the focus of the study. In addition to Genencor wastewaters, selected toxic compounds (organic and inorganic) and wastewaters from other sources were tested to validate the protocol developed.

1.4 Basic Concepts and Definitions of Terms

1.4.1 Inhibition and toxicity

Inhibition occurs when the presence of a chemical reduces the rate of microbial growth and substrate utilization. The inhibition impact increases with increasing toxicant

concentration. It is typically a reversible process, meaning that the biomass activity can fully recover when the toxic compound is removed (Grady *et al.*, 1999). Toxicity occurs when the concentration of the inhibitor becomes sufficiently high to stop the microbial activity. It is typically an irreversible process, meaning the microbial activity cannot fully recover even when the toxic compound is removed (Grady *et al.*, 1999). Unfortunately, literature has not always made a clear distinction between inhibition and toxicity; therefore, the two terms should not be interpreted too strictly.

1.4.2 Acclimation

Acclimation is the physiological adjustment by an organism to environmental change. Acclimation is required when bacteria are confronted with a substrate, which requires additional enzymes, metabolic pathways, or environmental conditions not encountered prior to the phase of bacterial growth (Speece, 1996). The length of the acclimation period varies considerably from hours to months. For instance, when casein was fed to an anaerobic system with no previous exposure to casein, several days were required for the biomass to reach the maximum degradation rate (Perle *et al.*, 1995). It was reported that with acclimation, the threshold toxicity concentration could be increased as much as ten-fold (Speece, 1996).

1.4.3 Biosensors and bioassays

"Biosensors are defined as devices that produce quantifiable response based on the action or reaction of a biological element, which is integrated with or located immediately adjacent to a physical/chemical transducer detection system" (Love and Bott, 2000). Common examples of biological elements include enzymes, antibodies, and whole cells. Physical and chemical transducers can be electrochemical, optical, or acoustical (Rogers and Mascini, 2000). A bioassay, however, includes a biological element detected by physical or chemical transducer that is not intimately integrated with the active biological element.

1.4.4 On-line and off-line

An on-line device does not require operator intervention to sample, analyze, or manually record the output, whereas an off-line device requires operator intervention at any point during the sampling, analysis, and recording. On-line devices require periodic maintenance and results interpretation by the operator (Love and Bott, 2000).

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

This review summarizes the recent literature on the use of aerobic respirometric and anaerobic techniques in the development of upset early warning systems for biological wastewater treatment. A brief review on the upset events and types of inhibition effects is included at the beginning of this section followed by a review on the available respirometric biosensors used as upset early warning systems. In addition, anaerobic bioassay techniques used for the quantification of inhibition effects are included. Finally, an evaluation on the available respirometric techniques used as early warning systems and the need for this research are presented.

2.2 Upset Events

Proper operation of wastewater treatment facilities is often endangered by influent disturbances such as the sudden discharge of toxic effluent. Upset events encountered by treatment plants may include poor BOD removal, poor nitrification, foaming, bulking, and others. Love and Bott (2000) present a source-cause-effect relationship (Table 1), which helps to illustrate the stages of an upset event.

Table 1. Stages of an upset event (Love and Bott, 2000)

Source	Cause	Effect
• BOD shock load • Toxic shock load • Wet weather flow • Internal recycle	• Biochemical mechanism • Physiochemical mechanism	• Poor BOD removal • Poor Nitrification • Deflocculation • Foaming • Bulking

2.3 Types of Inhibition Effects

There are three major types of inhibition effects on the biodegradation process as proposed by Volskay and Grady (1990).

1. Substrate inhibition – Toxicants can be inhibitory to their own biodegradation through substrate inhibition. Phenol is a good example of this type of inhibitor (Allsop *et al.*, 1990).

2. Inhibition to inhibitor-degrading microorganism – Toxicants may affect the biodegradation rate of biogenic organic matters by inhibitor-degrading microorganisms.

3. Inhibition to non-inhibitor-degrading microorganism – Toxicants may affect the biodegradation rate of biogenic organic matters by non-inhibitor-degrading microorganisms.

Modeling of type 2 inhibition is fairly difficult as complex interactions occur among toxicants, biogenic matter, and microorganisms (Santiago and Grady, 1990). Studies on type 3 inhibition have also been limited (Volskay and Grady, 1990).

There are several models used to represent the inhibition response of biomass. In particular, situation where the specific growth rate of the microorganisms reaches a maximum and reduces as the substrate concentration is increased can be modeled with Andrews equation as shown in Equation 1 (Grady *et al.*, 1999).

Equation 1. Andrews Equation

$$\mu = \hat{\mu} \frac{S_S}{K_S + S_S + \frac{S_S^2}{K_I}}$$

Where μ = Specific growth rate (T^{-1})

$\hat{\mu}$ = Maximum specific growth rate (T^{-1})

S_s = Substrate (inhibitor) concentration (ML^{-3})

K_s = Half saturation coefficient for substrate (ML^{-3})

K_I = Inhibition coefficient (ML^{-3})

In addition to Andrews equation, four models are commonly used in classifying inhibitor types (Table 2). The equations are expressed as the ratio of the respiration rate of the test sample to the respiration rate of the control (no inhibitor). They are classified according to the way they influence the maximum substrate removal rate (q_m) and the half-saturation coefficient (K_S) (Volskay *et al.*, 1988). Identification of the inhibition types is fairly important especially in understanding the response of a reactor towards an inhibitory load. For instance, inhibition acting in a competitive manner can be reversed by increasing the substrate concentration, whereas a mixed inhibitor is the worst type as it affects the growth rate regardless of the substrate concentration (Grady *et al.*, 1999).

Table 2. Inhibitor types (Volskay *et al.*, 1988)

Inhibitor Type	Effect on q_m	Effect on K_S	Respiration rate as a fraction of control
Competitive	None	Increase	$\frac{1+S/K_S}{1+I/K_S+S/K_S}$
Noncompetitive	Decrease	None	$\frac{1}{1+I/K_I}$
Uncompetitive	Decrease	Decrease	$\frac{1+K_S/S}{1+I/K_I+K_S/S}$
Mixed	Decrease	Increase	$\left(\frac{1}{1+I/K_I}\right)\left(\frac{1+S/K_S}{1+I/K_S+S/K_S}\right)$

Where I = Inhibitor concentration (ML^{-3})

S = Substrate concentration (ML^{-3})

K_s = Half saturation coefficient for substrate (ML^{-3})

K_I = Inhibition coefficient (ML^{-3})

2.4 Aerobic Respirometry Used As an Upset Early Warning System (UEWS)

Respirometry, the measurement and interpretation of the respiration rate of microorganisms (Spanjers *et al.*, 1996), is a relatively simple concept that can easily be interpreted especially when data acquisition is facilitated with a computer. The respiration rate is measured as the concentration of oxygen (typically mg/L) consumed by the microorganisms per unit time. Early development of respirometry was aimed at replacing the standard 5-day BOD tests. More commonly, respirometers are used to assess the biodegradation kinetics of a specific chemical and industrial treatability; to evaluate the impact of various wastes and chemicals on wastewater treatment plant (WWTP) performance; and to study factors affecting growth of microorganisms in various environmental settings (Young, 1996).

Respirometry technology has been widely commercialized. To date, a large database has been established with off-line respirometry screening of suspected toxicants and industrial wastewaters. The dose-response relationship of a broad spectrum of inhibitory and toxic substances is well documented. Besides, a respirometric system is fairly flexible in that it can be easily modified to detect the inhibitory and toxic effects on carbon oxidation and nitrification (Love and Bott, 2000). A review of respirometry used as upset early warning systems are included in the following sections.

2.4.1 Respirometry for general toxicity

The RODTOX respirometer (Rapid Oxygen Demand and TOXicity Tester), developed at the Laboratory of Microbial Ecology University of Gent, Belgium, is an open respirometric biosensor for rapid determination of potential toxicity. It is commercially

available from KELMA bvba, Niel, Belgium and commonly used for on-line BOD and toxicity detection, off-line IC_{50} estimation, and off-line respiration inhibition kinetics analysis. The RODTOX system consists of a biological system, peripheral equipment, and an electronic component. The biological system is based on an open and aerated respiration chamber (10 liters) operating in batch mode. Calibrated water (20 g COD/L acetic acid and acetic salt, and 2 g/L ammonia-N) and potentially toxic wastewater are injected to the respirometers. The dissolved oxygen profile (respirogram) is recorded continuously. Typically, the sludge in the RODTOX vessel is fed with wastewater every 30 minutes with periodic calibration in every 1 to 3 hours. Three respirometric parameters, the maximal peak slope (PS), peak height (PH), and peak area (PA), are measured from the respirogram to evaluate the inhibition effect. Percent inhibition is calculated by comparing the parameters of the calibration respirograms before and after the injection of wastewater (Vanrolleghem *et al.*, 1996; Temmink *et al.*, 1993).

Geenens and Thoeye (1998) observed that the inhibition profiles using RODTOX respirometer were sufficient for early warning toxicity at the WWTP of Deurne-Schijnpooort in Europe. IC_{10} was estimated and the obtained data showed that 10% respiration inhibition did not result in deterioration of the plant's effluent. However, substantial solids washout was detected for a 43% inhibition. Therefore, with IC_{10} estimation, the RODTOX biosensor was able to screen the WWTP catchment's area for inhibitive sources.

Vanrolleghem *et al.* (1996) investigated the performance of the RODTOX on a full-scale wastewater treatment plant with simulated toxicity. Creoline disinfectant was deliberately discharged at concentration of 380 mg/L. The experiment results showed that the detection was in sufficient time (50 minutes) to protect the plant. The addition of the toxic wastewater was interrupted at a creoline concentration of 5 mg/L in the aeration tank. Eleven days after the first run, a second toxicity experiment was conducted using similar wastewater. This time, the addition of toxic wastewater was interrupted by the RODTOX system at a creoline concentration of 25 mg/L, which resulted in significant effluent deterioration. The differences in toxicity detection might be due to the changed sludge characteristics as the sludge in RODTOX vessel changes only every 2 weeks. The toxicity of

the wastewater was compared off-line with Microtox, an acute aquatic toxicity detection system used for influent toxicity analysis. The analytical method of Microtox is based on the change in light output resulting from the bioluminescence decay of marine bacteria (*Photobacterium phosphoreum*). Influent toxicity is indicated by the bioluminescence reduction and is measured with a luminometer. Though Microtox has been proven to be more sensitive than the RODTOX bacterial cultures, there was a case when the Microtox gave a false alarm because it detected 75% light output reduction where in fact it was due to a red colored influent (Vanrolleghem *et al.,* 1996).

Temmink *et al.* (1993) compared the RODTOX system with the RA-1000 system, developed at the Department of Environmental Technology of the Agricultural University of Wageningen, Netherlands. The RA-1000 system is a closed chamber operating in a continuous mode with continuous fresh sludge and wastewater supply. A solenoid system periodically reverses the flow direction enabling the use of one DO probe to measure the DO of the incoming and outgoing flow. The RA-1000 vessel is designed as a small and highly loaded aeration tank, which results in short response time (15-30 min). However, the critical level of the ratio between wastewater and sludge flow into the test vessel must be determined to avoid insensitivity of the device to the variation in substrate concentrations at a high substrate loading. For instance, a preliminary experiment was performed at the AKZO-Botlek treatment plant to find the critical loading above which a maximum respiration rate was measured. In addition, the decrease in the respiration rate due to substrate deficiency and toxic spills must be differentiated in the RA-1000 system. From Temmink's studies, it was shown that both RODTOX and RA-1000 gave rapid indication of the potential toxicity of the influent.

The systems described so far used activated sludge as the test biomass. Nirmalakhandan *et al.* (1996) evaluated the suitability of a commercially available surrogate test culture, Polytox, in estimating toxicity of Synthetic Organic Chemicals (SOCs) to activated sludge using a respirometric technique. Polytox (Polybac Corporation, Bethehem, PA, U.S.A.) is a commercial blend of 12 strains of microorganisms isolated from activated sludge available in freeze dried form. A strong correlation ($r^2 = 0.922$) between the Polytox

and activated sludge was reported in experimentally determined IC_{50} values. Polytox was reported as an easy-to-use surrogate test culture, and yielded more consistent results than the activated sludge organisms (Nirmalakhandan *et al.*, 1994).

Kong *et al.* (1993) compared respiration inhibition using the Microtox and RODTOX systems. In general, higher sensitivity was detected using Microtox test. However, RODTOX was found more sensitive than Microtox test in the case of cyanide.

To evaluate the inhibition response of the biomass more accurately, kinetic analysis methods are used. The respiration inhibition kinetic analysis (RIKA) has been developed by Volskay and Grady (1990) to quantify the effect of the degradation rate of a biogenic organic compound by the non-inhibitor degrading population. Monod kinetic parameters describing the biodegradation of the biogenic substrate (butyric acid) are measured in the presence of toxicants with three inhibitory concentrations. Two or three pulses of butyric acid are injected during the exposure time of the biomass to the toxicants since the maximum sensitivity has been observed for actively metabolizing bacteria. However, this RIKA procedure is very time-consuming and laborious as a total of 19 pulses of different substrate concentrations are injected consecutively. Kong *et al.* (1994) presented a faster and automated RIKA method, named ARIKA, to quantify the inhibitory effect of toxicants on the biodegradation of biogenic organic matter using RODTOX in the laboratory. The complete characterization of the toxic effects could be done within 3 hours because the number of experiments was reduced to 4-5 instead of 57 (3 times 19). This was possible by applying a non-linear parameter estimation algorithm coupled with a model-based approach (see Kong *et al.*, 1994) and the kinetic parameters were calculated by automated data interpretation software.

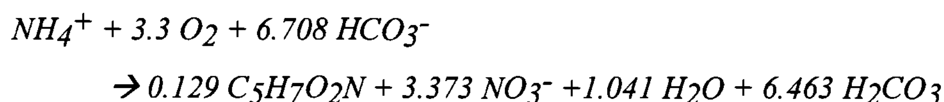
Though the respirometric techniques are aimed at detecting the toxicity effect of the influent wastewater in a relatively short period of time, a minimum retention time is needed to allow sufficient contact time between sludge and wastewater to take effect on the respiration rate. Tanlinli and Tokta (1994) suggested that prolonging the exposure time of inhibitor and the activated microorganisms would lead to more reliable results in terms of determining inhibition types. In their study, the inhibitory effects on the microorganisms

after a contact time of 3 and 18 hours were studied using a modified OECD 209 method. The OECD 209 method involves the measurements of oxygen uptake rates of activated sludge microorganisms exposed to different concentrations of inhibitors. From the results, it was observed that short sludge and wastewater contact time might cause misleading evaluations of inhibitory effects and behaviors. Temmink *et al.* (1993) reported a 15-minute contact time to adequately detect the inhibition effects of the biomass when RA-1000 system was studied.

2.4.2 Respirometry for nitrification inhibition

Nitrification represents a sensitive process in biological treatment because nitrifying bacteria are highly susceptible to inhibition by a large number of compounds (Hockenbury and Grady, 1977; Richardson, 1985; Blum and Speece, 1991). Nitrifiers are autotrophs that use inorganic materials as their carbon source. Heterotrophs, on the contrary, utilize organic materials as their carbon source. Nitrifiers obtain energy by oxidizing reduced nitrogen, NH_3 or NH_4^+, and the process is called nitrification. In this redox reaction, NH_4^+ serves as the electron donor while O_2 serves as the electron acceptor. The stoichiometric equation of nitrification is shown as follows.

Equation 2. Nitrification (in gram)



$$NH_4^+ + 3.3\ O_2 + 6.708\ HCO_3^- \rightarrow 0.129\ C_5H_7O_2N + 3.373\ NO_3^- + 1.041\ H_2O + 6.463\ H_2CO_3$$

Nitrifiers have a low growth yield and are very sensitive to the variation in pH. They engage in restricted energy yielding metabolism and synthesize all cell components from CO_2 (Grady et al., 1999). Nitrification has little impact on the quantity of biomass but large impact on the oxygen and alkalinity concentrations. As a result, sufficient buffering capacity

and dissolved oxygen are required during the test to optimize the performance of the microorganisms. Table 3 lists some of the typical parameters for nitrification.

Table 3. Typical parameters for nitrification (Grady, 1999)

Alkalinity consumed	8.62 mg HCO_3^-
O_2 demand	4.33 mg O_2/mg NH_4^+-N
Yield	0.166 mg biomass/mg NH_4^+-N
Optimum pH range	7.5 - 8.5
Ks (NH_3) for *Nitrosomonas*	0.06 - 5.6 mg/L as N (typical = 1.0 mg/L)
Ks (NO_2^-) for *Nitrobacters*	0.06 - 8.4 mg/L as N (typical = 1.3 mg/L)
Maximum specific growth rate coefficient (NH_3)	0.014 - 0.092 (typical = 0.032/hr)
Maximum specific growth rate coefficient (NO_2^-)	0.006 - 0.06 (typical = 0.034/hr)

Several attempts have been made to evaluate the nitrification inhibition in respirometers. Kroiss *et al.* (1992) used a two-step strategy to identify the source of nitrification inhibition based on respiration tests. A new mathematical inhibition model was used to describe the inhibition effect. A nitrification inhibitor, allylthiourea (ATU), was used to determine the heterotrophic and autotrophic respiration rates separately. A reaction time of 10 minutes and an ATU concentration of 10 mg/L and 30 mg/L respectively for complete inhibition of *Nitrosomonas* and *Nitrobacters* were suggested. The maximum autotrophic oxygen uptake rate was calculated by subtracting the heterotrophic oxygen uptake rate from the total oxygen uptake rate. A maximum ammonium chloride concentration of 50 mg/L was added to avoid ammonia inhibition.

A new method using a Double-Monod mathematical model and nonlinear parameter estimation algorithm for simultaneous determination of inhibition kinetics on both carbon oxidation and nitrification with the RODTOX biosensor was developed by Kong *et al.* (1996). This was a modified ARIKA method, which consisted of choosing a proper toxicant concentration range for simultaneous determination of the inhibitory effect of a toxicant on the degradation of multiple biogenic substrates (acetic acid and ammonium chloride) within a workday. The time for complete determination of the inhibition kinetics was approximately

8 hours. In this experiment, the best C: N ratio for the defined substrate mixture was determined to achieve similar degradation rates for the determination of carbon oxidation and nitrification inhibition. It was reported that the maximum autotrophic growth rate was not in the range of study due to the deficiency of the software used in distinguishing the nitrification biomass from the total biomass. The authors also reported the difficulty in estimating biokinetic parameters when the nitrification was inhibited more than 80% due to numerical inaccuracy

A system using a gas-static liquid batch assay method was developed to detect nitrification inhibition (Gernaey *et al.*, 1997b). The respirometric system was commercialized as the Nitrification Toxicity Tester (NITROX). Nitrification inhibition could be determined within 10 minutes in this system. In addition, inhibition of *Nitrosomonas* and *Nitrobacters* could be differentiated.

Hayes *et al.* (1998) used the AmTox system, a rapid (30-minute assay time) online response system, to determine the inhibition of nitrifying bacteria. An ammonia probe was used for the measurement of ammonia uptake rate over a 30°C bioreactor with a dense immobilized nitrifying population of approximately 15 g/L as total suspended solids (TSS).

2.5 Anaerobic Techniques

In addition to the aerobic evaluation of potentially toxic wastewaters, early detection and quantification of anaerobic inhibition is of equal significance for treatment facilities with both aerobic and anaerobic treatment units. The anaerobic test may also facilitate the detection of inhibitory influent, as the anaerobic cultures are more sensitive to certain compounds than the aerobic cultures. Prior to the establishment of anaerobic biosensors or bioassays, an understanding of the biotransformation of the substrates in an anaerobic environment is important. From Figure 1, it can be observed that the reaction pathways become more complex when a complex organic such as a toxic organic chemical is used. Unlike acetate and ethanol, a large number of acetogenic intermediates can be produced when toxic organic chemicals are used.

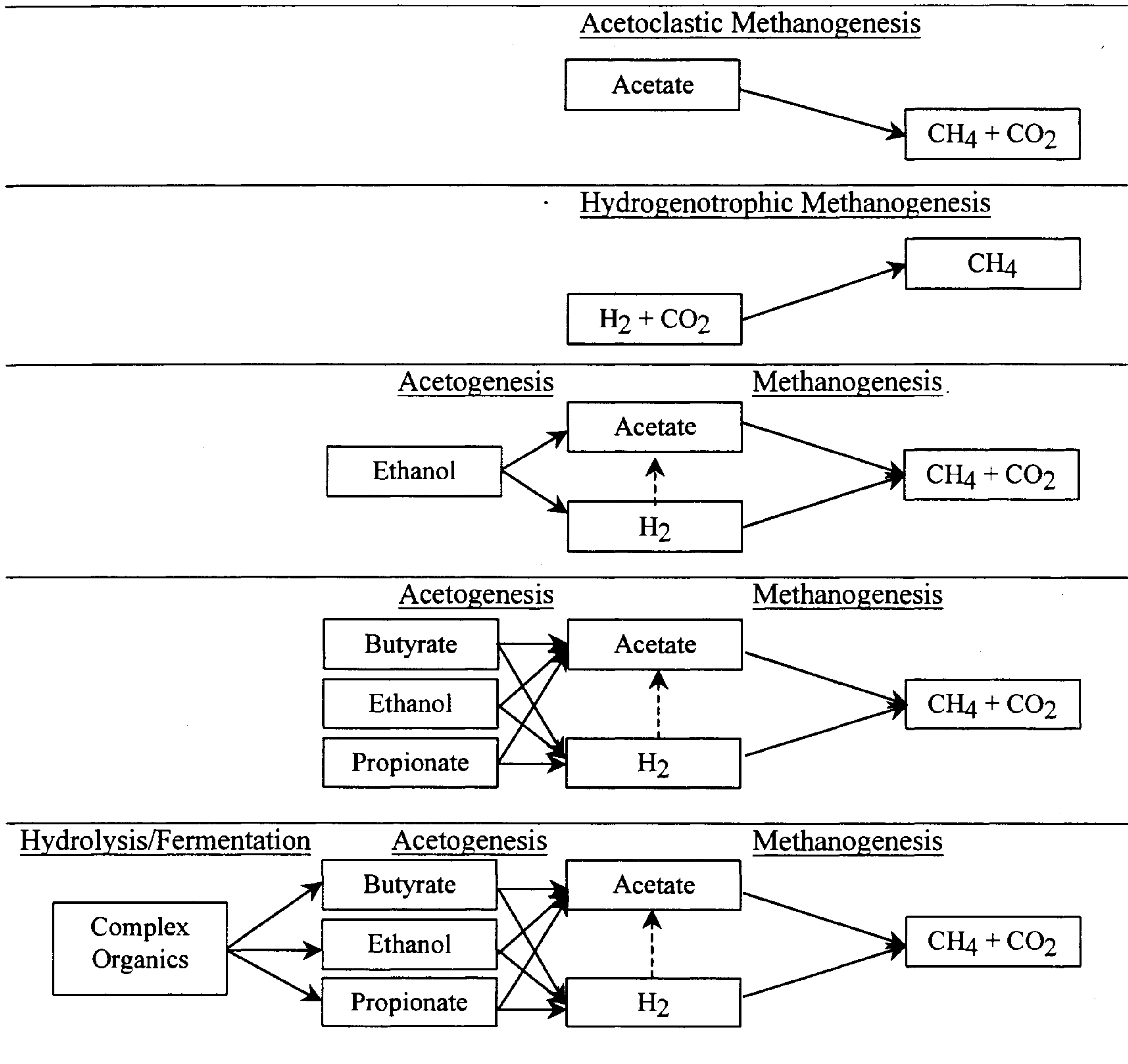


Figure 1. Anaerobic transformation of organic substrates (Young and Tabak, 1993)

2.5.1 Anaerobic upset early warning systems

Overall, less attention has been paid to anaerobic respirometry as upset early warning systems at full-scale wastewater treatment systems than aerobic respirometry (Love and Bott, 2000). There are basically two types of anaerobic respirometers. The first type measures gas production during anaerobic reaction by detecting the changes in pressure. A pressure transducer and valve are commonly used for this type of anaerobic respirometer (N-CON Systems, Inc.). The second type measures the gas production by evacuation of small volume increments (0.05 to 0.5 mL) to maintain a constant pressure in the reaction vessel. It includes counting the small gas bubbles produced in an anaerobic batch bottle as they pass through a specially designed flow cell (ANR-100 and ANR-200, Challenge Environmental Systems, Inc.) (Young, 1996).

Khandaker (1996) reported a number of case studies using anaerobic respirometers based on the anaerobic treatability screening protocol developed by Young. In the paper, the authors showed that the respirometers (Challenge ANR-200) could effectively be used for anaerobic treatability assessment of industrial wastewaters. The anaerobic respirometric method could serve as a cost-effective alternative to the pilot-scale studies.

Rozzi *et al.* (1997) evaluated the potential of a new biosensor, the RANTOX (Rapid ANaerobic load and TOXicity tester) to detect toxic loads in wastewaters. The development of the RANTOX biosensor was based on the monitoring of the metabolism of acetoclastic methanogens, the most sensitive microorganisms in anaerobic digestion, in the presence of toxicants. The wastewater that had the potential to induce an overload or contained a toxicant was tested in advance on a small "upstream" digester (RANTOX). The RANTOX biosensor was made of a laboratory-scale reactor and its working cycle was controlled by a personal computer. The instrument was equipped with a biogas flow meter, a temperature probe and a pH electrode for monitoring. While the RANTOX biosensor was fed with the same wastewater and organic loading rate, the acetate was added periodically (two hours) to rapidly detecting the inhibition effects on acetoclastic methanogens. Inhibition was assessed by comparing the gas productions of the biosensor before, during, and after the addition of inhibitory compounds. The authors mentioned the difficulty of directing process control due

to the complexity of the metabolic pathways and the difficulty of detecting and monitoring process instability in a short time. In other words, the consequent control actions were possible only after the instability had affected the operation of the reactor, especially when the toxicants were building up slowly and irreversibly.

Barnett *et al.* (1992) examined an expert system for anaerobic digestion process operation, which used if-then rules as the basic form of knowledge representation. The overall design of the expert system consists of monitoring, state assessment, and control decision modules. A mathematical model developed by Graef and Andrews (1974) was used for the development of the expert system. The configuration of the expert system consisted of three modules: monitoring module, state assessment module (SAM), and control decision module (CDM). The SAM contained rules for distinguishing different types of upset. Hydraulic upset, for instance, was characterized by high loading rate and low detention time while organic overload was characterized by a high organic loading at a normal detention time. Though the expert system can be an important component for current and future computer-based systems for the operation of anaerobic digestion, thorough understanding of the operations of the expert system and anaerobic digestion system is required to avoid process deficiency.

2.5.2 Anaerobic inhibition/toxicity protocols

Owen et al. (1979) developed a batch anaerobic toxicity assay (ATA) technique to measure the adverse effect of a compound on the rate of total gas production. Assay bottles were prepared with defined media, seed inocula, and samples. A "spike" containing acetate and propionate was added and ratios between respective rates for the samples and the controls (designated the maximum rate ratio, MRR) were computed. A possible inhibition was suggested by a MRR value of less than 0.95 and a significant inhibition was suggested by a value of less than 0.9. Sample decomposition and varying ratios of carbon dioxide and methane production could complicate the analysis. Nonetheless, it could be confirmed by semi-continuous studies.

Young (1991) developed an anaerobic treatability screening protocol for different industrial wastewaters. Treatability was based on the computation of the rate and extent of biodegradation, identification of the presence of toxic substances, and dilution factors. The treatability protocol consisted of two phases. Phase I involved batch serum bottle tests while phase II used semi-continuous bench scale reactors. The batch serum bottle tests studied the response of a single dose of test waste while the bench-scale semi-continuous reactor tests showed the response of anaerobic cultures to long-term feeding of test waste. Good agreement of gas production from the test reactors and a control reactor indicated a good potential for using anaerobic processes. However, a sufficient amount of time (3-5 days) was needed to determine the extent of biodegradation and the presence of the inhibitory/toxic constituents.

The fate and effect of toxic organic chemicals in the anaerobic treatment processes using a multilevel protocol was developed by Young and Tabak (1993). Level I was a relatively rapid 3-step screening protocol for assessing toxicant effect on specific anaerobic reactions. It was designed to identify threshold toxicant concentrations causing inhibition of acetogenic and methanogenic reactions. In fact, Step I of the Level I protocol was a modification of the basic anaerobic toxicity assay (ATA) by Owen *et al.* (1979). Level II was a cosubstrate kinetics protocol to reveal the effect of toxic organic chemicals on the kinetics of acetogenic and methanogenic transformation. Level III was a toxicant degradation kinetics protocol, which required the use of an acclimated culture to determine the kinetic parameters for the degradation of toxicants. The procedures provided a consistent means of determining the fate and effect of toxic organic chemicals and also the intrinsic parameters for describing the anaerobic reaction.

2.6 Evaluations and Conclusion

When using a calibrated substrate, the time lag must be minimized to avoid a significant amount of toxicity entering the treatment facility. In the RODTOX system, a calibrated substrate is injected at 1-2 hours frequency. It is likely that the toxic wastewater might have entered the treatment plant between the calibration injections and deteriorated the

plant performance. No calibration substrate was used in the respirometric protocol developed here, but the oxygen uptake rate (OUR) values before and immediately after injection of sample were compared to determine the percent inhibition. This minimized the time lag period and an overall reaction time of approximately 30 minutes could be achieved.

It is important to ensure that fresh biomass is used in the respirometric devices when detecting and evaluating the influent toxicity. This is critical to ensure unbiased interpretations of the respirometric results by accounting for the changing sludge characteristics. For instance, the biomass concentration in the vessel might have increased as a result of substrate utilization in a 1 to 2-week period. In the RODTOX vessel, activated sludge is typically refreshed every 1 to 2 weeks. Inconsistency in detecting the creoline wastewater's toxicity was observed when tested at different time periods by Vanrolleghem *et al.* 1996. In our testing, specific normalized oxygen uptake rate (NOURc) is calculated to account for the changing sludge characteristics. The NOURc term takes dilution and the differences of biomass concentration into consideration and gives unbiased accounts of the inhibition effects. Moreover, the closed oxygenated respirometers used in this research give a quicker response when compared to the RODTOX system. A total of 15-30 minutes, which includes time for oxygenation, endogenous respiration, and sample injection and response, is sufficient for the detection of the inhibition response.

Size is another consideration that weighs in favor of the respirometer used in this study. The respirometric system used in this study consists of four parallel respirometric vessels each of 250 mL with a total volume of 1 liter. The size is relatively smaller than the 10-liter open chamber used in the RODTOX system. It can be easily maneuvered from site to site for inhibition testing. Furthermore, the respirometric technique used here is a direct measurement of inhibition effects on activated sludge, which is a more representative protocol than the use of a surrogate text culture such as Microtox or Polytox, which has the potential to overestimate the inhibition effect.

The RA-1000 respirometric system measures inhibition on the basis of maximum respiration rates, which requires a preliminary study to determine the critical loading rate of the wastewater. With the determined critical loading rate, the maximum respiration rate can

be achieved without causing insensitivity to variation in substrate concentrations at high substrate concentration. In our measurements, a model-based approach was used. This helps avoiding the extra time spent on conducting a preliminary test for the determination of the critical loading rate. The model-based approach allows us to have a more accurate assessment and interpretation of the inhibition phenomenon, which avoids the use of peak slope, peak height, and peak area (PS, PH, and PA) in the classical approach of RODTOX system.

The Double-Monod mathematical model and nonlinear parameter estimation algorithm for simultaneous determination of inhibition kinetics of carbon oxidation and nitrification inhibition described by Vanrolleghem *et al.* (1996) showed many complications. A large number of parameters such as Y_i, μ_{max}, X_i, i_{XB}, f_I, b_i, and $K_{m,i}$ (see definitions in Vanrolleghem's paper) are to be estimated. Instead of using one chamber to quantify the kinetics of nitrification and carbon oxidation inhibition, two separate respirometric tests were used in our studies to simplify the parameter estimation.

The expert system used for the anaerobic digestion system shows much complexity. Successful operation of the expert system is a knowledge-intensive task, which requires complete understanding of the expert system. It is a highly mathematical and computer based system, and inadequate knowledge of the process can lead to system deficiency. Moreover, it is difficult to determine how a given rule's form changes with more complex behavior, which is commonly encountered in the actual biological wastewater treatment plant.

The four basic inhibition models reviewed in the beginning of this section are often found inadequate to describe the actual situation (Kroiss *et al.*, 1992) due to the complexity of the biological treatment system. In practice, none of these models can describe the inhibition kinetics accurately. Activated sludge systems contain a mixture of compounds in the influent wastewater and in the biomass that complicate the modeling process. In this study, an inhibition model that combines the Andrews equation and one of the four basic models described is used to address both the inhibitor- and non-inhibitor degrading portions of the biomass used, whereas RIKA and ARIKA methods focused only on the type III

inhibition (inhibition on the non-inhibitor degrading population). Furthermore, the best C:N ratio of a defined substrate mixture has to be determined in the ARIKA method to achieve similar degradation rates of carbon oxidation and nitrification inhibition in one chamber.

In review of the existing protocols developed, a model-based and more simplified version of the protocol is adopted. It includes the respirometric assessment of carbon oxidation and nitrification inhibition and the anaerobic inhibition batch study of wastewaters for future development of an anaerobic respirometric system.

CHAPTER 3. MATERIALS AND METHODS

The materials and methods section is divided into five parts: general testing methods, samples used, microorganism types, aerobic respirometric test, and anaerobic batch test.

3.1 General

Chemical oxygen demand (COD) and total and volatile suspended solids (TSS and VSS) tests were conducted in accordance with the Standard Methods for the Examination of Water and Wastewater (19th Edition, 1995). A closed reflux titrimetric method was adopted for the COD test, and details can be found in section 5220 C. Solids tests (TSS and VSS) were conducted according to the procedures described in section 2541 D and E with glass fiber filters (42.5 mm diameter, Whatman GF/C). Electronic pH meters (Accumet Model 10 pH meter, Fisher Scientific; Model 05669-20 pH meter, Cole Palmer) were used for the measurement of sample pH.

3.2 Samples Tested

Two potentially toxic industrial wastewaters and selected organic and inorganic compounds were investigated in this study.

3.2.1 Industrial wastewaters

Wastewaters from a biotechnology company (Genencor International) and a food processing company (Quaker Oats) treated at Cedar Rapids Water Pollution Control Facility (WPCF) were tested.

3.2.1.1 Cedar Rapids WPCF

Cedar Rapids WPCF is located on approximately 40 acres in the southeastern quadrant of Cedar Rapids, Iowa. It currently serves the cities of Cedar Rapids, Marion, Hiawatha, and Robins, Iowa. The plant operates separate stages of activated sludge for

carbonaceous BOD and nitrogenous BOD removal. It is treating 40% municipal and 60% industrial wastewaters including Genencor International and Quaker Oats. It is a pure oxygen activated sludge plant with a relatively short SRT (carbonaceous) that ranges from 1.5 to 3 days. The average flow to the facility was 38 million gallons per day (MGD) from January to August 2001.

3.2.1.2 Genencor International

Genencor International, located at 1000 41st Avenue Drive, S.W. Cedar Rapids, Iowa, is a biotechnology company focused mainly on the development of enzymes that catalyze chemical reactions for applications in health, agriculture, and industrial chemical markets. Examples of such applications include treating textiles, cleaning contact lenses, processing paper, brewing low-calorie beer, and converting plants such as corn to chemicals (http://www.genencor.com).

The Genencor wastewater is discharged at Cedar Rapids WPCF at an average flow of 0.55 MGD, which is diluted approximately 70 times at the plant. Periodic upsets were reported previously during both lab-scale (at ISU) and pilot-scale (at Cedar Rapids) treatment of Genencor International wastewater. It was suspected that the wastewater contained toxic constituents that are inhibitory to the microorganisms commonly found in biological treatment systems. Twenty-four hour composite Genencor wastewater samples were collected and shipped to our laboratory in a cooler biweekly. The samples were kept in a 4°C refrigerator to minimize any physiological changes. Genencor wastewater samples collected were studied with both aerobic respirometric and anaerobic batch techniques.

3.2.1.3 Quaker Oats

The Quaker Oats Company is well known for its grain and oats based products such as cereal, oatmeal, rice cake, and granola bars. The company also is involved in the production of furfural, which can be produced commercially by the dehydration of pentose sugars from the byproduct of oats and oatmeal production such as oat husks.

Quaker Oats wastewater samples were collected from the Cedar Rapids WPCF and brought to the environmental laboratory at ISU. Samples were preserved at low pH (~3) at room temperature and were used as feed solution for the aerobic yeast-culturing reactors developed at ISU. It was suspected that the furfural in the wastewater was inhibitory to the yeast culture (*Candida utilis*) since an acclimation period of up to 2-week was observed. Since no comparison study was made with different wastewaters, the inhibition effect of the Quaker Oats sample to the yeast culture was not known. In this study, the inhibition effects of the Quaker Oat wastewater sample on carbon oxidation, nitrification, and anaerobic digestion were investigated.

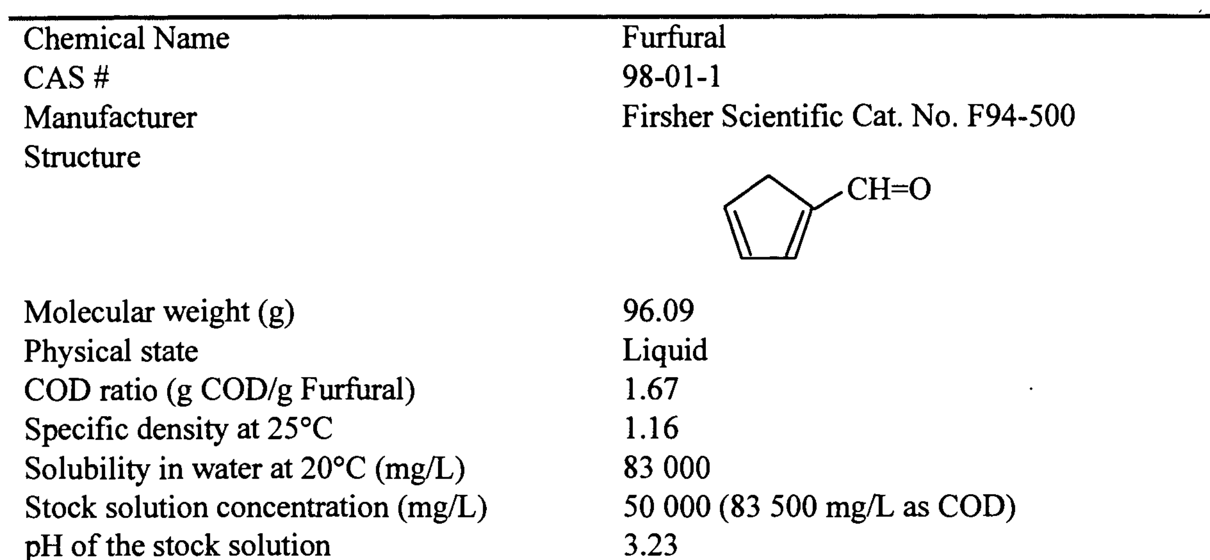
3.2.2 Organic compounds: Furfural and Phenol

In order to examine the applicability of the protocol to a broader spectrum of inhibitory compounds, two known toxic organic compounds (phenol and furfural) were tested in addition to the wastewaters. Both chemicals are listed on the Toxic Substances Control Act (TSCA) inventory and classified as hazardous substances under the Clean Water Act (CWA) according to the Material Safety Data Sheet (MSDS) by Fisher Scientific.

3.2.2.1 Furfural ($C_5H_4O_2$)

Furfural (2-furaldehyde) is a colorless to light yellow oily liquid that has an almond-like aromatic smell. Upon exposure to air, it turns dark brown in color. The chemical and physical properties are listed in Table 4. A stock solution of 50 000 mg/L was prepared and stored in a 4°C refrigerator for testing. Furfural is mainly used as a feedstock for furfuryl alcohol production that in turn is used in the production of furan resins for foundry sand binders. It is also widely used as a refining solvent in the manufacture of synthetic rubber and nylon. It is produced commercially by the dehydration of pentose sugars from agricultural wastes such as corncobs, oat husks and peanuts (http://www.levulinic.com/furfural-for-sale.htm).

Table 4. Chemical and physical properties of furfural

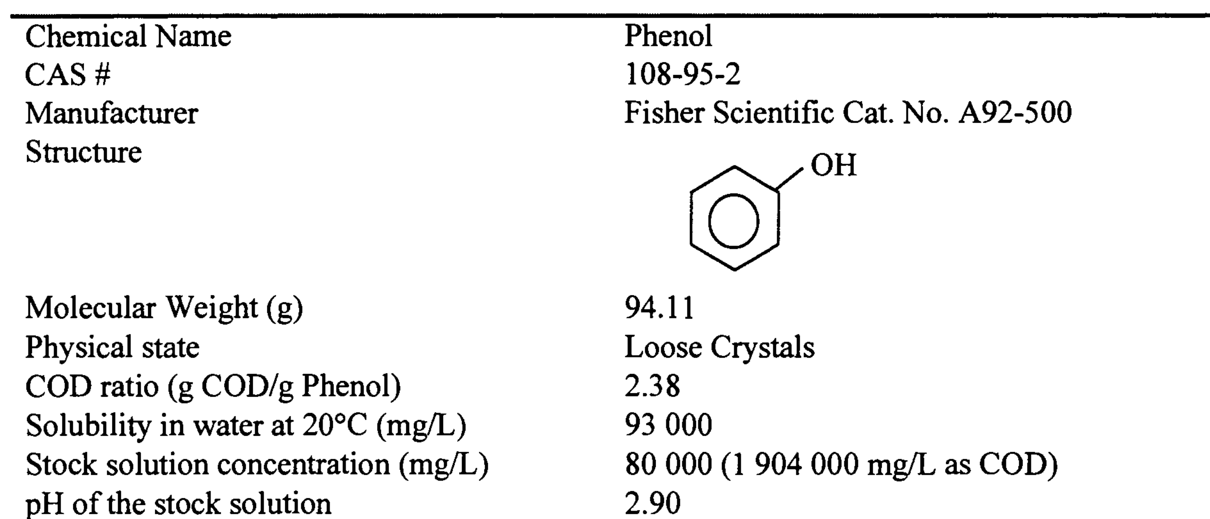


Chemical Name	Furfural
CAS #	98-01-1
Manufacturer	Firsher Scientific Cat. No. F94-500
Structure	CH=O
Molecular weight (g)	96.09
Physical state	Liquid
COD ratio (g COD/g Furfural)	1.67
Specific density at 25°C	1.16
Solubility in water at 20°C (mg/L)	83 000
Stock solution concentration (mg/L)	50 000 (83 500 mg/L as COD)
pH of the stock solution	3.23

3.2.2.2 Phenol (C_6H_5OH)

Phenol is a colorless liquid with sweet and irritating odor. It is commonly used in making plastics, caprolactam (for nylon and other man-made fibers), bisphenol A (for epoxy and other resins), and other uses (http://www.eco-usa.net/toxics/phenol.shtml). A stock solution of 80 000 mg/L was prepared and stored at 4°C. Table 5 shows the characteristics of phenol.

Table 5. Chemical and physical properties of phenol



Chemical Name	Phenol
CAS #	108-95-2
Manufacturer	Fisher Scientific Cat. No. A92-500
Structure	OH
Molecular Weight (g)	94.11
Physical state	Loose Crystals
COD ratio (g COD/g Phenol)	2.38
Solubility in water at 20°C (mg/L)	93 000
Stock solution concentration (mg/L)	80 000 (1 904 000 mg/L as COD)
pH of the stock solution	2.90

3.2.3 Inorganic compound: NaCl

In addition to organic compounds, an inorganic compound that is potentially inhibitory to the microorganism was studied. In particular, NaCl was tested to evaluate the inhibition effect on the nitrifying population. A client from Fox Engineering Associates, Inc. was concerned about chloride inhibition on ammonia removal at a nearby wastewater treatment plant. The reported chloride content was as high as 4 g/L, which is equivalent to 6.6 g/L of NaCl. Table 6 lists some of the physical and chemical properties of NaCl.

Table 6. Chemical and physical properties of NaCl

Chemical name	Sodium chloride
CAS #	7647-14-5
Manufacturer	Fisher Scientific
Physical state	Crystalline
Solubility in water at 20°C	360 g/L
Stock solution concentration	250 g/L
pH of the stock solution	7.08

3.3 Microorganisms

To increase the sensitivity of the biosensor developed, both aerobic and anaerobic cultures were tested to study the inhibitory effects of the wastewaters and toxic chemicals. Activated sludge with appreciable amount of nitrifiers and heterotrophs, and anaerobic microorganisms cultured from a master culture reactor (MCR) were used.

3.3.1 Activated sludge (AS)

3.3.1.1 Biomass characteristics

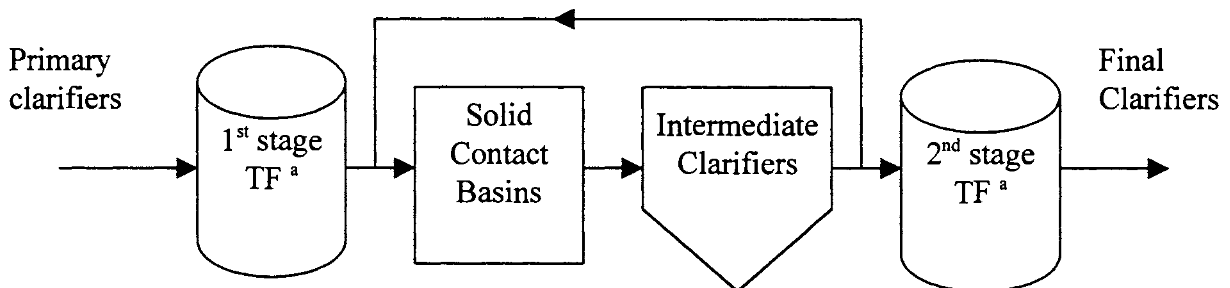
Activated sludge with substantial amount of nitrifiers and heterotrophs was collected from the solids contact basins at the Ames WPCF. It was stored in the laboratory refrigerator at 4°C for aerobic respirometric testing within 72 hours. Total and volatile suspended solids (TSS and VSS) concentrations were measured as an assessment of the active biomass

concentration. The average TSS, VSS, and % VSS of the biomass were 2984 mg/L, 2217 mg/L, and 74% respectively.

3.3.1.2 Plant characteristics

The Ames WPCF uses two-stage trickling filters with solids contact basins and intermediate clarifiers in their biological treatment system. The average wet-weather flow was about 12 MGD with both influent BOD and TSS of approximately 160 mg/L. The reported influent ammonia nitrogen was around 27 mg/L. Wastewater from the primary clarifiers flows to the first-stage trickling filters of 80 feet diameter towers filled with 26 feet depth of plastic media and then to the solids contact aeration basins. This phase can be described as a short-term activated sludge step (http://www.city.ames.ia.us/waterweb). The flow diagram of the trickling filters system is illustrated in Figure 2.

The flow continues to the 100 feet diameter intermediate clarifiers for the settling of finer solids. Most of the activated sludge from the intermediate clarifiers is recycled back to the solid contact basins, which returns viable organisms, improves treatment efficiency, and helps reducing the nuisance from odors and flies (Metcalf and Eddy, 1991). Additional ammonia reduction is achieved later in the second stage filter with the effluent flowing into the final clarifiers.



[a] TF = Trickling Filter

Figure 2. Flow diagram of the two-stage trickling filters system at Ames WPCF

3.3.2 Anaerobic granules

The inhibitory effects of the test samples were further evaluated with anaerobic inocula. In order to provide cultures that have identifiable and repeatable properties, a master culture reactor (MCR) was used. Figures 3 and 4 show the photo of the MCR and the schematic diagram of the MCR setup respectively. The culture reactor was a modification of the MCR described by Young and Tabak (1993), and was operated for nearly two months at 35°C in a constant temperature room. Instead of feeding once per day and mixing with a magnetic stir bar as described by Young and Tabak (1993), the reactor was fed three times per day with recirculation at an organic loading rate of 500 mg COD/L. Table 7 summarizes the operational parameters of the MCR. Steady state was reached after approximately 20 days of operation. The operating data of the MCR during the startup period is included in Appendix A.

The anaerobic seed sludge was obtained from an Upflow Anaerobic Sludge Blanket (UASB) reactor at Heileman's Brewery in LaCrosse, Wisconsin, which had been stored in the laboratory refrigerator at 4°C for up to a year. The seed granule was transferred into a 0.25 inches thick Plexiglas reactor with 2.5 inches diameter. Sufficient nutrient, buffer, and mineral (NBM) solution was added according to Table 8. To minimize oxygen contamination, sufficient nitrogen flushing of the solution (~10 to 15 minutes) and headspace (~5 minutes) were needed.

The ethanol acclimated granules were used in the anaerobic toxicity tests (ATA) to evaluate the inhibition effects imposed by the test sample. Ethanol was used as the base substrate because it is a convenient solvent for a large number of toxic organic chemicals as reported by Smith and McCarty (1989). In addition, the stoichiometry of its conversion to methane gas and intermediates are known (Smith and McCarty 1989). Ethanol is neutral in pH and preferable over acetate acid in the anaerobic batch procedure.

Table 7. Operating parameters of the Master Culture Reactor (MCR)

Operating temperature	Mesophilic (35°C)
Mode of operation	Semi-continuous with recirculation
Hydraulic Retention Time (HRT)	20 days
Organic Loading Rate (OLR)	500 mg COD/L-d
Reactor volume	4 L
Working volume	3 L
Granule volume	2 L
Feedstock composition	10 000 mg COD/L of ethanol + NBM solution
Feed/effluent flow rate	150 mL/d (~5% of the working volume)
Feed/effluent cycle	Every 8 hours
Recirculation cycle	Every 4 hours (1 minute mixing)

Figure 3. Photo of the Master Culture Reactor (MCR)

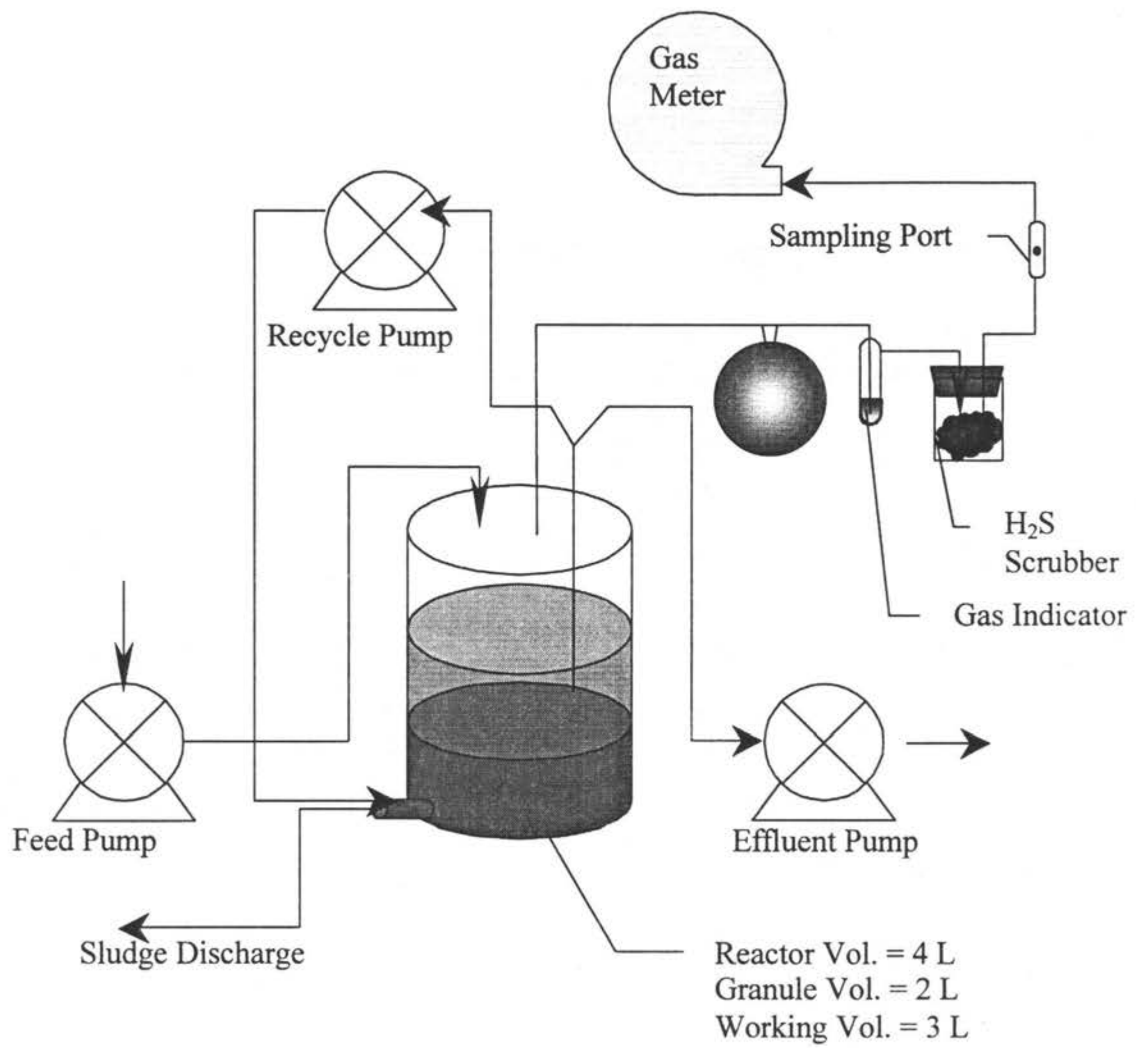


Figure 4. Schematic diagram of the Master Culture Reactor (MCR)

Table 8. Composition of Nutrient/Buffer/Mineral (NBM) medium[a]

Medium	Compound	Test Culture (mg/L)
Nutrients	KH_2PO_4	500
	Na_2SO_4	150[b]
	NH_4Cl	530
	Cysteine	100[c]
Buffer	$NaHCO_3$	6000
Minerals	$CaCl_2.2H_2O$	150
	$MgCl_2.6H_2O$	200
	$FeCl_2.4H_2O$	20
	$MnCl_2.4H_2O$	0.50
	H_3BO_3	0.25
	$ZnCl_2$	0.25
	$CuCl_2.2H_2O$	0.15
	$Na_2MoO_2.2H_2O$	0.05
	$CoCl_2.6H_2O$	2.50
	$NiCl_2.6H_2O$	0.25
	Na_2SeO_4	0.25

[a] Source: Young and Tabak (1993)
[b] 150 mg/L Na_2SO_4 in the NBM solution provides 5 mg SO_4^{2-}/1000 mg COD or 0.5% of the COD load
[c] 100 mg/L cysteine in the NBM solution provides 5 mg cysteine/1000 mg COD or 0.5% of the COD load

3.4 Experimental Design

3.4.1 Aerobic inhibition test

The respirometric testing was divided into two phases. The first phase involved the injection of sample into an NH_4Cl saturated biomass obtained from the Ames WPCF solids contact basins. In the NH_4Cl saturated condition, a maximum background autotrophic respiration rate (nitrification) was achieved. Effect on the nitrification rate was studied in this phase by comparing the oxygen uptake before and after the injection of sample. For the second phase, no NH_4Cl was injected. Inhibition on the exogenous respiration of the biomass was studied in this section.

A total of six to seven levels of the toxicants (or potentially toxic wastewaters) were studied. The injected volume varied from sample to sample depending on the inhibitory nature and concentration of the sample. Both carbonaceous and nitrogenous phases of the testing were studied for each test sample.

3.4.2 Anaerobic inhibition test

3.4.2.1 General

Seed obtained from the MCR was used for the ATA test. Both sample and base substrate (1000 mg COD/L ethanol) were injected to the test bottle and an inhibition was indicated by a decrease in total biogas produced. Seed blanks and controls (without toxicant) were included in the ATA test in addition to the sample bottles. Seed blanks provided a basis for correcting the background gas production while the control was used for comparing the effects with and without the addition of test sample. Table 9 lists the compositions of blank, control, and sample bottles in the ATA test. All samples were measured in duplicate for quality control.

Table 9. Composition of blank, control, and sample bottles in the ATA test

Bottle ID	Composition
Blank, B	Seed + NBM + Anaerobic H_2O
Control, E	Seed + NBM + Anaerobic H_2O + Ethanol
Sample without ethanol, S	Seed + NBM + Anaerobic H_2O + Sample
Sample with ethanol, SE	Seed + NBM + Anaerobic H_2O + Ethanol + Sample

3.4.2.2 Screening test

A screening test was performed prior to the actual inhibition testing of the potentially inhibitory wastewater samples. This was done to give a general idea of the inhibition level of the samples tested. In the screening test, only one sample concentration (typically 1000 mg/L as COD) was injected, and the relative activity of each sample was determined. Relative activity (RA) is the ratio of the cumulative gas produced by the test bottle to the cumulative gas produced by the control bottle at a selected reaction time (Equation 10). The same amounts of COD equivalents were added to the sample and control bottles. An inhibition response was indicated by a RA value of less than 100%.

3.4.2.3 Inhibition test

Four different toxicant levels were tested in the inhibition test. The concentrations selected depended on the inhibitory nature of the samples. Generally, lower injection concentrations were chosen for samples with higher toxicity. For instance, 30, 90, 270, and 810 mg COD/L of the furfural samples were selected while 500, 1000, 1500, and 2000 mg/L of the Genencor sample were used. The RA values at a 6-hour incubation time were plotted against sample concentrations to determine the sample concentration causing 50% inhibition.

3.5 Aerobic Respirometric Test

A modification of the respirometric technique developed by Ellis *et al.* (1996) was adopted for assessing the inhibition effects of the test samples. A photo of the respirometers

is shown in Figure 5. Descriptions of the equipment, testing procedure, and data analysis method are discussed in the following sections.

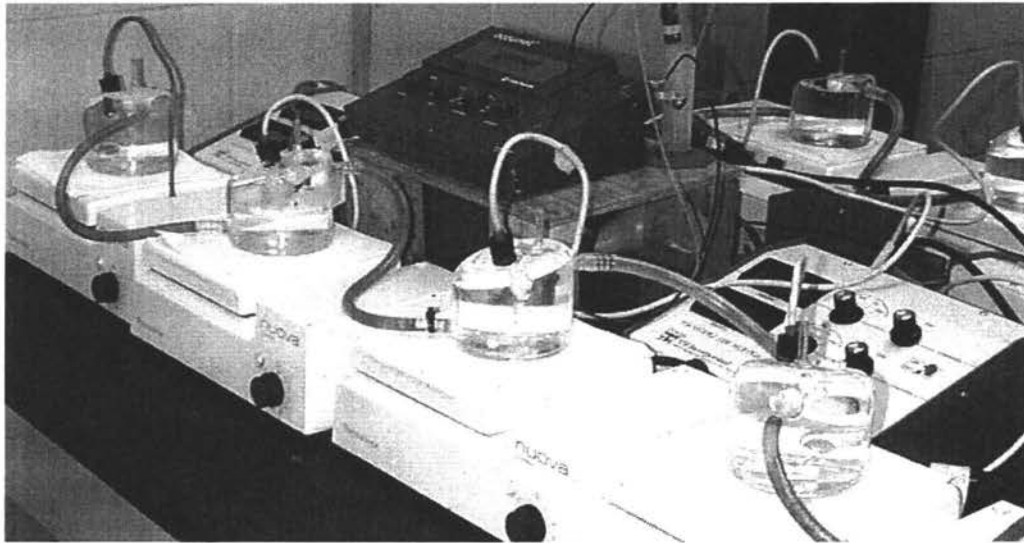


Figure 5. Photo of the aerobic respirometers

3.5.1 Equipment descriptions

The batch vessel used in the respirometric test has an internal volume of approximately 250 mL (Tudor Glass Co., Belvedere, SC). The ports found on the slanted top of the vessel were used for the insertion of polarographic oxygen probe (YSI Model 5331, Yellow Springs Instrument Co., Inc., OH). The vessel was water-jacketed and maintained at 25°C throughout the test. Both oxygen gas and test samples were injected through the small diameter tubulation on top of the vessel as depicted in Figure 6. The reactor content was continuously stirred at a consistent speed on a stir plate (Thermolyne Nuova II Stirrer, Model S18525, Dubuque, Iowa) with a magnetic bar throughout the entire testing procedure.

Dissolved oxygen (DO) was monitored by the DO probe connected to the biological oxygen monitor (YSI model 5300 biological oxygen monitor, YSI inc., OH) that was interfaced with a personal computer (PC). The PC has a data acquisition board (Computer

Board, Middlebrow, MA) installed, and the DO data were recorded at a rate of 10 Hz. In addition, the data acquisition was facilitated by an integrated data acquisition software Labtech Notebook LE by Laboratory Technologies Corporation in Wilmington, Mass.

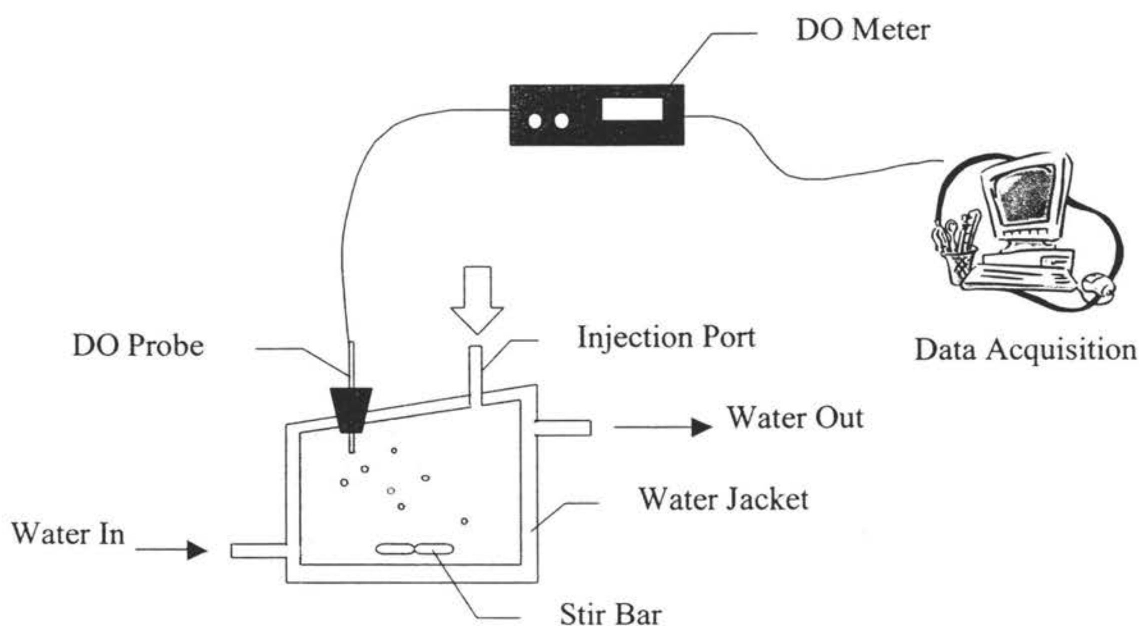


Figure 6. Schematic diagram of the aerobic respirometers

3.5.2 Preparations

3.5.2.1 Equipment preparation

Prior to the testing (24 hrs), the oxygen probe was cleaned and replaced with a new membrane (YSI 5776 Oxygen probe kit, YSI, Inc., OH). Calibration was done by inserting the probe into the vessel filled with tap water. Water was stirred continuously to saturate

with the atmospheric oxygen. Once the DO readings were stabilized, the DO meter was set to 100% saturation.

3.5.2.2 Biomass preparation

The activated sludge previously stored in a 4°C refrigerator was aerated for at least an hour prior to the testing to remove any residual substrates. During the aeration, the temperature was brought up to the room temperature. Phosphate buffer was added to the biomass to maintain an optimum pH range of 6.5 - 7.5. Approximately 4 - 6 mL of buffer solution was injected per 250 mL of biomass. Two types of buffer solution were used depending on the purpose of the test. Buffer II is used when nitrification is intended. The buffer solutions were prepared according to section 4-67 in the Standard Methods for the Examination of Wastewater (1995). The composition of the buffer solutions are listed in Table 10.

Table 10. Composition of the phosphate buffer solutions used in the respirometric test

Phosphate buffer	pH at 25°C	Composition (for 1 liter solution)
I	6.86	3.4 g KH_2PO_4 + 6.7 g Na_2HPO_4
II	7.42	1.2 g KH_2PO_4 + 4.3 g Na_2HPO_4

3.5.3 Testing procedure

The following summarizes the testing procedure in general. The section marked by * applied only to the nitrification inhibition test.

1. The tap water was removed from the respirometer vessel after the DO meter was calibrated. Newly obtained biomass was injected into the vessels. In order to maintain homogeneity among vessels, the biomass was distributed evenly from vessel to vessel.

2. The port was sealed with a rubber stopper and the biomass was oxygenated until the DO concentration reached 20 mg/L. The oxygenation procedure took about 10 seconds depending on the flowrate of the oxygen. During the oxygenation, a capillary tubing connected to the oxygen cylinder was inserted into the small diameter tube as shown in Figure 6.

3. When the oxygenation was completed, additional biomass was injected to the vessel until the injection port was filled to the top. Air bubbles were removed by tilting the vessel repeatedly.

4. *Ammonium chloride solution (5 000 mg/L as NH_4Cl) was added to the reaction chamber once an endogenous respiration rate was obtained (represented by a constant DO slope). The volume of NH_4Cl injected was determined prior to the test to achieve a maximum autotrophic respiration rate.

5. Test sample was injected into the vessel using a needle syringe once a straight and stable slope was obtained.

6. The test was terminated once a constant response slope was obtained. It was important to note that the DO should not go down to 2 mg/L during the test to prevent oxygen deprivation. DO data were later retrieved from the computer with a time interval of 4 seconds.

3.5.4 Determination of NH_4Cl volume

The amount of ammonium chloride required to achieve a maximum background nitrification rate differed from biomass to biomass. Therefore, a screening test was conducted on the test biomass prior to the actual inhibition test. An ammonium chloride solution of 5000 mg/L as NH_4Cl was used. The NH_4Cl solution was injected to the respirometer containing biomass at a rate of 0.5 mL per 3-5 minutes until a maximum slope was obtained. Figure 7 shows the changes in oxygen uptake rate (OUR) over time with five

injections of 0.5 mL NH_4Cl solution. The slope increased with increasing NH_4Cl volume. A constant slope was obtained after the third injection indicated that the solution was saturated with NH_4Cl, and a maximum background respiration rate was reached.

Next, the specific normalized oxygen uptake rate (NOURc, see section 3.7.1.1) was plotted against NH_4Cl volume as shown in Figure 8. It was found that a concentration of 30 to 40 mg/L as NH_4Cl (approximately 8 to 11 mg/L as N) was sufficient to achieve a maximum autotrophic respiration rate for the Ames WPCF biomass. The ammonia concentration had to be high enough to achieve maximum autotrophic respiration but low enough to prevent ammonia inhibition as reported by Anthonisen *et al.* (1976). The half saturation constant, K_{NH}, was generally smaller than 0.5 mg/L as ammonia-N using activated sludge from the authors' experience.

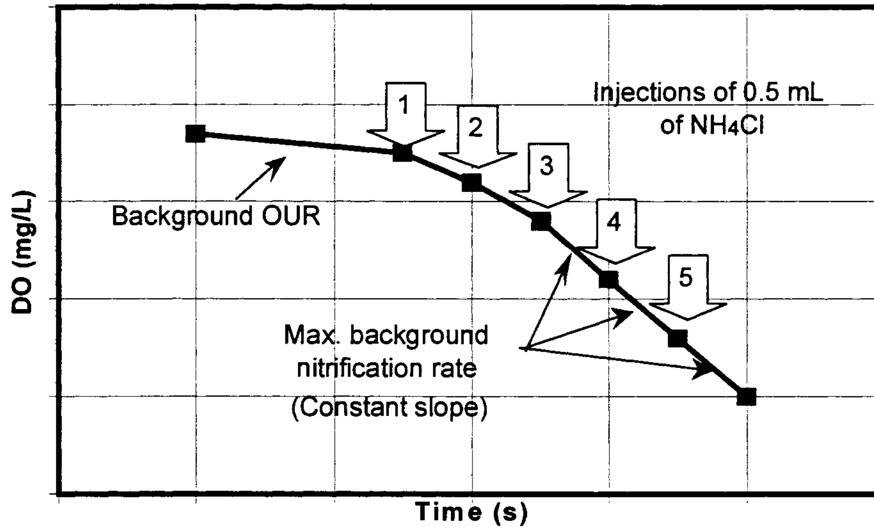


Figure 7. Nitrification rates at different injections of NH_4Cl

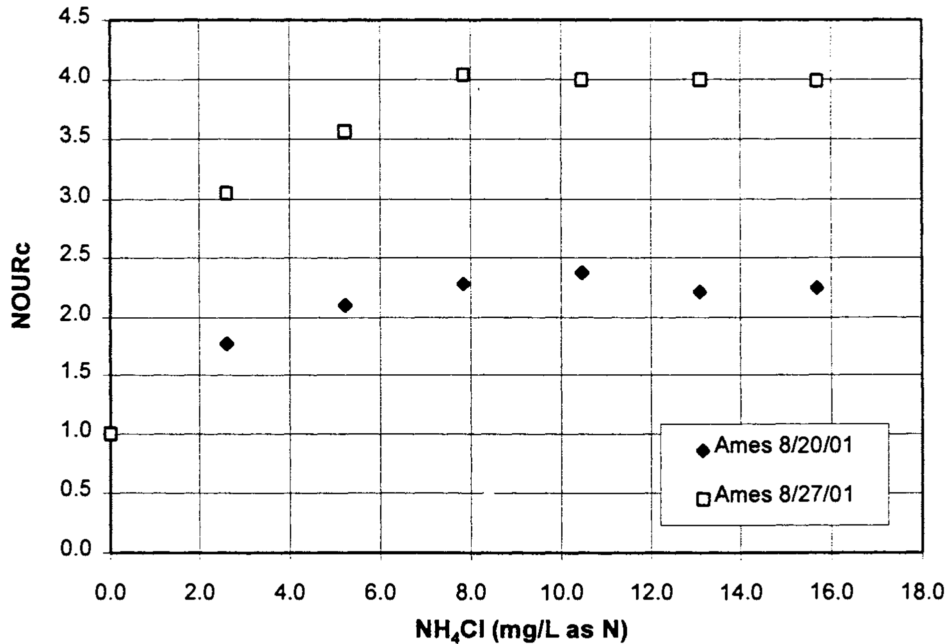


Figure 8. NOURc versus NH_4Cl concentration

3.6 Anaerobic Toxicity Assay (ATA)

The anaerobic toxicity assay (ATA) was used to evaluate the inhibitory effect of toxic compounds (or inhibitory wastewaters) on anaerobic cultures. It was conducted in the presence of excess substrate such as acetate or ethanol to achieve a non-substrate limited condition. Under this condition, a maximum substrate utilization rate was achieved in the absence of inhibitory constituents. Consequently, if the sample tested was inhibitory to the inocula, a reduced initial rate of the gas production resulted (Speece, 1996).

It is important to note that the production rate instead of the total volume of the biogas was critical in the toxicity assay. With sufficient acclimation time, it was possible for the biomass with an injected inhibitory sample to produce an equal amount of biogas as the one without the inhibitory sample. Therefore, the initial production rate of biogas was determined.

The ATA technique employed here was a modification of the ATA described by Young and Tabak (1993) and the Specific Methane Activity (SMA) by Rinzema *et al.* (1988). The modified ATA test was run for 3-5 days at mesophilic condition. Seed acclimated with ethanol was obtained from the MCR and ethanol was used as the base substrate. Prior to the sample injections, test bottles that contained seed inocula, NBM solution, and anaerobic water were incubated approximately 24 hours in advance at 35°C to stabilize the transferred seed.

The following parts describe the experimental design, equipment descriptions, testing procedures, gas measurements, and data analysis for the modified ATA test.

3.6.1 Equipment description

250 mL serum bottles and rubber serum caps were used in the ATA tests. The liquid volume of each bottle was maintained at 150 mL to allow adequate headspace. The test bottles were incubated in an incubator shaker (Controlled Environment Incubator Shaker, Series 25, New Brunswick Scientific CO. Inc., Edison, N.J., U.S.A.) at 35°C and 150 rpm.

3.6.2 Testing procedures

Following are the procedures used for the modified ATA test. A seed volume of 20 to 30 mL per serum bottle was used as it was determined to be the optimum range from the previous study.

Day 1

1. Anaerobic seed granules were obtained from the MCR and transferred to the serum bottles with pipette. The seed granules were covered during the transferring process to minimize oxygen contamination.

2. The NBM stock solution was added to the serum bottle to achieve a final concentration listed in Table 8 (same as the MCR) followed the addition of

anaerobic water. The anaerobic water was prepared by flushing nanopure water with nitrogen gas to lessen the oxygen contamination.

3. Once seeded, NBM solution, and anaerobic water were added, the liquid was flushed with nitrogen gas for approximately 5 minutes followed by the headspace flushing of about 30 seconds.

4. Then, the serum bottle was sealed with the rubber serum cap followed by the addition of 0.5 mL of 0.25 M Na_2S for reducing environment as recommended in the SMA test.

5. All the test bottles were incubated at 35°C in an incubator shaker for 24 hours at 150 rpm.

Day 2

1. After 24 hours of incubation, the desired volume of the sample was injected to the serum bottle. The pH of the solution was adjusted to approximately 7.0-8.0 with 0.1 N NaOH or H_2SO_4.

2. The headspace was flushed with N_2 for about 30 seconds. A cable tie was applied and tightened around the bottle's neck on the serum cap to minimize gas leakage.

3. Finally, the bottles were put inside the incubator shaker for an hour. The pressure in the headspace was corrected by withdrawing the extra gas out an hour after the incubation. This was done to correct the pressure buildup of the N_2 as a result of over flushing and expansion of the gas volume under mesophilic conditions. The reaction time was counted after the pressure correction.

3.6.3 Gas measurements

3.6.3.1 Total gas

The total gas production of each sample was measured manually every 3-5 hours after the injection of sample. Lubricated glass syringes (5 - 50 mL) equipped with 20-gauge needles were used. Prior to each measurement, the syringe was lubricated with nanopure water to minimize the friction between the interacting surfaces. All the readings were taken at the incubation temperature (35°C) and corrected for the background gas production and STP condition (Equation 3). The syringe was held horizontally and measurements were made by allowing the syringe plunger to move. The plunger was twirled gently to equilibrate between the bottle and atmospheric pressures (Owen, 1978). The gas in the syringe was removed for wasting after each measurement. Cumulative gas production was plotted against reaction time at different concentrations of the toxicant.

Equation 3. Net total gas produced at STP condition

$$V = (V_S - V_B)\left(\frac{273}{273 + 35}\right)\left(\frac{29.92}{P}\right)$$

Where

V = Net gas produced at STP
V_S = Gas produced by sample at 35°C
V_B = Gas produced by blank at 35°C
P = Atmospheric pressure (inches Hg)

3.6.3.2 Methane contents

The biogas composition for each serum bottle was measured every 3 - 5 hours with a gas chromatograph (GOW-MAC Instrument Co., Model 69-350 Thermal Conductivity Gas Chromatography, Bridgewater, N.J.). The methane content of each sample was plotted

against reaction time in addition to the total gas production to cross check the inhibition response. The sample size for each injection was 0.5 mL. Standard gas containing 30% N_2, 30% CH_4, and 15% CO_2 was used. The settings of the GC are summarized in Table 11. Biogas composition was calculated by comparing the methane peak of the sample with the methane peak of the standard gas. Equation 4 shows the calculation of the CH_4 content as a percentage.

Table 11. Settings of the gas chromatography (GC)

Injection port temperature	160°C
Detector temperature	200°C
Column temperature	70°C
Bridge current	200 mV
Carrier gas (Helium) flowrate at GC outlet	60 mL/min

Equation 4. CH_4 content

$$\% CH_4 = \frac{h}{H} \cdot (\% CH_4 \text{ of standard gas})$$

Where

h = Methane peak height of sample

H = Methane peak height of standard gas

3.7 Data Analysis

3.7.1 Aerobic respirometric test

3.7.1.1 Specific normalized oxygen uptake rate ($NOUR_c$)

Determination of NOUR. Normalized oxygen uptake rate (NOUR) is the ratio of the OUR of tested biomass immediately (~10 seconds) after the injection of sample to the background OUR before the injection of sample as shown in Equation 5 (Ellis *et al.*, 1996).

The purpose of determining NOUR instead of OUR was to account for the differences in biomass concentrations between samples. In other words, biomass response expressed in NOUR was independent of the variation in biomass concentration. The inhibition response was measured immediately after the injection of sample to give a true account of short-term inhibition response. When sufficient dissolved oxygen and reaction time were allowed for complete substrate utilization, endogenous respiration rate was followed. In addition, attention must be paid to differentiate the responses due to dilution and to inhibition (or substrate utilization) to avoid misinterpretation. Figure 9 illustrates the different responses.

In this study, carbonaceous and nitrogenous NOURs were measured. Carbonaceous NOUR is the ratio of the OUR after sample injection to the endogenous respiration rate of biomass (heterotrophs and autotrophs). Nitrogenous NOUR is the ratio of the OUR after sample injection to the respiration rate of biomass when a maximum autotrophic respiration rate was achieved.

Equation 5. Normalized oxygen uptake rate (NOUR)

$$\text{NOUR} = \frac{\text{OUR}_{\text{after injection of sample}}}{\text{OUR}_{\text{before injection of sample}}}$$

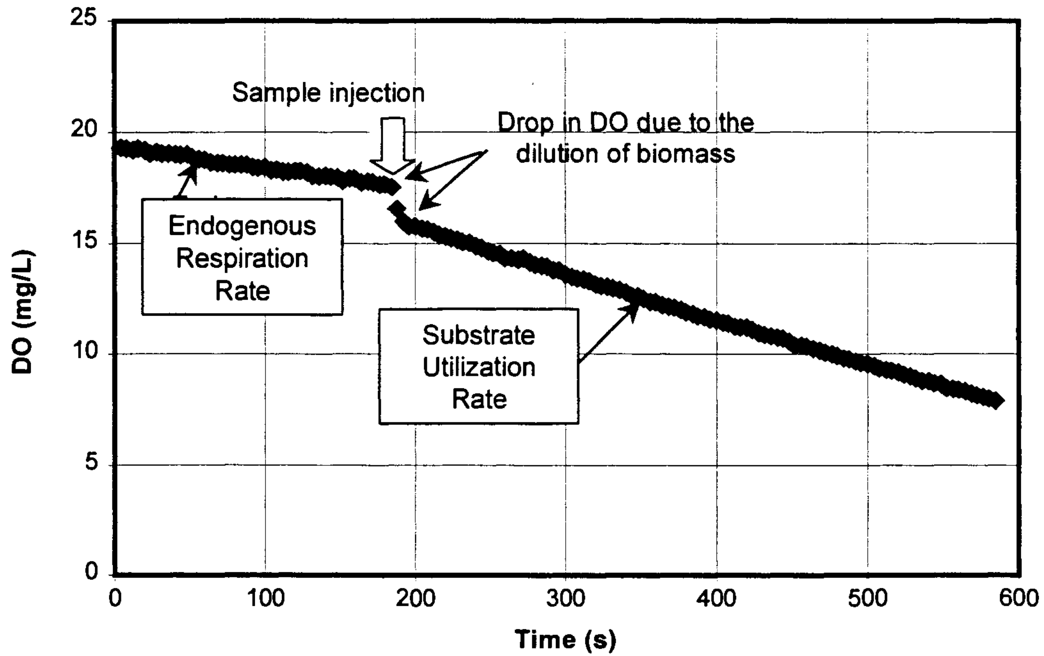


Figure 9. Dilution response of biomass due to sample injection (in a respirometer)

Determination of NOURc. Although NOUR accounts for the variation in biomass concentrations, it does not account for the dilution effect of biomass due to sample injection. To take dilution into considerations, specific normalized oxygen uptake rate (NOURc) was determined (Equation 6).

Equation 6. Specific normalized oxygen uptake rate (NOURc)

$$\mathrm{NOURc} = \mathrm{NOUR} \cdot f = \mathrm{NOUR} \cdot \left(1 + \frac{\mathrm{Vs}}{(250 - \mathrm{Vs})}\right)$$

Specific NOUR was calculated as the ratio of specific OUR after the injection of sample to the specific endogenous respiration rate. The relation was derived as follows.

$$
\begin{aligned}
NOURc &= \frac{\left(\dfrac{OURs}{Xb \cdot Vb'}\right)}{\left(\dfrac{OURe}{Xb \cdot Vb}\right)} = \frac{\left(\dfrac{mg/L.s}{mg/L \cdot mL}\right)}{\left(\dfrac{mg/L.s}{mg/L \cdot mL}\right)} \\
&= \left(\frac{OURs}{OURe}\right)\left(\frac{Vb}{Vb'}\right) \\
&= NOUR \cdot \frac{250}{(250 - Vs)} \\
&= NOUR \cdot \left(1 + \frac{Vs}{(250 - Vs)}\right)
\end{aligned}
$$

Where OURe = Endogenous respiration rate (mg/L.s)

OURs = OUR after the injection of sample (mg/L.s)

NOUR = Normalized oxygen uptake rate

NOURc = Specific normalized oxygen uptake rate

Xb = Concentration of active biomass, MLVSS (mg/L)

Vs = Volume of the injected sample (mL)

Vb = Volume of the biomass before the injection of sample (250 mL)

Vb' = Volume of the biomass after the injection of sample (mL)

f = Correction factor for the dilution

Significance of NOURc. When a non-inhibitory biodegradable sample was injected to a respirometer filled with biomass, the oxygen uptake rate was expected to be greater than the background respiration of the microorganisms due to substrate utilization. That is, the value of NOURc would be greater than 1. Therefore, an inhibition response can be deduced when the NOURc was less than 1. This is true when the sample concentration injected was high enough to affect the endogenous respiration rate of the microorganisms. However, there were cases when the sample itself did not, or the sample concentration was not high enough to, affect the endogenous respiration rate of the microorganisms. In this case, the NOURc value was not less than 1, and it might not necessarily mean that the sample was non-

inhibitory. To determine the degree of inhibition in a more accurate way, a dose-response curve (NOURc versus sample concentration) was plotted, and inhibition parameters were calculated using models.

3.7.1.2 Determination of inhibition parameters

Inhibition models. The inhibitory responses of the aerobic cultures tested were described using Models I and II as illustrated in Equations 7 and 8. Model I describes the inhibition effect on both inhibitor-degrading and non-degrading portions of the biomass (Ellis *et al.*, 1996). The biodegrading portion has the same form as the Andrews expression (Grady *et al.*, 1999) while the non-degrading portion depends on the inhibitor types (Volskay *et al.*, 1988). For instance, the left term of Model I is the biodegradable portion of the biomass, while the right term represents the noncompetitive portion.

In addition to Model I, another inhibition model was used as shown in Equation 7. This is a noncompetitive inhibition model without the biodegradation term. A modification was made by including an "n" term in the model. It was found in this study that the inclusion of the "n" term improved the model fit for furfural. To check the statistical significance of the "n" term, more samples will have to be tested in the future.

Equation 7. Inhibition model I (Andrews and noncompetitive model)

$$\mathrm{NOURc} = \left(\frac{\mathrm{N\hat{O}URc} \cdot \mathrm{S}}{\mathrm{K_S} + \mathrm{S} + \dfrac{\mathrm{S}^2}{\mathrm{K_I}}} \right) + \left(\frac{1}{1 + \dfrac{\mathrm{S}}{\mathrm{L_I}}} \right)$$

Where

$$\frac{\mathrm{NOURc}^*}{\mathrm{N\hat{O}URc}} = \frac{1}{2\left(\dfrac{\mathrm{K_s}}{\mathrm{K_I}}\right)^{0.5} + 1}$$

$\hat{NOURc}$ = Theoretical maximum NOURc of inhibitor-degrading population, analogous to $\hat{\mu}$ in the Andrews equation, dimensionless

NOURc* = Observable maximum NOURc of inhibitor-degrading population, analogous to μ^* in the Andrews equation, mg/L as COD

S = Substrate (inhibitor) concentration, mg/L as COD

K_S = Half saturation concentration, mg/L as COD

K_I = Inhibition coefficient for inhibitor on the inhibitor-degrading population, mg/L as COD

Equation 8. Inhibition model II (modified noncompetitive model)

$$NOURc = \frac{1}{\left(1 + \frac{S^n}{L_I}\right)}$$

Where

L_I = Inhibition coefficient for inhibitor on the total biomass, mg/L as COD

n = Order of inhibition ($n > 0$), dimensionless

Estimation of the inhibition parameters. Experimentally determined NOURc values were plotted against sample concentration and fitted with the non-linear regression models as described above. The fitting process was facilitated with the SOLVER program in Microsoft Excel based on the least squares method. NOURc*, the maximum observable NOURc, was determined from the dose-response curve as shown in Figure 10. Three parameters, K_S, K_I, and L_I, were fitted with SOLVER when Model I was used and two (L_I and n) were fitted with SOLVER when Model II was used. The spreadsheets used for the model fitting are included in Appendix B. The relationship between the experimentally

determined data and model fit data was calculated in correlation coefficient (r^2) using CORREL function in Microsoft Excel.

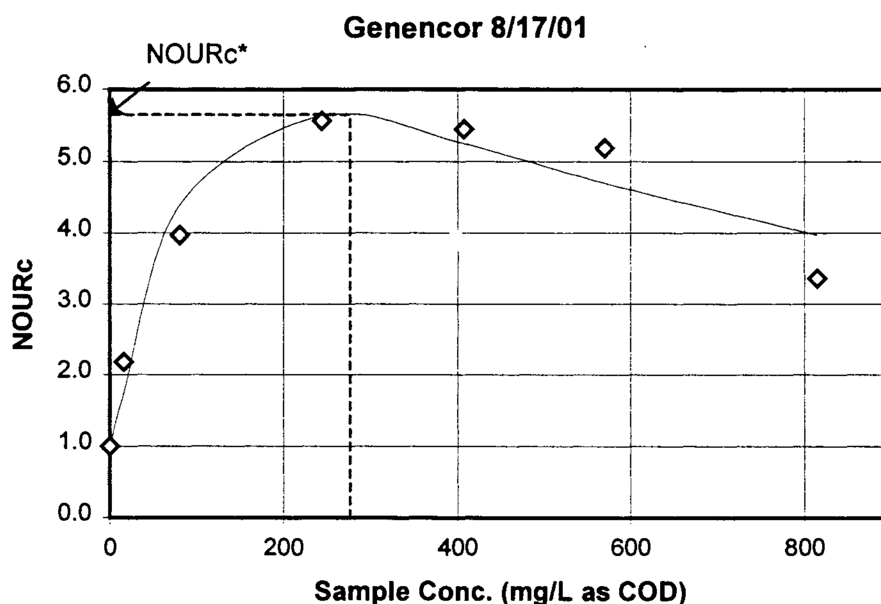


Figure 10. Determination of NOURc* from experimental data
(Experimental data: ◊; Model fitted line: —)

Significance of the inhibition parameters. To determine the degree of inhibition between samples, several parameters were determined: $p(K_S/K_I)$, L_I, n, IC_{50}, and $I\hat{C}_{50}$. The ratio K_S/K_I, (analogous to the K_S/K_I in Andrews equation) was used to determine the degree of inhibition on the inhibitor-degrading population. The larger the K_S/K_I value, the smaller the NOURc* value relative to the $N\hat{O}URc$ value, and hence greater was the degree of inhibition. In this study, the negative logarithmic value of K_S/K_I was calculated instead of K_S/K_I as the log value was more convenient for comparison especially when the differences between samples were large. The inhibition effect was lower for higher $p(K_S/K_I)$ value. L_I

and n were used to describe the degree of inhibition on the total biomass. Greater inhibition was indicated by lower L_I and higher n values.

Once K_S, K_I, L_I, and n were fitted, IC_{50} could be calculated using the model described above. Greater inhibition was indicated by lower IC_{50}. For instance, chlorobenzene is more toxic than dibromomethane because the reported IC_{50} values were 155 mg/L and 1572 mg/L respectively with activated sludge (Sun *et al.*, 1994).

In situations where the NOURc reached a maximum level and then reduced with increasing concentration, $I\hat{C}_{50}$ was determined in addition to IC_{50}. IC_{50} is the inhibitor concentration that reduces 50% of the microorganisms' endogenous respiration rate, while $I\hat{C}_{50}$ is the inhibitor concentration that reduces 50% of the maximum exogenous respiration rate. In other words,

IC_{50} = Inhibitor concentration causing 50% reduction in NOURc, and
$I\hat{C}_{50}$ = Inhibitor concentration causing 50% reduction in NÔURc.

$I\hat{C}_{50}$ was a more reasonable and sensitive parameter than IC_{50} for the evaluation of the degree of inhibition as it measured the effect of the inhibitor on the maximum possible performances that can be achieved. Both IC_{50} and $I\hat{C}_{50}$ were expressed in logarithmic value for the reasons stated before. Table 12 summarizes the types and applications of the inhibition parameters.

Table 12. Types of inhibition parameters

Inhibition parameter	Degree of inhibition increases as the value	Application
$p(K_S/K_I) = -\log(K_S/K_I)$	Decreases	Inhibitor-degrading population
$\mathrm{Log}(IC_{50})$	Decreases	Total biomass
$\mathrm{Log}(I\hat{C}_{50})$	Decreases	Total biomass
L_I	Decreases	Total biomass
n	Increases	Total biomass

3.7.2 Anaerobic toxicity assay test

Plots of total gas and CH_4 content alone do not quantify the degree of inhibition. In addition, the "relative activity " described by Young and Tabak (1993) was calculated as shown in Equation 9. Relative activity (RA) compares the cumulative gas production of the test samples with the control. It serves as a meaningful parameter for assessing the impact of inhibitory substances (or toxicants). To study the response of the anaerobic cultures, RA values were plotted against the sample concentration. RA at a 24-hour incubation was used by Young and Tabak (1993). In this study, RA at 6 or 12-hour incubation time was used to determine the inhibition effect at earliest possible stage of incubation. An inhibitory sample was indicated by a RA value of less than 100%. From the RA plot, the concentration causing 50% RA (IC_{50}) was determined.

Equation 9. Relative activity (RA)

$$RA\,(\%) = \frac{Vs,t}{Vc,t} \cdot 100\%$$

Where

Vs,t = Cumulative gas production in sample bottle at time t

Vc,t = Cumulative gas production in control bottle at time t

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Genencor

4.1.1.1 Wastewater characteristics

The variations in flow, pH, total organic carbon (TOC), 5-day carbonaceous biological chemical oxygen demand ($CBOD_5$), TSS, TKN, and NH_3-N from December 19, 1999 to September 17, 2001 are illustrated in Figures 11 to 15. Table 13 summarizes the average values for each of the sample characteristics parameters.

Table 13. Average characteristics of Genencor wastewater (12/19/99 – 9/17/01)[a]

Parameters	Average value ± standard deviation	Range
Flow (MGD)	0.53 ± 0.12	0.15 – 1.09
pH	10.2 ± 0.825	7.7 – 12.7
TSS (mg/L)	901 ± 922	42 – 6990
TOC (mg/L)	1947 ± 614	208 – 5000
$CBOD_5$ (mg/L)	3124 ± 987	431 – 8050
TKN (mg/L)	472 ± 160	13.8 – 1264
NH_3-N (mg/L)	135 ± 63.3	2.47 – 732

[a] Data obtained from the Cedar Rapids WPCF

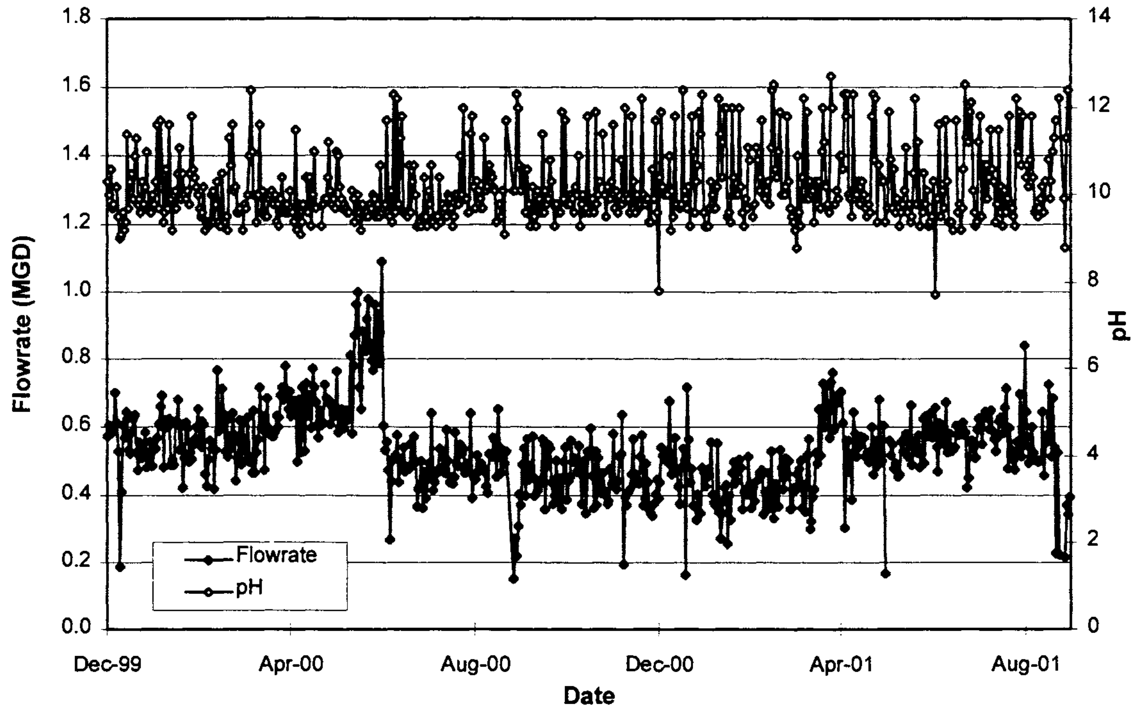


Figure 11. Daily flow and pH of Genencor wastewater[a]

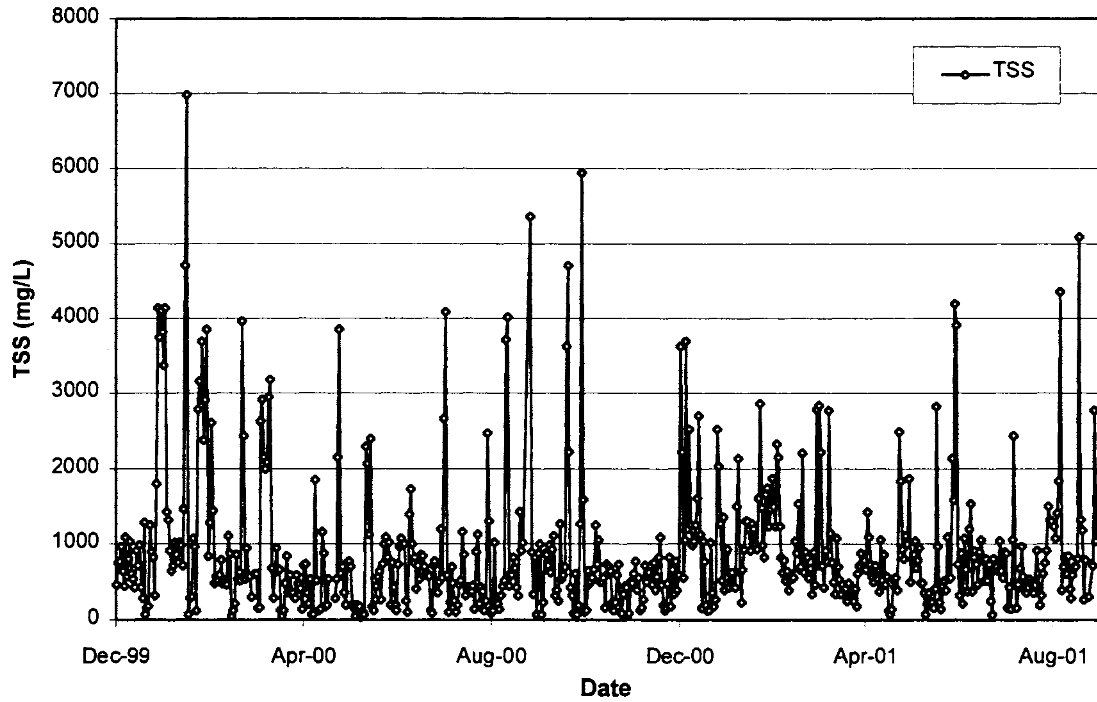


Figure 12. Daily TSS content of Genencor wastewater[a]

[a] Data provided by Cedar Rapids WPCF personnel

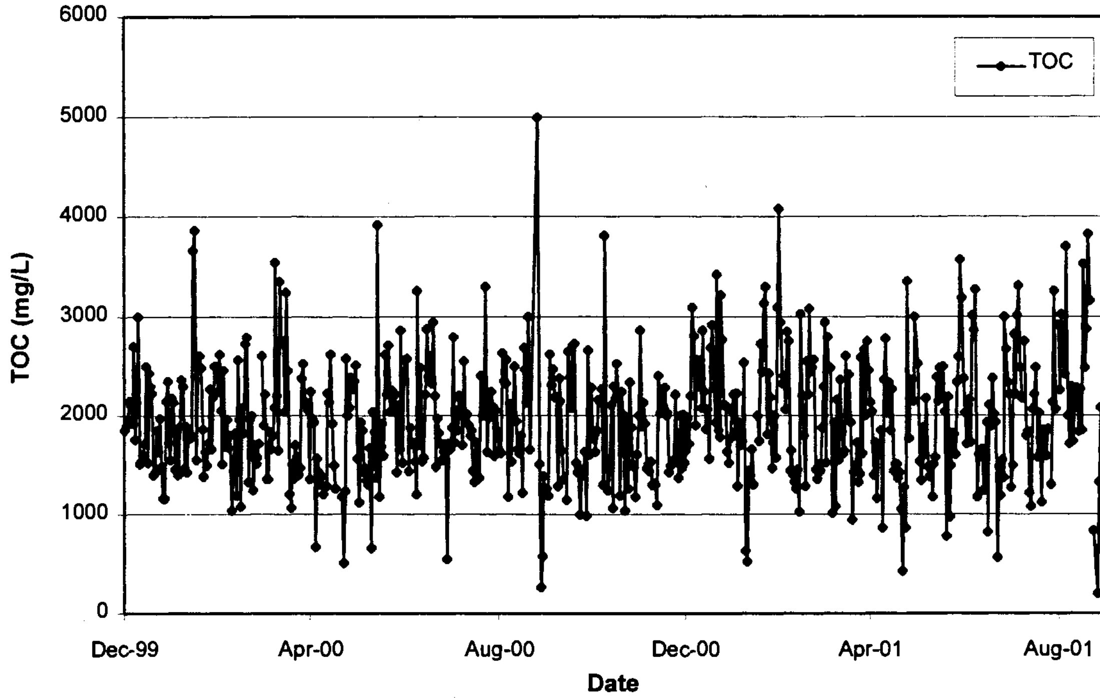


Figure 13. Daily TOC content of Genencor wastewater[a]

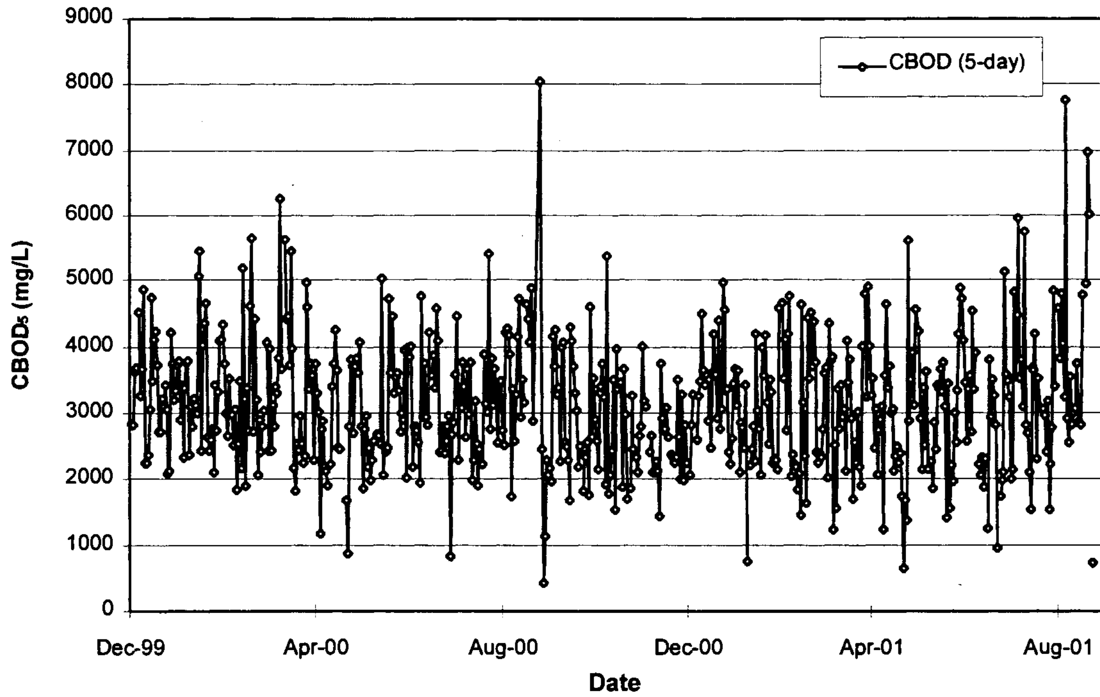


Figure 14. Daily $CBOD_5$ content of Genencor wastewater[a]

[a] Data provided by Cedar Rapids WPCF personnel

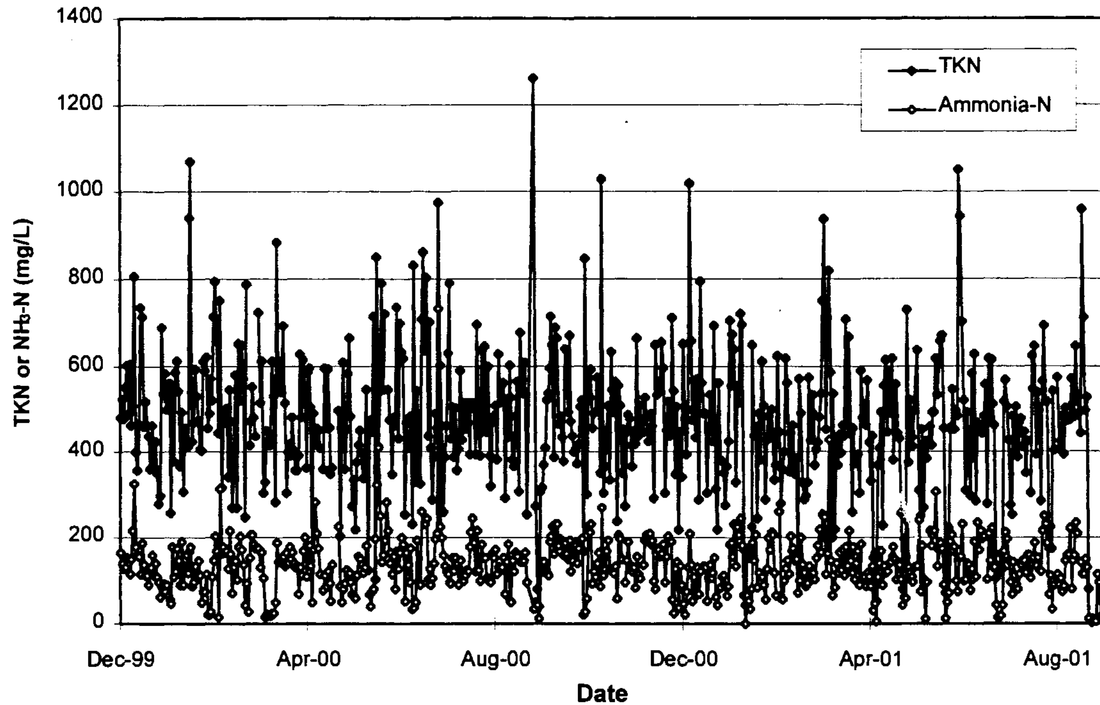


Figure 15. Daily TKN and Ammonia-N contents of Genencor wastewater[a]

[a] Data provided by Cedar Rapids WPCF personnel

4.1.1.2 Respirometric results

Genencor wastewater samples were investigated with the respirometric technique developed. The extensive study was conducted over a period of one year during which the characteristics of the wastewater showed significant variation. Table 14 lists the characteristics of the selected samples.

The NOURc response curves are illustrated in Figure 16. Both experimentally determined and model fit data are shown in the figure. The experimentally determined NOURc values were fitted using Model I. Inhibition parameters and correlation coefficients between the experimental data and model fit data were calculated (Table 15). From these results, it was observed that the experimental NOURc values were explained well by Model I. The average r^2 value was 0.981 ± 0.012 (standard deviation) for carbon oxidation, and 0.982 ± 0.015 for nitrification. Several observations can be deduced from the NOURc plots.

First, none of the experimentally determined NOURc values dropped below 1.0 at a maximum injection volume of 50 mL (20% of the respirometer vessel). Second, no inhibition effect was observed (as shown by the dashed lines in Figure 16) at actual concentrations of Genencor wastewater samples at the treatment plant (lowest dilution = 40 times). This suggested that the Genencor wastewater samples tested could be degraded at the Cedar Rapids WPCF in the carbonaceous and nitrogenous BOD removal systems.

Table 14. Characteristics of selected Genencor wastewater samples

Sample	pH	COD (mg/L)	$CBOD_5$ (mg/L)	$CBOD_5$/COD Ratio	TKN (mg/L)	NH_3-N (mg/L)	Sulfate (mg/L)	TSS (mg/L)
02/22/01	10.8	4072	2740	0.67	398	98.4	403.6	1220
03/13/01	10.2	4966	3770	0.76	534	117.0	297.6	552
04/18/01	10.2	5671	4010	0.71	422	109.0	129.2	788
05/11/01	11.9	1493	1310	0.87	196	61.4	327.6	2490
06/11/01	8.3	2783	1960	0.70	457	206.0	1224.0	1090
07/08/01	6.6	5443	3270	0.60	510	131.0	291.6	736
08/14/01	12.1	2686	2220	0.83	226	113.0	248.8	900
08/17/01	9.7	8014	3410	0.43	403	89.4	169.2	1300

Table 15. Inhibition parameters of selected Genencor wastewater samples

A. Carbon Oxidation

Sample	$p(Ks/K_I)$	$\hat{N}OURc$	$\log(I\hat{C}_{50}{}^{a})$	r^2
02/22/01	16.80	4.20	18.00	0.962
03/13/01	2.40	5.41	4.00	0.981
04/18/01	2.78	4.98	4.18	0.970
05/11/01	1.57	5.97	2.87	0.991
06/11/01	5.10	3.94	6.00	0.984
07/08/01	3.02	4.23	4.02	0.997
08/14/01	0.09	10.82	2.34	0.986
08/17/01	0.66	10.85	2.84	0.973

B. Nitrification

Sample	$p(Ks/K_I)$	$\hat{N}OURc$	$\log(I\hat{C}_{50}{}^{a})$	r^2
02/22/01	2.15	2.81	3.54	0.953
03/13/01	8.26	2.40	10.00	0.991
04/18/01	1.97	3.14	3.90	0.970
05/11/01	1.78	2.64	3.17	0.991
06/11/01	2.48	2.79	3.18	0.985
07/08/01	3.81	2.34	4.40	0.999
08/14/01	1.94	3.03	3.54	0.991
08/17/01	2.60	3.80	4.30	0.976

[a] in mg/L

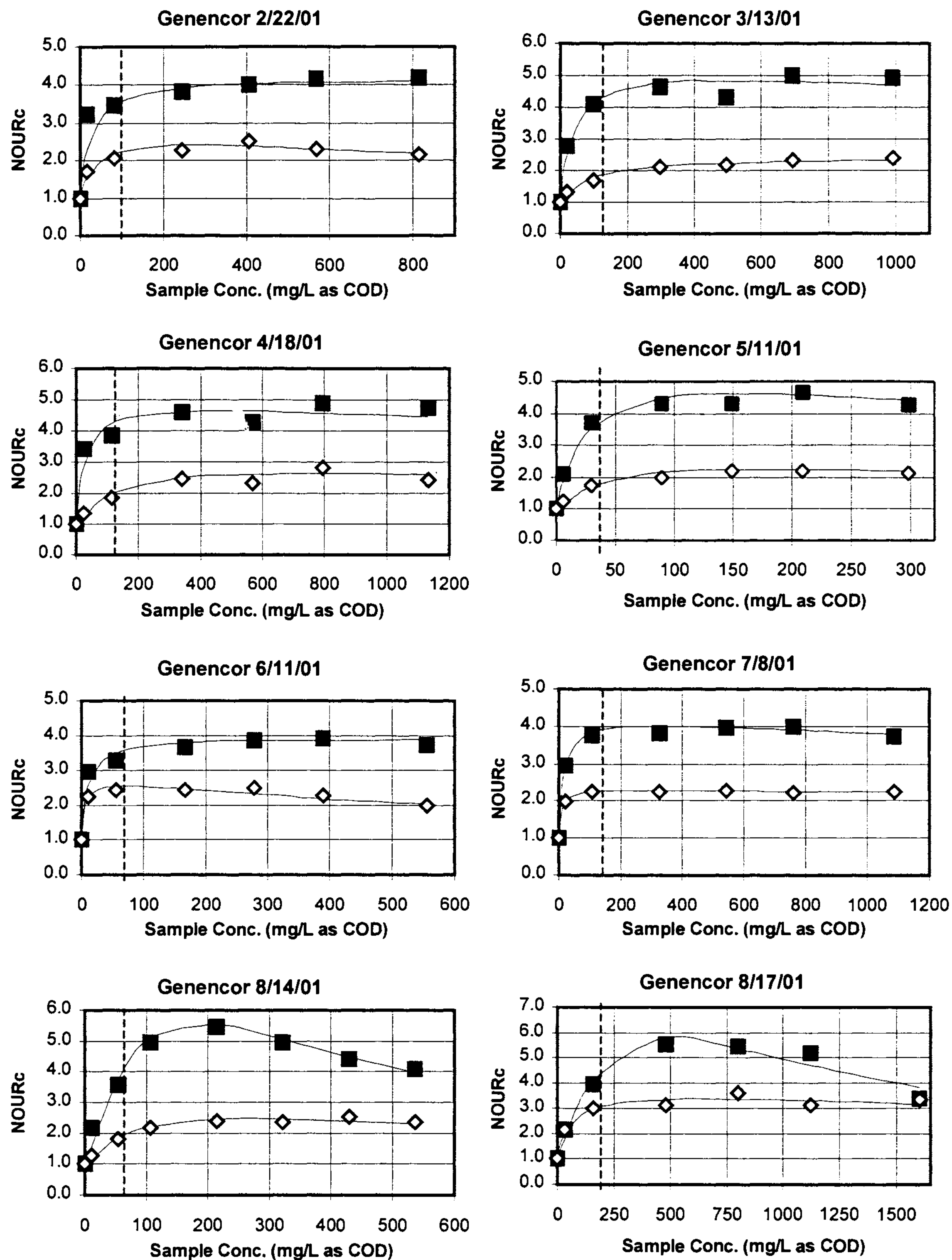


Figure 16. NOURc with increasing concentrations of Genencor samples

(Experimental data: ■ Carbonaceous, ◊ Nitrogenous; Model Fit: —; Concentration at 40 times dilution: ----)

4.1.1.3 ATA results

Screening test results. The results of the screening test for selected Genencor wastewaters samples are shown in Figures 17 and 18. The total gas production and CH_4 contents of the samples were plotted over a 5-day incubation period. The relative activity (RA) of each sample over the 5-day reaction time is illustrated in Figure 19. From the total gas plots, it was observed that most of the samples' initial rates of gas production were close to the control's except for Genencor sample of 5/11/01 and 6/11/01. In particular, the RA values of the two Genencor wastewater samples were 61.5% and 74.1% respectively (Table 16). The gas production rates were later recovered as shown by RA values of greater than 100% when sufficient incubation time (1.5 to 2.0 days) was allowed.

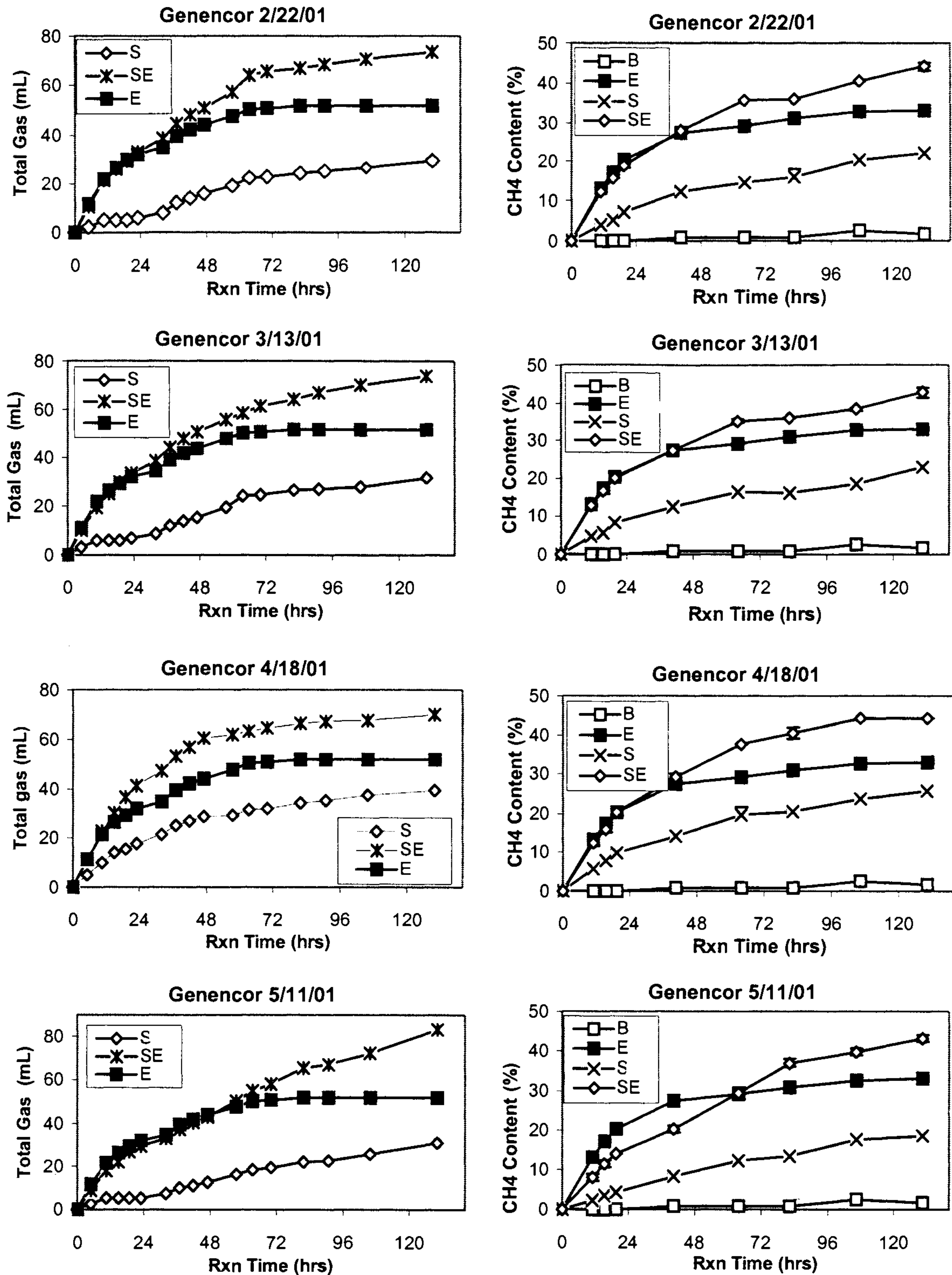


Figure 17. Total gas and CH_4 content of Genencor samples (2/22/01 - 5/10/01)

(B=Blank; E=Ethanol at 1000 mg COD/L; S=Sample at 1000 mg COD/L; SE = Sample and Ethanol both at 1000 mg COD/L)

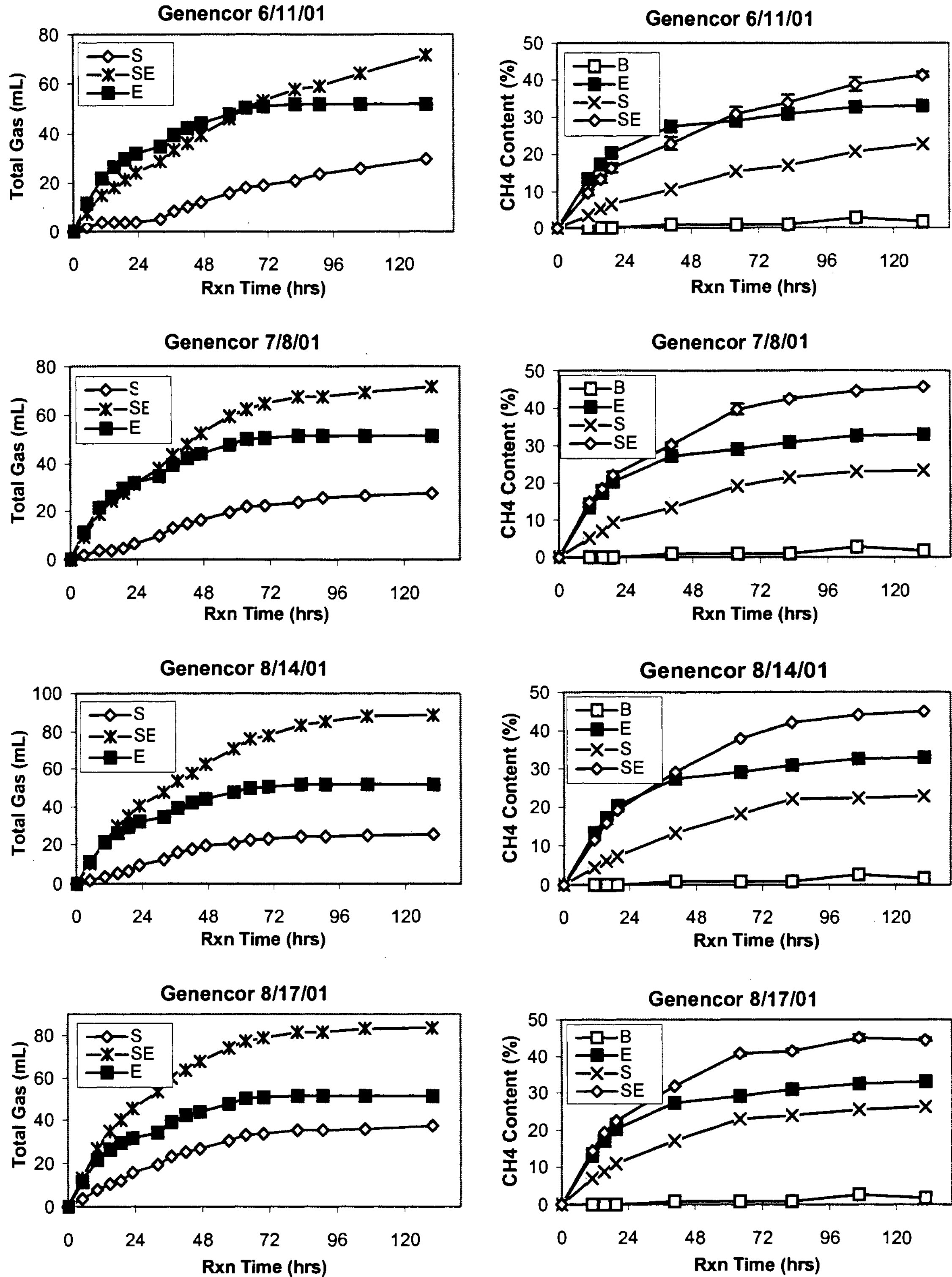


Figure 18. Total gas and CH_4 content of Genencor samples (6/11/01 - 8/17/01)

(B=Blank; E=Ethanol at 1000 mg COD/L; S=Sample at 1000 mg COD/L; SE = Sample and Ethanol both at 1000 mg COD/L)

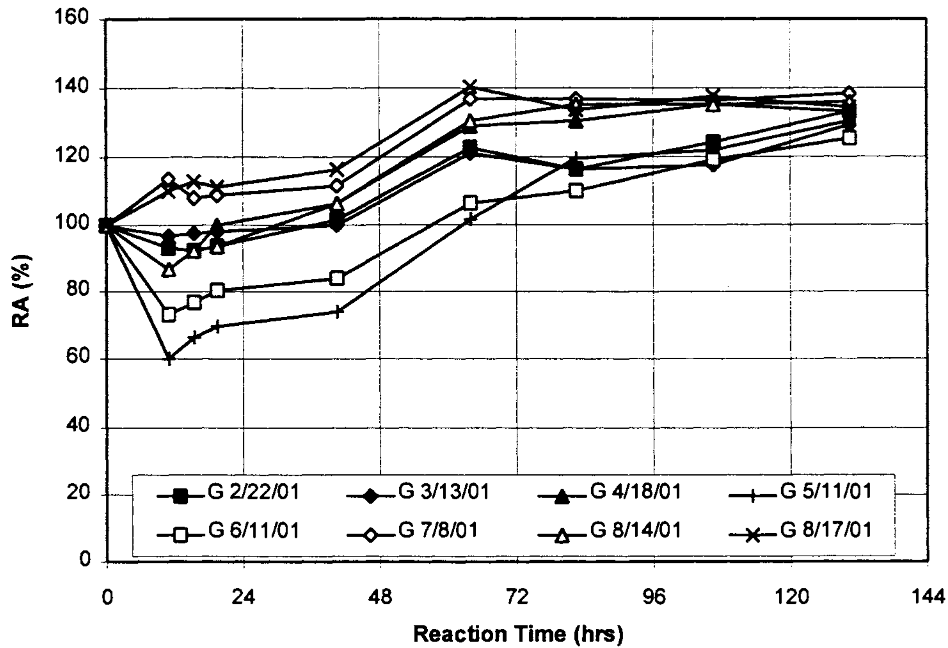


Figure 19. RA of selected Genencor wastewater samples over 5-day reaction time

(All sample bottles contained 1000 mg COD/L of ethanol; RA values were measured at a 12-hour incubation time)

Table 16. RA of selected Genencor wastewater samples at a 12-hour reaction time

Sample	RA (%)
2/22/01	93.1
3/13/01	96.8
4/18/01	93.1
5/11/01	61.5
6/11/01	74.1
7/08/01	112.1
8/14/01	87.9
8/17/01	110.6

Inhibition test results. The Genencor sample from 5/11/01 was tested in the inhibition test to establish a dose-response relationship. Specific total gas, methane content,

and relative activity of the 5/11/01 Genencor wastewater sample at concentrations of 500, 1000, 1500, and 2000 mg COD/L were plotted (Figure 20). RA values greater than 100% were observed at Genencor sample concentrations of less than 1000 mg/L as COD. This was an indication that the Genencor sample was biodegradable by the ethanol acclimated culture at low concentrations. However, increasing sample concentration lowered the %RA value and nearly 20% inhibition (80% RA) was observed at concentration of 2000 mg/L as COD (Table 17).

Table 17. RA of 5/11/01 Genencor wastewater sample

Sample concentration (mg/L as COD)	% RA at a 6-hour incubation
0	100.0
500	108.6
1000	99.7
1500	92.2
2000	80.5

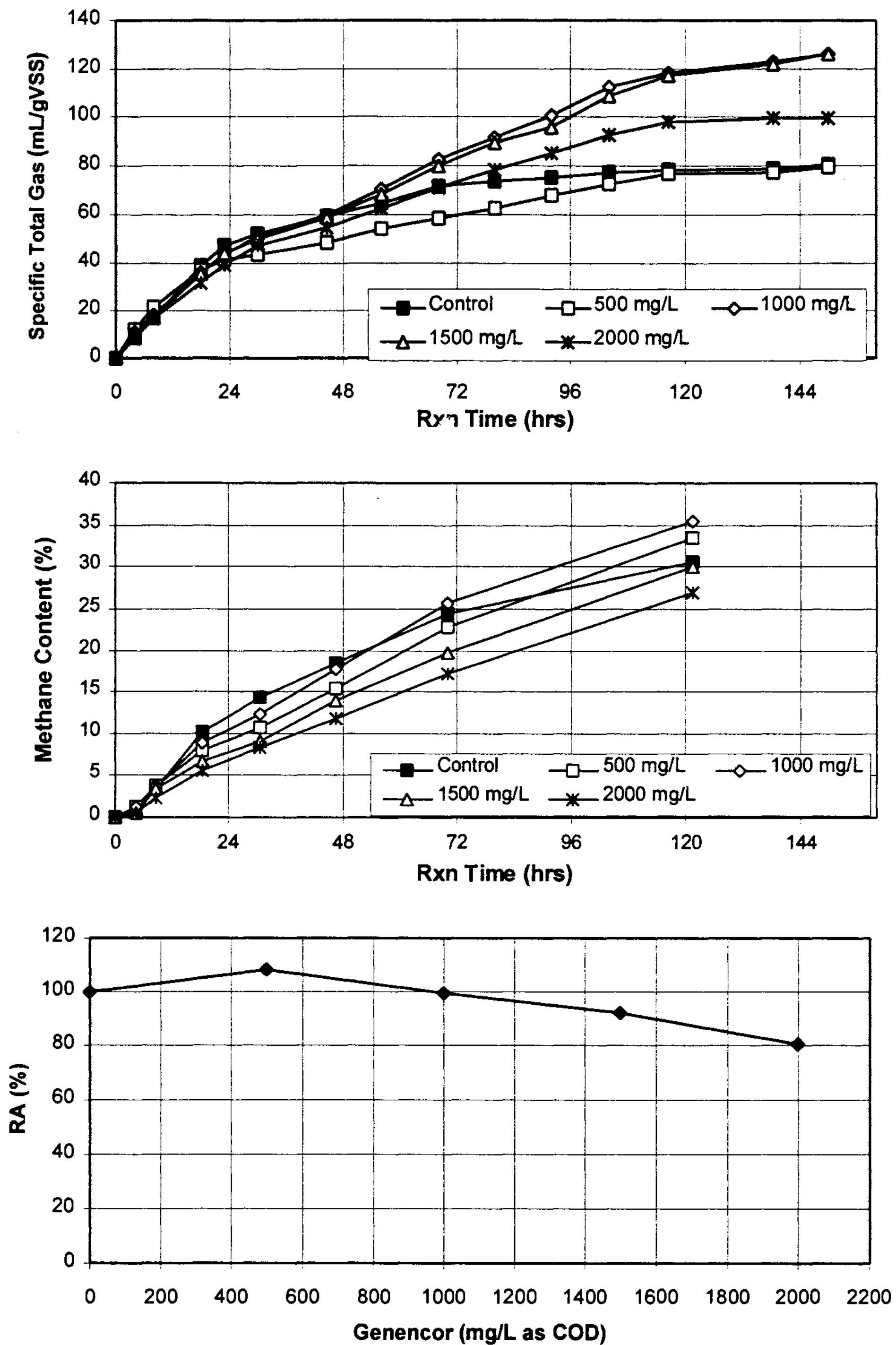


Figure 20. Total gas, methane content, and %RA of 5/11/01Genencor sample

(All test bottles contained 1000 mg COD/L of ethanol; Control = 0 mg/L of Genencor sample)

4.1.2 Quaker Oats

4.1.2.1 Wastewater characteristics

Sample characteristics such as pH, soluble COD, and furfural content of the Quaker Oats sample collected on June 27, 2001 are summarized in Table 18. On average, the Quaker Oats wastewater has a flowrate of 0.1 MGD ranging from 0.018 to 0.162 MGD based on the data obtained from January 1 to March 19 (2001). This indicated more than 250 times dilution was expected when the wastewater was discharged at the Cedar Rapids WPCF. The reported sulfate content of the Quaker Oats wastewater was lower than 50 mg/L.

Table 18. Characteristics of 6/27/01 Quaker Oats wastewater sample

Parameter	Average value
pH	3.08
SCOD [a] (mg/L)	15 000
Furfural (mg/L as COD)	408

[a] SCOD = Soluble Chemical Oxygen Demand

4.1.2.2 Respirometric results

The plot of NOURc value versus Quaker Oats sample concentration is shown in Figure 21. The calculated inhibition parameters are listed in Table 19. From the plot, Quaker Oats wastewater sample was found to have a greater impact on carbon oxidation rate than the nitrification rate. The $\hat{IC}_{50}$ values for nitrification were found to be 6 times higher than the carbonaceous $\hat{IC}_{50}$ values. However, this was not the case for the IC_{50} values. Examination of the difference confirmed that $\hat{IC}_{50}$ was more representative and sensitive than IC_{50} since it predicted the inhibition response more accurately. Consequently, an inhibition response would not be expected at the Quaker Oats concentration equal to the actual concentration of the wastewater at the treatment plant (250 times dilution).

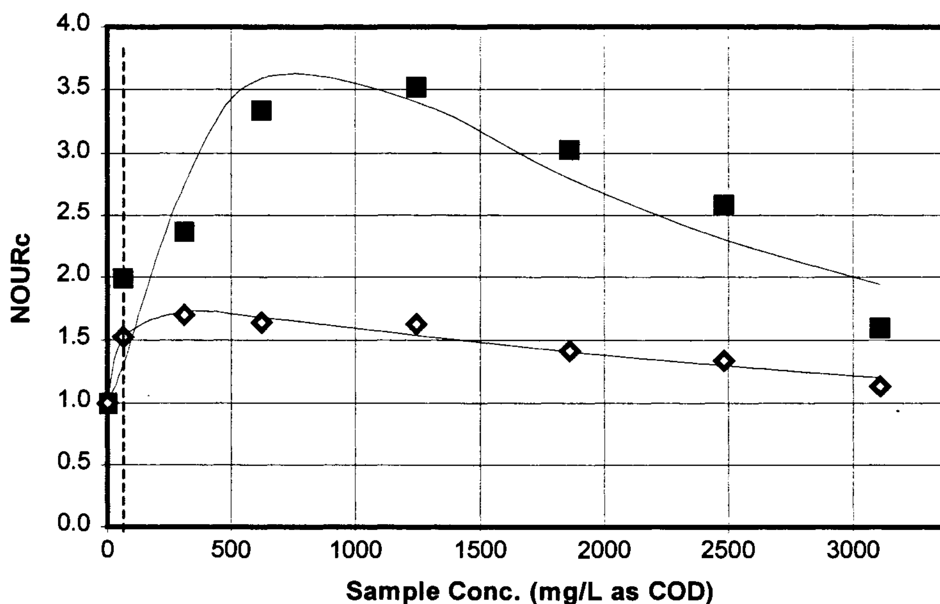


Figure 21. NOURc with increasing concentration of 6/27/01 Quaker Oats

(Experimental data: ■ Carbonaceous, ◊ Nitrogenous; Model Fit: —; Concentration at 250 times dilution: ----)

Table 19. Inhibition parameters of 6/27/01Quaker Oats wastewater sample

Types of test	$p(K_S/K_I)$	L_I [a]	$\hat{NOURc}$	IC_{50} [a]	$\hat{IC}_{50}$ [a]	r^2
Carbonaceous	0.61	40	7.07	17400	832	0.870
Nitrogenous	1.82	60	2.05	15700	5012	0.975

[a] in mg/L

4.1.2.3 ATA results

The anaerobic batch test results of Quaker Oats samples are shown in Figure 22 and Table 20. From the total gas production plot, a low gas production rate was observed at the initial incubation time. Approximately 30% inhibition was observed at a sample concentration of 1000 mg/L at a 6-hour incubation. However, the gas production rate increased after approximately 48 hours of incubation. This suggests that the Quaker Oats

wastewater sample was biodegradable at high COD concentrations (>2000 mg/L) when sufficient incubation time was given.

Table 20. RA of 6/27/01 Quaker Oats sample

Sample concentration (mg/L as COD)	% RA at a 6-hour of incubation time
0	100.0
250	74.5
500	75.0
1000	71.6
2000	66.7

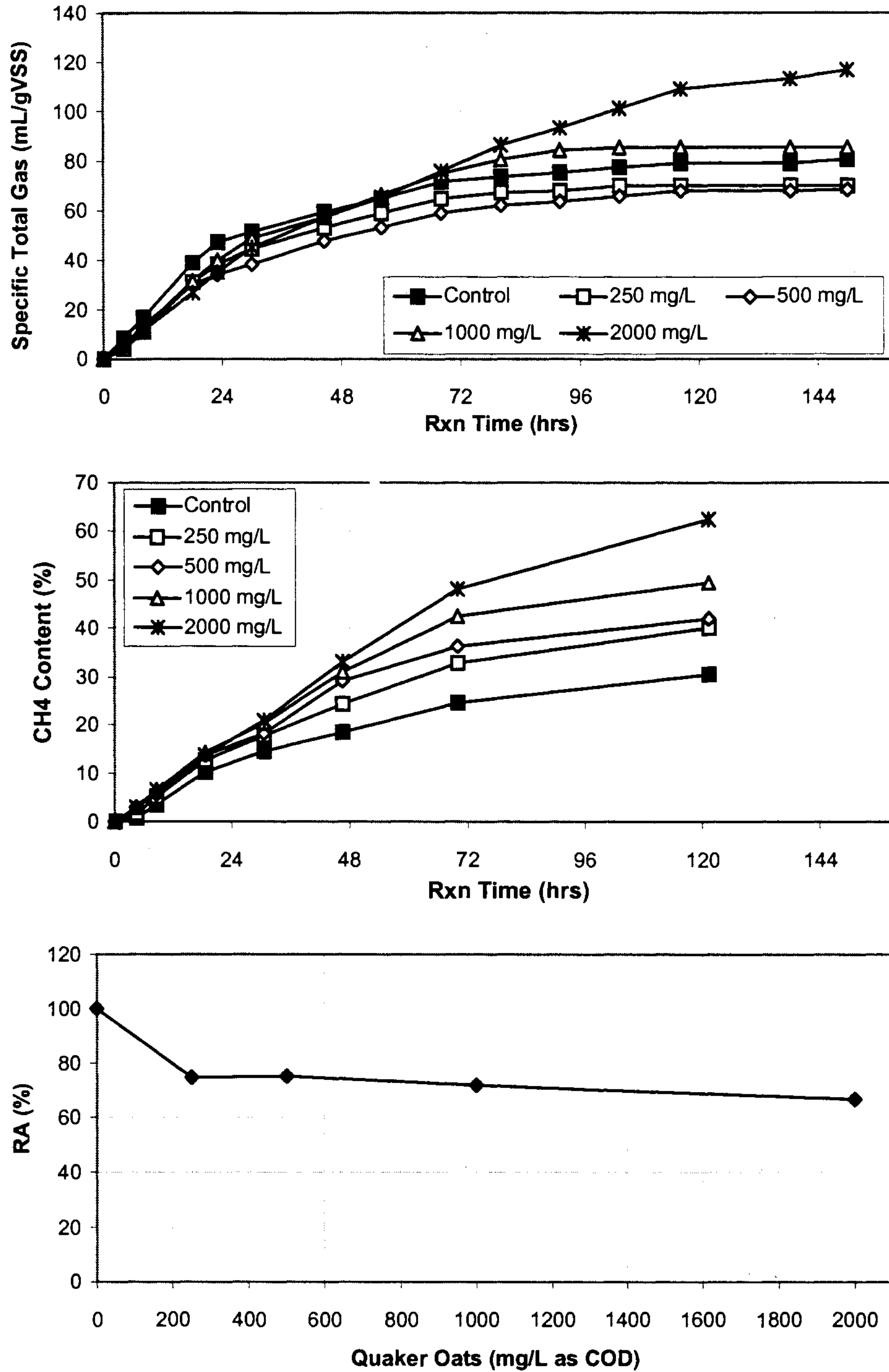


Figure 22. Total gas, methane content, and %RA (6/27/01 Quaker Oats sample)

(All test bottles contained 1000 mg COD/L of ethanol; Control = 0 mg/L Quaker Oats sample)

4.1.3 Organic Compounds: Furfural and Phenol

4.1.3.1 Respirometric results

The impact of increasing concentration of furfural and phenol on NOURc was illustrated in Figure 23. The calculated inhibition parameters are listed in Table 21. Model I was used to estimate the $I\hat{C}_{50}$ on carbon oxidation while Model II was used to fit the nitrification data since no biodegradation was observed (NOURc<1). Several observations can be made from the results.

1. In general, nitrification inhibition was found to be more severe than carbon oxidation inhibition for both furfural and phenol.

2. Phenol had a greater inhibition on carbon oxidation than furfural. The estimated $I\hat{C}_{50}$ for phenol was 3 times smaller that for furfural. However, both compounds showed a similar degree of inhibition on nitrification (13% difference in IC_{50}).

Table 21. Inhibition parameters of fufural and phenol

A. Carbon oxidation (Model I)

Sample	$p(Ks/K_I)$	L_I (mg/L)	$N\hat{O}URc$	$I\hat{C}_{50}$ (mg/L)	r^2
Furfural	2.70	80	1.20	10300	0.962
Phenol	5.51	2	1.21	3200	0.987

B. Nitrification (Model II)

Sample	L_I (mg/L)	n	IC_{50} (mg/L)	r^2
Furfural	80	0.56	2503	0.973
Phenol	2850	1.00	2860	0.972

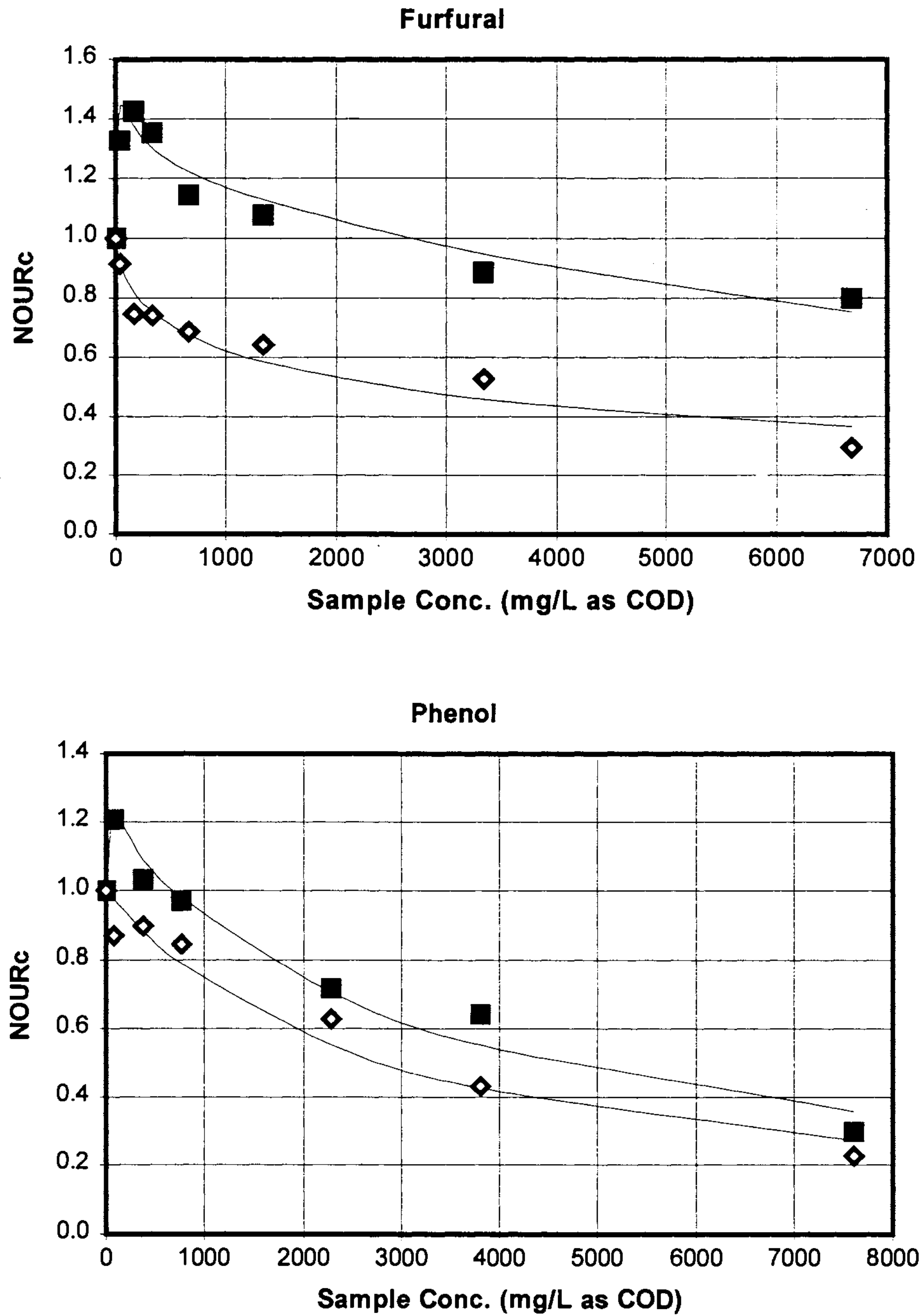


Figure 23. NOURc with increasing Fufural or Phenol concentration

(Experimental data: ■ Carbonaceous, ◊ Nitrogenous; Model Fit: —)

4.1.3.2 ATA results

Furfural and phenol were studied in the ATA test with concentrations of 30, 90, 270, and 810 mg COD/L. The ATA results were shown in Figure 24, Figure 25, Table 22, and Table 23. From these results, it was observed that both furfural and phenol were highly toxic to the MCR culture since more than 50% reduction in RA was observed at the end of 5-day incubation period. Furfural had a greater impact on the initial gas production rate than phenol at low concentrations since 40% inhibition was observed with 30 mg COD/L of furfural, but only 14% inhibition was seen with phenol at the same concentration. However, almost similar % inhibition values (50% for furfural and 62.5% of inhibition) were observed after 5 days of incubation at a concentration of 30 mg COD/L. This might suggest that the anaerobic culture had a relatively faster recovery rate for the furfural compound.

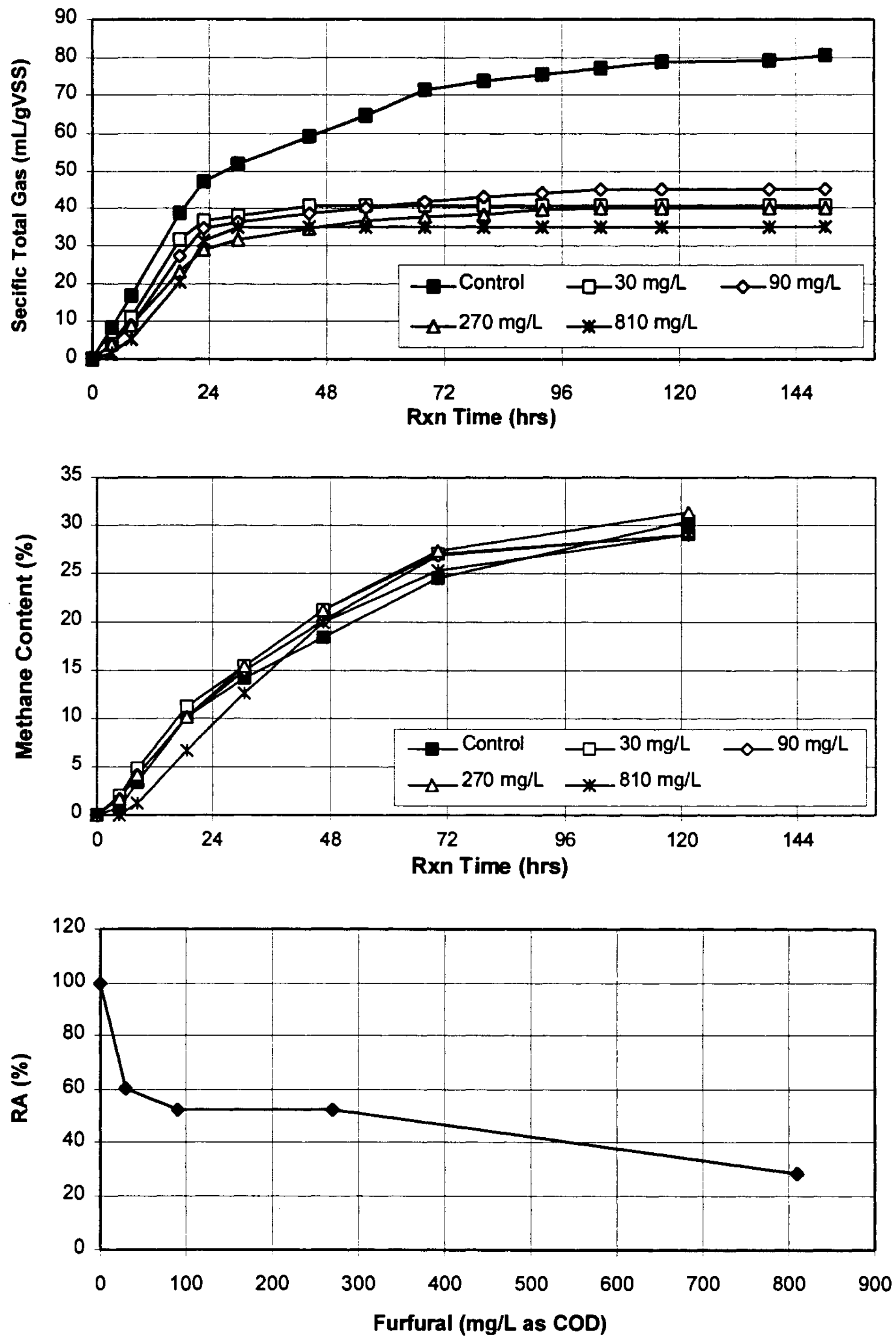


Figure 24. Total gas, methane content, and %RA of furfural

(All test bottles contained 1000 mg COD/L of ethanol; Control = 0 mg/L of furfural)

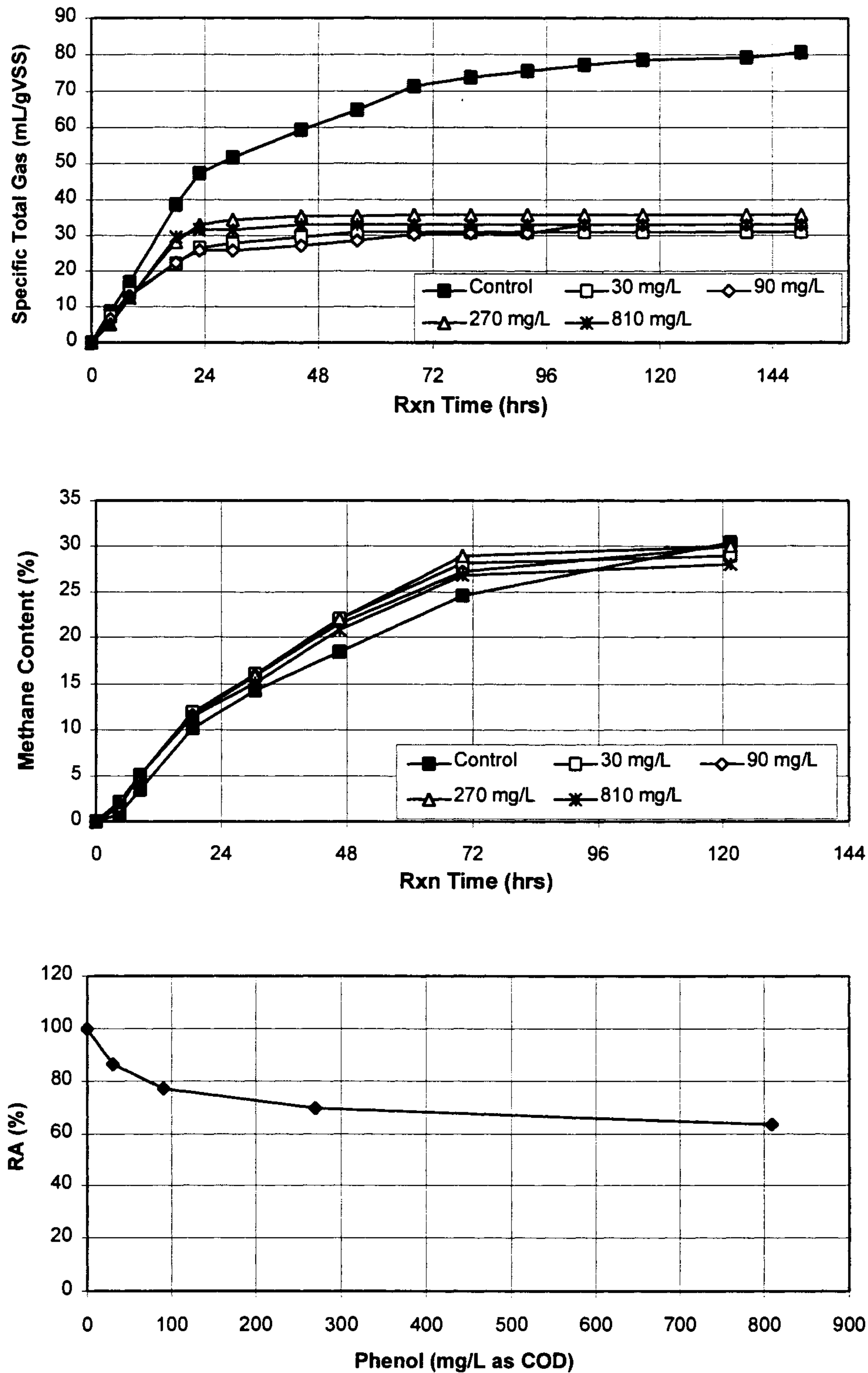


Figure 25. Total gas, methane content, and %RA of phenol

(All test bottles contained 1000 mg COD/L ethanol; Control = 0 mg/L of phenol)

Table 22. RA of furfural

Sample concentration (mg/L as COD)	% RA at a 6-hour incubation
0	100.0
30	60.1
90	52.1
270	52.2
810	28.1

Table 23. RA of phenol

Sample concentration (mg/L as COD)	% RA at a 6-hour of incubation
0	100.0
30	86.2
90	76.9
270	69.6
810	63.3

4.1.4 Inorganic Compound: NaCl

The use of a nitrifying respirometer in assessing nitrification inhibition was demonstrated in this study. A client from Fox Engineering Associates, Iowa, has reported a salt content as high as 6.6 g/L as NaCl at a local wastewater treatment plant. To evaluate the inhibition effect of such salt concentration on the nitrification rate, five NaCl concentrations were studied. The results are shown in Figure 26. From the plot, it was observed that the nitrification rate, calculated as specific NOURc, decreased with increasing NaCl concentration. In particular, the maximum nitrification rate was reduced by 22% at 6.6 g/L of NaCl.

The inhibitory effect of NaCl was further confirmed by a decrease in pH change as shown in Table 24. The pH change dropped 45% from 0.74 at zero salt content to 0.41 at 10 g/L of sodium chloride. Since each vessel was saturated with equal amount of NH_4Cl, equal pH change was expected under non-inhibitory condition.

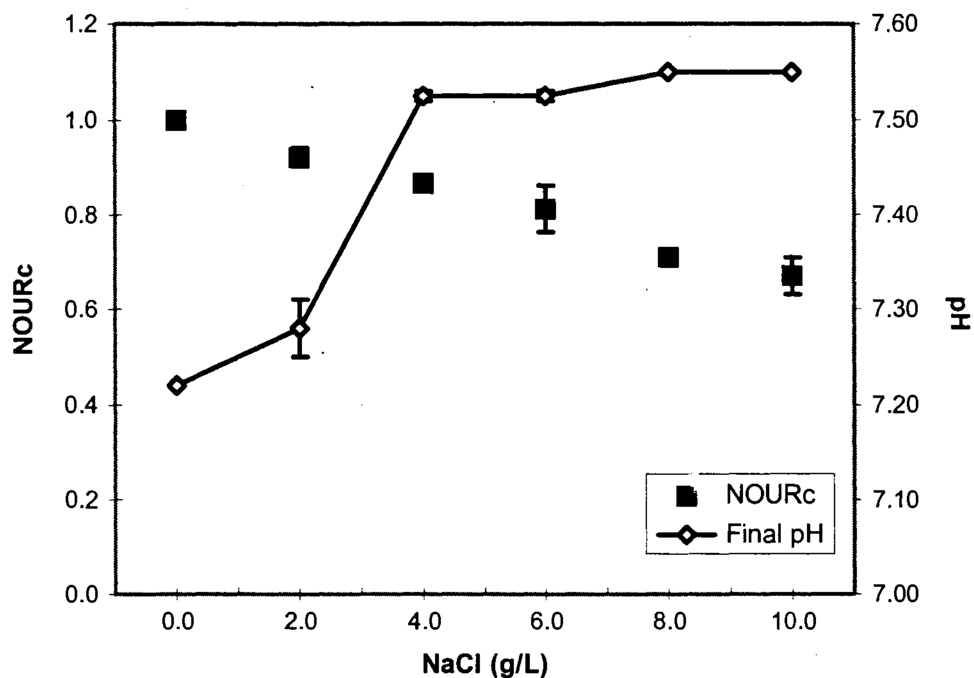


Figure 26. NOURc (nitrogenous) and final pH with increasing NaCl concentration

Table 24. NOURc and pH consumption with increasing NaCl concentration

NaCl (mL)	NaCl (g/L)	NOURc	Final pH	pH change[a]
0	0	1.00	7.22	0.74
2	2	0.92	7.28	0.68
4	4	0.87	7.53	0.44
6	6	0.81	7.53	0.44
8	8	0.71	7.55	0.41
10	10	0.67	7.55	0.41

[a] pH of the original biomass without the addition of NH_4Cl was 7.96

4.2 Discussion

4.2.1 Genencor

4.2.1.1 Sensitivity of C-, N-, and An- tests

The differences in microorganisms' sensitivity towards different Genencor samples are illustrated in Figure 27 and Table 25. From the screening results, it was shown that majority of the Genencor samples tested (samples of 3/13/01, 5/11/01, 7/8/01, 8/14/01, and 8/17/01) had the greatest impact on the carbonaceous biomass followed by nitrifiers and anaerobic biomass. Among those samples, samples of 3/13/01 and 5/11/01 showed almost similar effects on the carbonaceous and nitrogenous tests. The nitrogenous test (N-test) became the most sensitive when Genencor samples of 2/22/01, 4/18/01, and 6/11/01 were tested.

Table 25. Percent inhibition of C-, N-, and An- tests at 1000 mg/L Genencor samples

Genencor sample	Carbonaceous test[a] C-test	Nitrogenous test[a] N-test	Anaerobic test[b] An-test
2/22/01	1.4	23.5	6.9
3/13/01	11.8	4.2	3.2
4/18/01	7.8	16.4	6.9
5/11/01	57.3	40.5	38.5
6/11/01	27.7	40.0	25.9
7/08/01	9.5	4.2	0.0
8/14/01	74.5	30.2	12.1
8/17/01	54.8	8.6	0.0

[a] Percent inhibition = (NOURc / NÔURc) * 100% (see section 3.7.1 for the definitions of NOURc and NÔURc)
[b] Percent inhibition = (1 - RA) * 100% (see section 3.7.2)

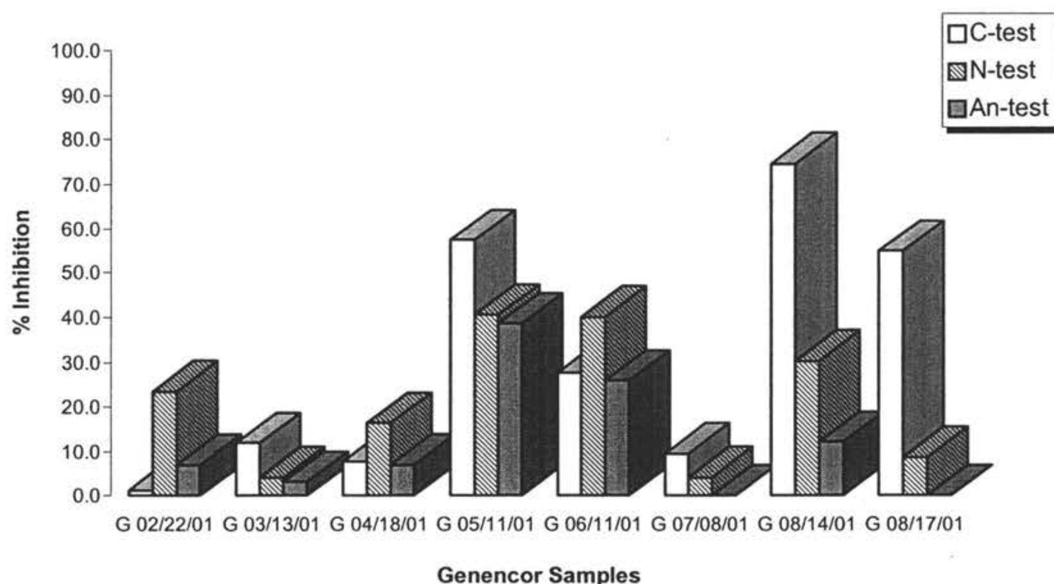


Figure 27. Comparison of the % inhibition values of selected Genencor samples
(The values of % inhibition were calculated at a sample concentration of 1000 mg COD/L; C-test: Carbonaceous test; N-test: Nitrogenous test; and An-test; Anaerobic test)

The % inhibition values of the C-test and N-test were plotted versus % inhibition values of the An-test at 1000 mg COD/L of Genencor wastewater samples to determine the correlation among the three tests (Figure 28). From the plot, a logarithmic relationship could be deduced between the N-test and An-test with a correlation coefficient of 93.5%. This suggested that the nitrifiers were more sensitive than the anaerobic microorganisms for the Genencor samples tested, which contradicted the common perception that methanogens are more sensitive to inhibitory substances than aerobic autotrophs. This logarithmic relationship, however, showed a limit to the sensitivity of the nitrifiers. In Blum and Speece's (1991) study of correlation among test organisms, the *Nitrosomonas* showed 10-fold greater toxicity than methanogens when a variety of organic chemicals (except chlorinated aliphatic group) were tested with a correlation coefficient of 0.60. This further confirmed that methanogens might not necessarily be more sensitive than aerobic cultures.

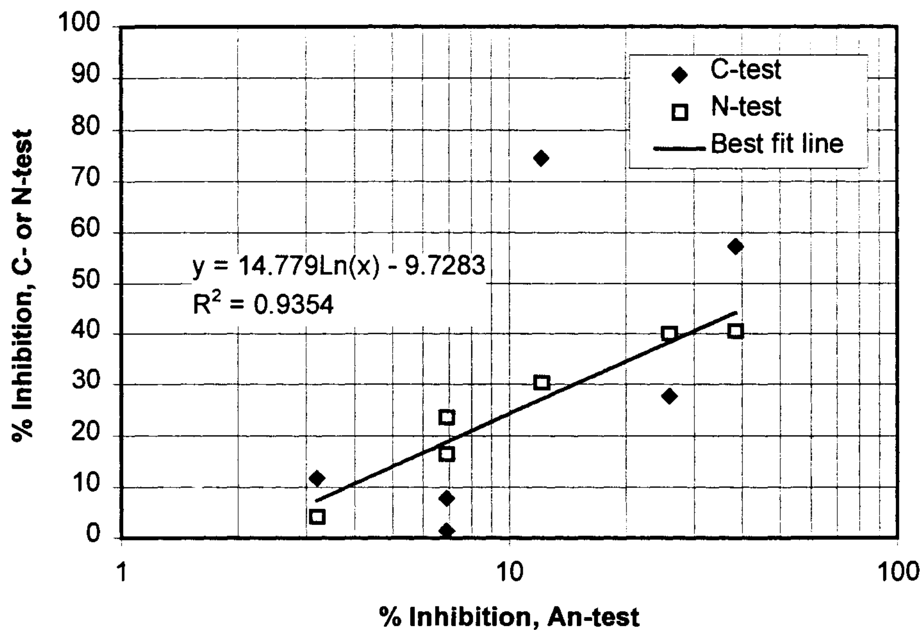


Figure 28. Correlation among C-, N-, and An- tests (at 1000 mg COD/L Genencor)

4.2.1.2 Correlation between sample characteristics and degree inhibition

To deduce the possible factors causing varying degrees of inhibition, inhibition parameters, log ($I\hat{C}_{50}$) and %RA, were plotted versus Genencor samples characteristics such as pH, TSS, sulfate, and etc. The plots are shown in Figures 29, 30, and 31. Several trends were observed from the figures.

1. Similar inhibition patterns were observed among the C-test, N-test, and An-test.

2. Increased inhibition effects (i.e., lower $I\hat{C}_{50}$ or/and %RA values) were observed with increasing pH, TSS, and sulfate.

3. Increasing TOC, CBOD, COD, and TKN concentrations did not reduce the biomass activity.

4. The effects of increasing CBOD/COD ratio, ammonia-N and ammonia-N/TKN ratio on the biomass tested were not distinctive.

From the trends observed above, it could be deduced that the inhibitory compounds found in the Genencor wastewater samples were high in pH, TSS, and sulfate content. Overall, no strong correlation coefficients were observed for the factors mentioned above ($r^2 < 0.8$, results not reported here). This was expected as the inhibition response was complicated by the interactions among factors such as COD, TKN, and TSS. Therefore, individual dose-and-response relationships were difficult to deduce.

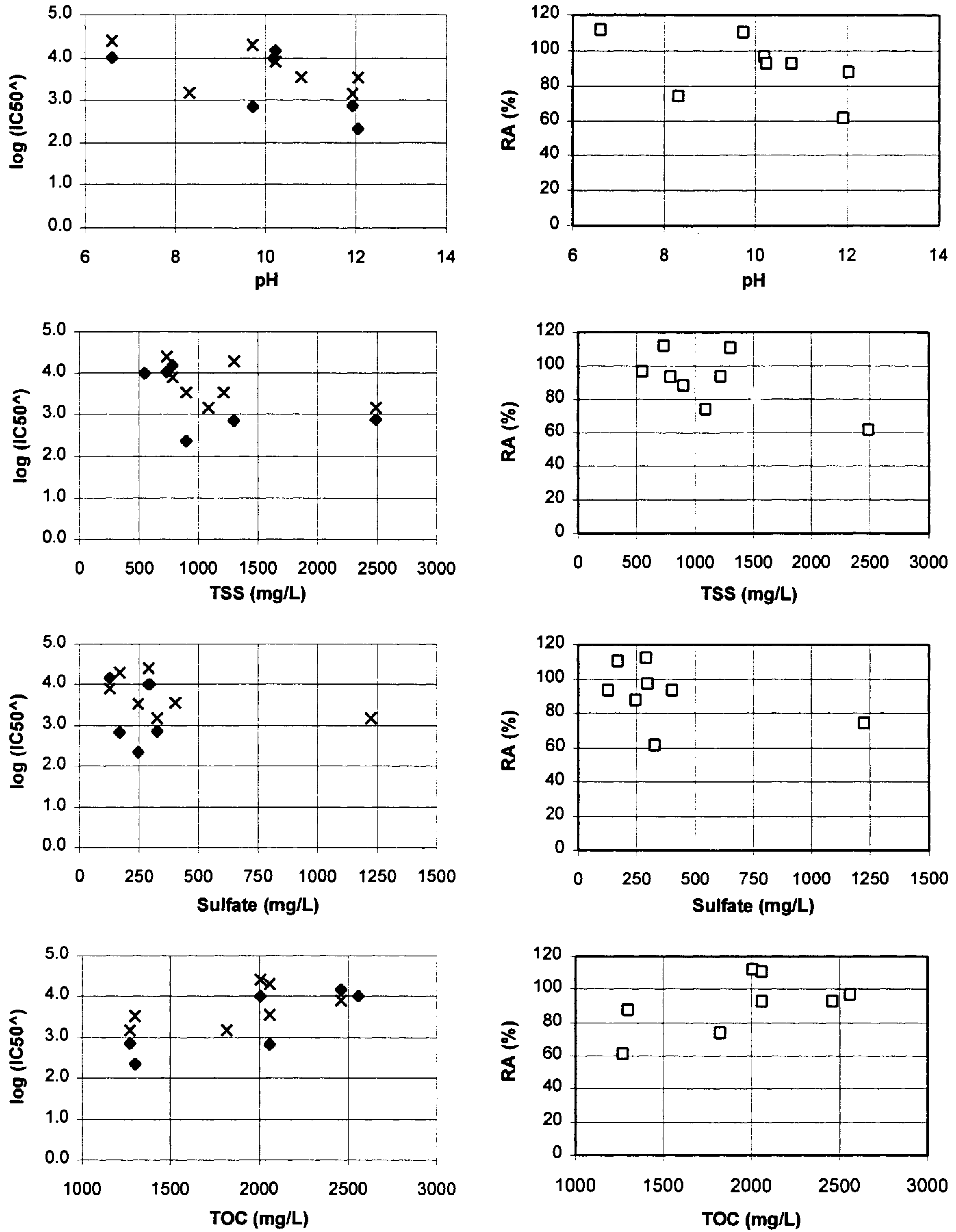


Figure 29. Log($I\hat{C}_{50}$) and %RA versus sample pH, TSS, sulfate, and TOC

(IC50^ = $I\hat{C}_{50}$; Carbonaceous: ♦; Nitrogenous: ×; Anaerobic: □)

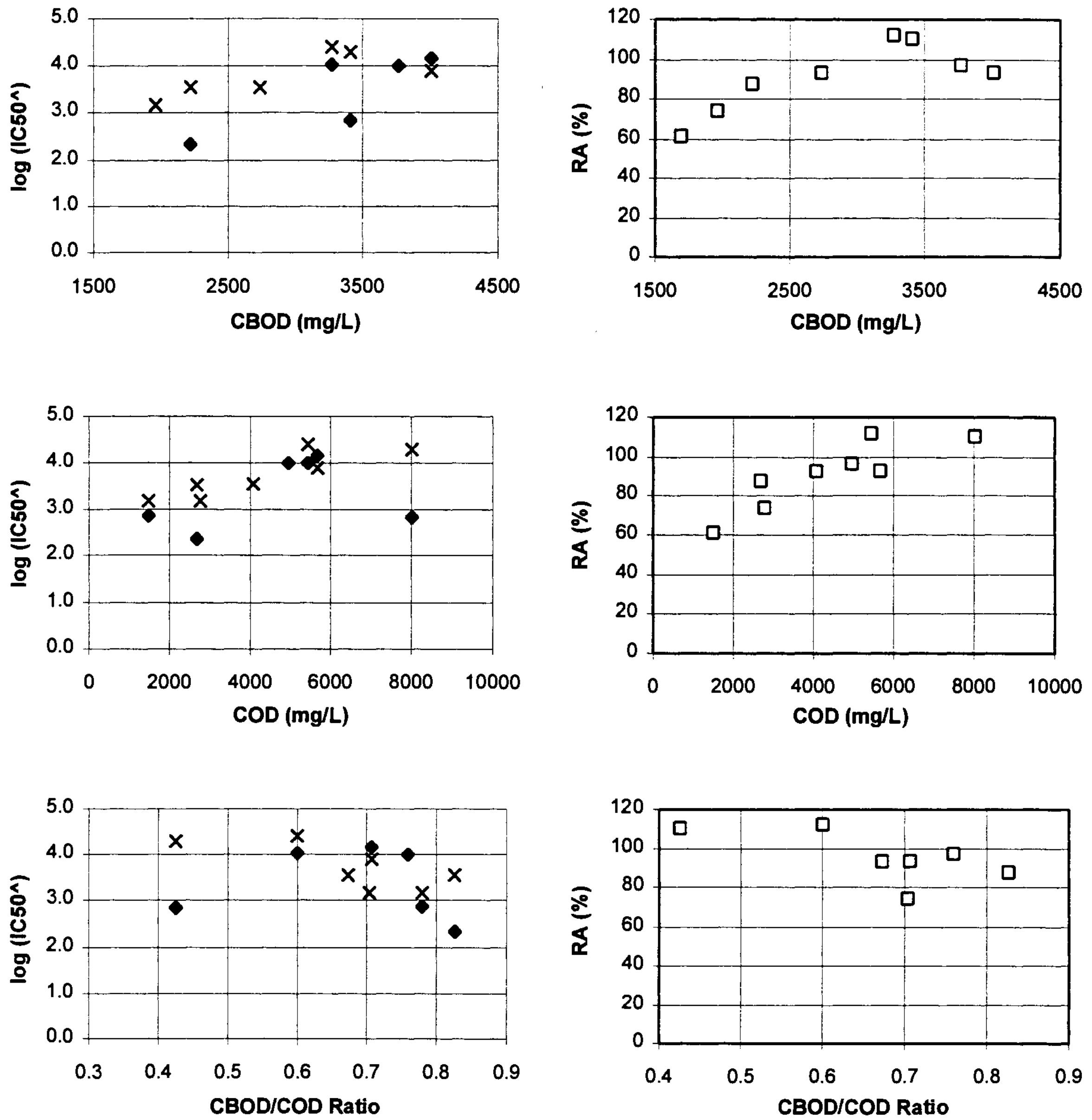


Figure 30. Log($I\hat{C}_{50}$) and %RA versus sample COD, $CBOD_5$, and $CBOD_5$/COD ratio

(IC50^ = $I\hat{C}_{50}$; Carbonaceous: ♦; Nitrogenous: ×; Anaerobic: □)

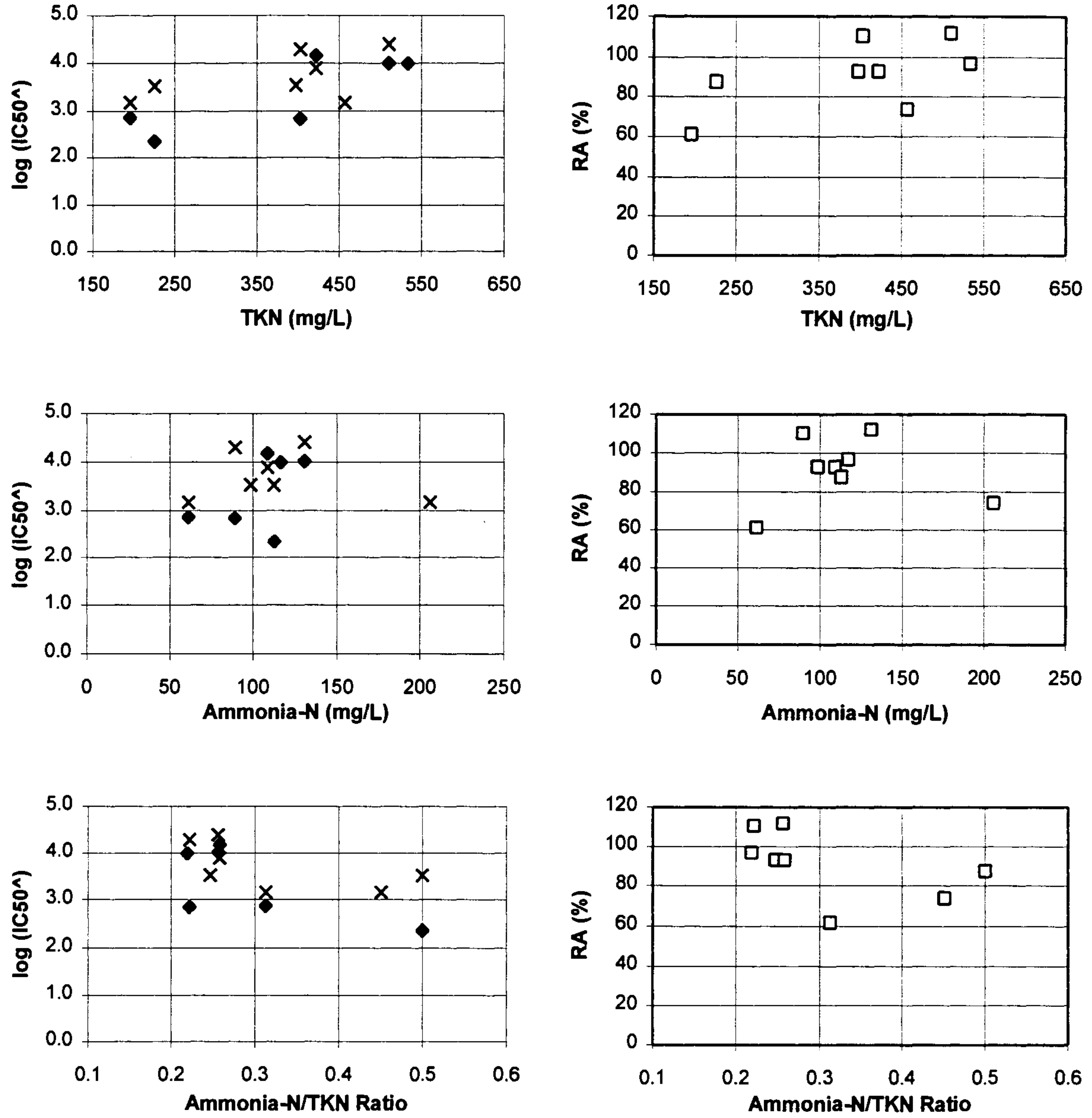


Figure 31. Log($\hat{IC}_{50}$) and %RA versus sample TKN, NH_3-N, and NH_3-N/TKN ratio

(IC50^ = $\hat{IC}_{50}$; Carbonaceous: ♦; Nitrogenous: ×; Anaerobic: □)

4.2.2 Comparison among wastewater samples and organic compounds

In order to observe the different responses of aerobic, anoxic, and anaerobic microorganism towards Genencor, Quaker Oats, furfural, and phenol samples, the % inhibition at the same concentration were compared as listed in Table 26. A concentration of 1000 mg COD/L was used for the wastewater samples while 500 mg COD/L was used for the organic compounds. The differential sensitivity among samples was further illustrated in Figure 32. It is interesting to note that the sensitivity of the tests changed from sample to sample, and from biomass to biomass. For instance, the anaerobic culture was observed to be the most sensitive when furfural and phenol were tested, followed by nitrifiers and heterotrophs. However, it was exactly the opposite for the Genencor wastewater sample of 5/11/01. These observations indicate that the 3-tier approach (C-, N-, and An- tests) is very important in designing the early warning detection system as no one test alone is going to give us the necessary sensitivity.

Table 26. Percent inhibition of Genencor, Quaker Oats, furfural, and phenol

	% Inhibition			
	Genencor[a]	Quaker Oats[b]	Furfural	Phenol
	at 1000 mg COD/L		at 500 mg COD/L	
C-test	57.3	51.6	13.3	13.2
N-test	40.5	21.2	28.9	14.9
An-test	8.2	28.4	58.1	33.1

[a] Sample of 5/11/01
[b] Sample of 6/27/01

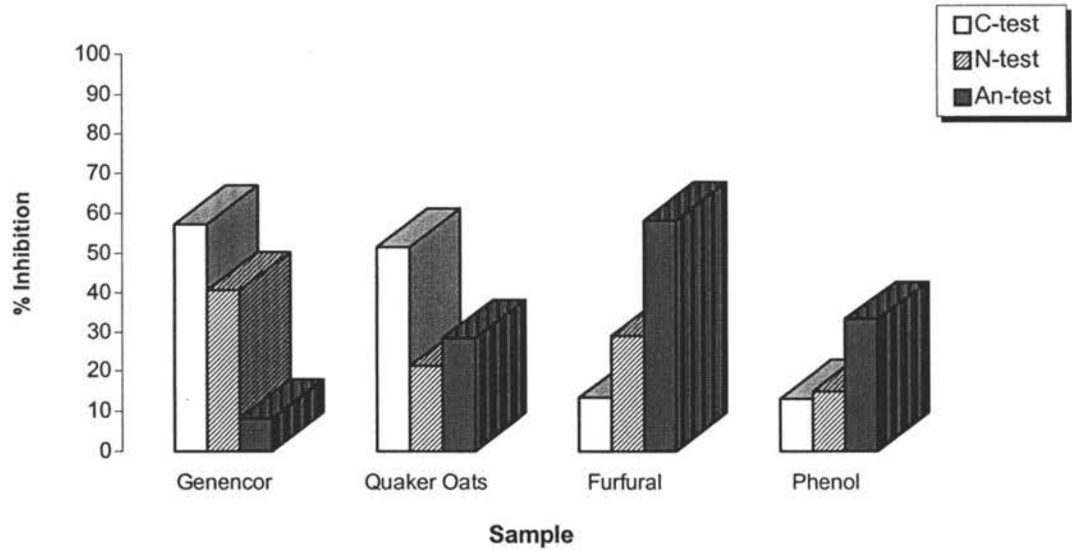


Figure 32. % inhibition of Genencor, Quakers Oats, furfural, and Phenol

(Genencor sample of 5/11/01 and Quaker Oats sample of 6/27/01 were used; Sample concentrations: Genencor and Quaker Oats at 1000 mg COD/L; Furfural and Phenol at 500 mg COD/L; C-test: Carbonaceous test; N-test: Nitrogenous test; and An-test: Anaerobic test)

CHAPTER 5. ENGINEERING SIGNIFICANCE

Over the past 20 to 30 years, the number of industries discharging wastes into domestic sewers has increased drastically (Metcalf and Eddy, 1991). Many municipal wastewater treatment plants (WWTPs) are now facing the challenge of maintaining efficient process performance to produce quality effluents. Transient upsets resulting from inhibitory industrial influent to the biological units of the WWTP often induce reductions in biological treatment efficiency. This may lead to undesirable effluent toxicity, which can adversely affect the ecology and lead to acute environmental health problems. Protection of the receiving waters at full-scale plant through upset early warning devices is, therefore, a must before substantial time and money is spent on the construction of pilot-scale plant and actual site work investigation.

The design strategies of an upset early warning system often require rapid detection and identification of the inhibitory source and response such as a reduction in the microorganism's respiration rate (Love and Bott, 2000). Since biodegradation is the key objective of secondary (biological) treatment systems, upset early warning systems using respirometry, which relates respiration rate to catabolic function, represents a rational approach. Respirometry gives rapid identification of the presence of influent disturbances such as toxicity and BOD shock loads. It has the ability to detect upset conditions and to enact appropriate and swift operational changes to protect the plant from process deterioration.

In this study, a protocol was developed to rapidly detect the transient inhibition of potentially inhibitory wastewaters using aerobic respirometry and an anaerobic bioassay. Respirometric techniques for both general inhibition and nitrification inhibition were developed. This is particularly important, as the treatment plants in the United States strive to meet the stringent ammonia discharge standards enforced by U.S. Environmental Protection Agency (EPA). In addition, nitrifying respirometry may represent a more sensitive tool to protect the CBOD system sooner.

Anaerobic evaluation of industrial wastewaters is equally important as the aerobic part. Inhibition effects on the anaerobic cultures were studied and inhibition parameters such

as relative activity (RA) were determined. Though future research has to be done on establishing an anaerobic respirometric system which is able to detect the process disturbance in a relatively short time, the anaerobic bioassay technique (batch toxicity test) used in this research can serve as the basis of the anaerobic respirometry setup.

To broaden the application of the protocol developed, an algorithm illustrated in Figure 33 can be adopted for rapid detection and evaluation of potentially inhibitory wastewaters. The design of the algorithm allows necessary sensitivity by using unacclimated sludge and incorporating aerobic, anoxic, and anaerobic tests for the detection and evaluation of the test samples. The inhibition effect detected by unacclimated biomass will be confirmed using acclimated sludge from the treatment plant. If significant inhibition effects (e.g. 20% inhibition) are detected using acclimated sludge and the concentration is critical to the plant, several process control actions, such as diverting the toxic wastewater to a separate basin, can be enacted within sufficient time to protect the plant.

As the popularity of upset early warning systems increases, problems associated with abrupt influent disturbances to the WWTP can be minimized. The treatment plants will have sufficient time to react and several mitigation actions can be employed. For instance, suspicious influent wastewater can be directed to a separate basin for further treatment and study. This allows for optimized conversion of potentially toxic byproduct streams from industrial effluents such as agribusiness and biotechnology industries to microbial biomass.

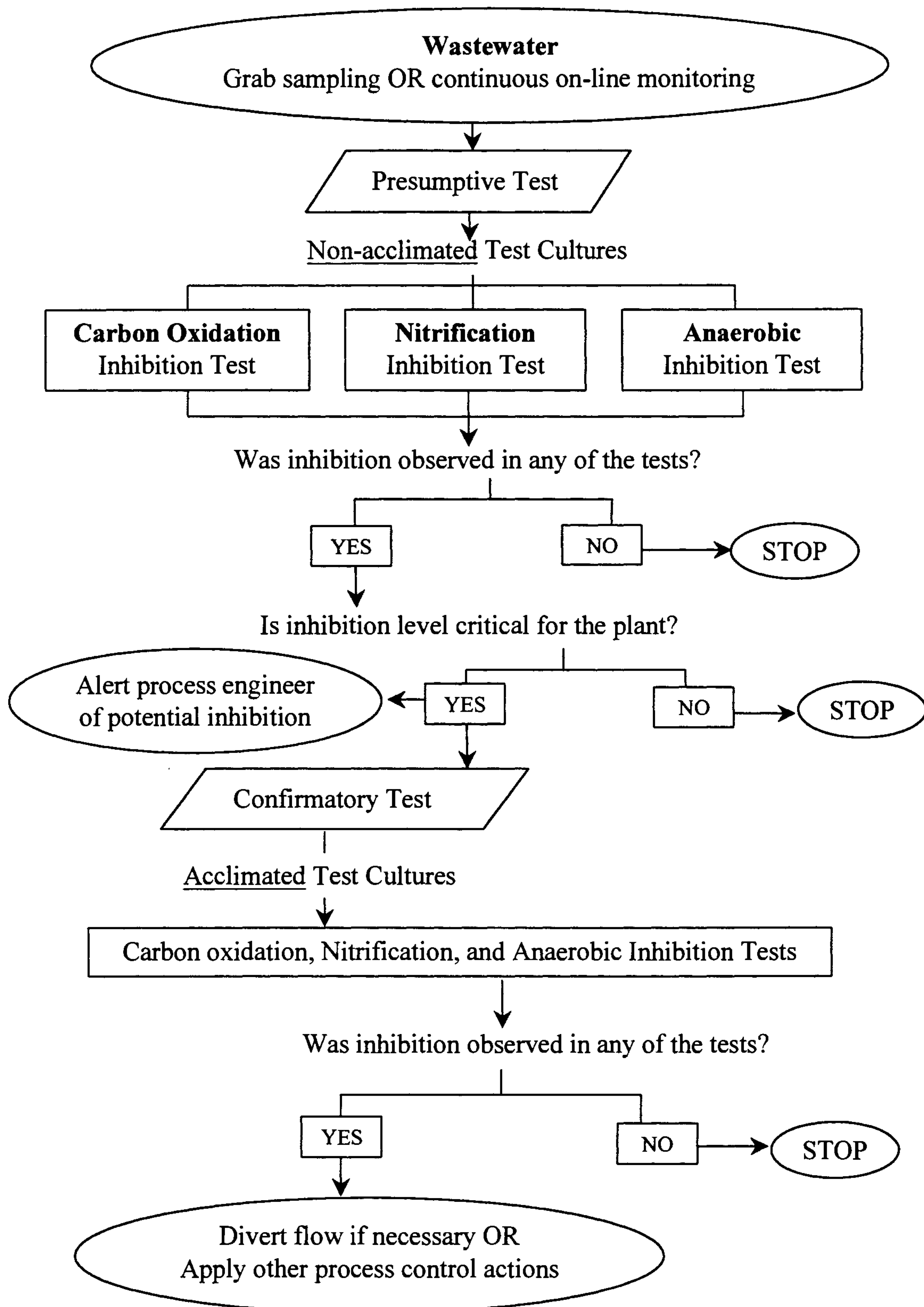


Figure 33. Algorithm used for early detection of influent toxicity

CHAPTER 6. CONCLUSIONS

6.1 Summary

A protocol for rapid detection and evaluation of the inhibitory characteristics of influent wastewaters was developed. The protocol was developed based on the wastewater samples, organic toxic compounds, and an inorganic compound tested in this study. Assessments of carbon oxidation, nitrification, and anaerobic inhibitions are included in the protocol to provide necessary sensitivity of the biosensors. Summary of the results are listed below.

1. Varying degrees of sensitivity were demonstrated in the study when different groups of microorganisms, wastewater samples, and chemicals were tested.

2. The majority of the Genencor wastewater samples showed greater impact on carbonaceous biomass than nitrifiers and anaerobic cultures. Nitrification was the most sensitive for some of the Genencor samples tested. When individual organic toxic compound (furfural or phenol) was tested, the anaerobic cultures became the most sensitive group.

3. None of the Genencor wastewater samples studied showed inhibition at the actual concentrations received at the Cedar Rapids WPCF. This observation, however, did not reject the existence of inhibitory compounds in the Genencor wastewater. Significant inhibition was observed at higher concentrations of some samples. For instance, nearly 75% inhibition (carbonaceous) was observed at 1000 mg COD/L of 8/14/01 Genencor wastewater sample.

4. Examination of the effect of factors such as pH, COD, and sulfate of the Genencor wastewater samples on the log ($I\hat{C}_{50}$) and RA values suggested that the inhibition became more profound with increasing pH, TSS, and sulfate concentrations. However, the correlations were not statistically significant

between the factors. Complications due to the complex interactions among factors might be the reason.

5. A logarithmic correlation ($r^2 = 0.953$) was observed between the percent inhibition values of N-test and An-test when Genencor wastewater samples were tested. Nitrifiers showed higher sensitivity than methanogens to the inhibitory substances in Genencor wastewater samples.

6. Sodium chloride inhibition was demonstrated using the nitrifying respirometers developed. An inhibition of nearly 20% was observed at a concentration of 6 g/L as NaCl, which represents a critical level in the nitrogenous BOD removal system at the actual treatment plant.

7. The models used in the study were found adequate in explaining the inhibition response of the aerobic biomass when wastewater samples and organic compounds were tested. An average r^2 value of 0.975 ± 0.026 (standard deviation) were found.

6.2 Recommendations for Future Research

Although most of the inhibition effects can be predicted by respirometric techniques, there are cases when toxicants deteriorate process performance at sublethal concentrations without significantly hampering the respiration rate (Bott and Love, In press). Therefore, it is important to define a clear source-cause-effect relationship in the respirometric system. Though the aerobic respirometric device is quite flexible in terms of switching from off-line to on-line implementation, future research is needed for applications of the respirometry system as part of a complete upset early warning system. To date, an anaerobic respirometric system that is capable of rapid inhibition detection has not been developed. The anaerobic batch results from the study could provide valuable information for the development of an automated anaerobic system. Recommendations for future research are listed below.

1. Clearly define the source, cause, and effect relationships of an upset phenomenon.
2. Determine the appropriate process control and alleviation actions to combat the upset detected by the upset early warning respirometric devices.
3. Develop a protocol for rapid detection and evaluation of inhibition on denitrification and biological phosphorus removal.
4. Develop a model for prediction of inhibition parameters and evaluation of inhibition response on anaerobic cultures.
5. Setup an automated anaerobic respirometric system with data acquisition system and analog pressure transducer.
6. Conduct a pilot-scale study of the respirometry system as an upset early warning system and cost assessment for the maintenance of respirometric biosensors used as upset early warning systems.

APPENDIX A. Operating data during startup period of MCR

MCR-startup period

Date (mm/dd/yy)	Time (day)	Pressure (in. Hg)	Total gas @ 35'C (L)	Total gas @ STP (L)	Methane (%)	pH Influent	pH Effluent	Note
08/08/01	0	29.02	0.00	0.00		8.20	7.94	
08/09/01	1	29.02	0.00	0.00			7.82	
08/10/01	2	29.22	0.04	0.04			7.70	
08/11/01	3	29.10	0.00	0.00			7.60	
08/12/01	4	29.20	0.00	0.00	2		7.66	
08/14/01	6	29.20	0.04	0.04			7.66	
08/16/01	8	29.11	0.08	0.07			7.73	
08/17/01	9	29.11	0.33	0.30	16		7.83	
08/18/01	10	29.11	0.03	0.03		8.00	7.89	Vent line choked
08/19/01	11	29.11	0.04	0.04			7.97	Vent line choked
08/20/01	12	29.11	0.35	0.32	30		7.97	
08/21/01	13	28.90	0.65	0.60			7.71	
08/22/01	14	28.90	0.63	0.58			7.75	
08/23/01	15	29.11	0.67	0.61			7.81	
08/24/01	16	29.04	0.67	0.61	66		7.88	
08/25/01	17	29.04	0.65	0.59			7.86	
08/26/01	18	29.13	0.56	0.51			7.85	
08/27/01	19	29.01	0.73	0.67			7.95	
08/28/01	20	29.10	0.71	0.65			7.91	
08/29/01	21	28.97	0.51	0.47			8.09	
08/30/01	22	28.95	0.73	0.67			8.01	
08/31/01	23	29.13	0.54	0.49			8.15	
09/01/01	24	29.07	0.5	0.46			8.35	
09/02/01	25	28.93	0.49	0.45		7.57	8.32	
09/03/01	26	29.01	0.47	0.43			7.92	
09/04/01	27	29.18	0.63	0.57	71		8.19	
09/05/01	28	29.16	0.69	0.63			7.99	
09/06/01	29	28.86	0.61	0.56			8.08	
09/07/01	30	28.73	0.54	0.50	72		8.08	
09/08/01	31	28.97	0.45	0.41		7.82	8.15	
09/09/01	32	29.10	0.33	0.30			8.01	
09/10/01	33	29.31	0.58	0.52			8.00	
09/11/01	34	29.20	0.77	0.70			8.05	
09/12/01	35	29.16	0.61	0.55			8.10	
09/13/01	36	29.39	0.48	0.43			8.06	
09/14/01	37	29.36	0.56	0.51			8.13	
09/15/01	38	29.27	0.62	0.56			8.14	
09/16/01	39	29.11	0.58	0.53			8.13	

APPENDIX B. Spreadsheets used for model fitting

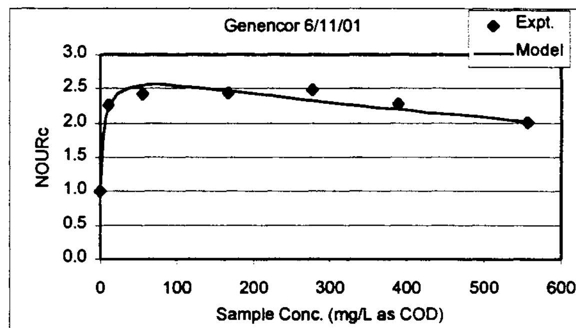
A. Model I

Nitrogenous

Gen 6'11'01			COD =	2783	mg/L		
Ks	Ki	Li	NOURs*	Ks/Ki	p(Ks/Ki)	IC50^ (mg/L)	NOURc^
5.0	1500.0	5.00	2.500	3.33E-03	2.48	1500	3

Andrews + Noncompetitve

S. Vol. (mL)	S. Conc. (mg/L)	Model	Expt.	SE
0.0	0.0	1.000	1.000	0.00E+00
1.0	11.1	2.224	2.261	1.32E-03
5.0	55.7	2.557	2.430	1.60E-02
15.0	167.0	2.473	2.441	1.02E-03
25.0	278.3	2.335	2.494	2.53E-02
35.0	389.6	2.204	2.286	6.78E-03
50.0	556.6	2.030	2.003	7.16E-04
			SSE	5.12E-02
			r2	0.9848



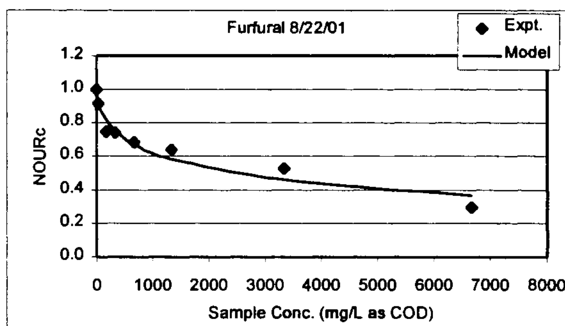
B. Model II

Nitrification

Furfural		COD =	83500	mg/L
Li	n	IC50 (mg/L)		
80.00	0.560	2503		

Andrews + Noncompetitve

S. Vol. (mL)	S. Conc. (mg/L)	Model	Expt.	SE
0.0	0.0	1.000	1.000	0.00E+00
0.1	33.4	0.918	0.915	9.71E-06
0.5	167.0	0.820	0.748	5.21E-03
1.0	334.0	0.755	0.742	1.82E-04
2.0	668.0	0.677	0.687	9.80E-05
4.0	1336.0	0.587	0.644	3.23E-03
10.0	3340.0	0.460	0.527	4.59E-03
20.0	6680.0	0.366	0.296	4.92E-03
			SSE	1.82E-02
			r2	0.9730



REFERENCES

1. Allsop, P.; Young, M.M.; and Sullivan, G.R. (1990) The Dynamics and Control of Substrate Inhibition in Activated Sludge. *CRC Crit. Rev. Environ. Control, **20**, 115-167.*

2. Anthonisen, A.C.; Loethr, R.C., Prakasam, T.B.S.; and Srinath, E.G. (1976) Inhibition of Nitrification by Ammonia and Nitrous Acid. *J. Water. Poll. Control Fed., **24**, 835-852.*

3. Barnett, M.W. and J. F. Andrews (1992) Expert System for Anaerobic Digestion Process Operation. *J. Env. Eng., **118** (6), 949-963.*

4. Blum, D. and Speece, R. (1991) A Database of Chemical Toxicity to Environmental Bacteria and Its Use in Interspecies Comparisons and Correlations. *Journal of Water Pollution Control federation, **63**, 198-207.*

5. Bott, C.B. and Love, N.G. (In press) The Immunochemical Detection of Stress Protein Expression in Activated Sludge Exposed to Toxic Chemicals. *Water Research.*

6. Ellis, T.G.; Barbeau, D.S.; Smets, B.F.; Grady, C.P. Leslie, Jr. (1996) Respirometric Technique for Determination of Extant Kinetic Parameters Describing Biodegradation. *Wat. Env. Research, **68**(5), 917-926.*

7. Geenens, D. and Thoeye, C. (1998) The Use of an Online Respirometer for the Screening of Toxicity in the ANTWERP WWTP Catchment Area. *Wat. Sci. Tech. 37(12), 213-218.*

8. Gernaey, K., Verschuere, L.,Luyten, L., and Verstraete, W. (1997b) Fast and Sensitive Acute Toxicity Detection with an Enrichment Nitrifying Culture. *Wat. Env. Research, **69**, 1163-1169.*

9. Grady, Jr., Leslie C.P.; Daigger, Glen T.; Lim, Henry C. (1999) *Biological Wastewater Treatment, 2nd Edition.* Marcel Dekker, Inc., New York.

10. Graef, S.P., and Andrews, J.F. (1974) Mathematical Modeling and Control of Anaerobic Digestion. *Water-1973, AICHe Symposium Series No. 136,* G.F. Bennett, ed., Am. Inst, of Chem. Engrs., New York, N.Y., ***70**, 101-113.*

11. Hayes, E., Upton, J., Batts, R., and Pickin, S. (1998) On-line Nitrification Inhibition Monitoring Using Immobilized Bacteria. *Wat. Science and Technology, 37, 193-196.*

12. Hemond, Harold F. and Fechner, Elizaberth J. (1994) *Chemical Fate and Transport in the Environment.* Academic Press, San Diego, California.

13. Hockenbury, C. and Plowman, R. (1997) Bioaugmentation: Put Microbes to Work. *Chemical Engineering, **104**, 74-84.*

14. Khandaker, N.R. (1996) Application of Anaerobic Respirometers for Treatability Assessment of Industrial Wastewaters. *51st Purdue Industrial Waste Conference Proceedings.* Ann Arbor Press, Inc., Chelsea, Michigan.

15. Kong, Z. P.; Vanrolleghem, P.; and Verstraete, W. (1993) Actiavted Sludge-based Biosensor for Rapid IC50 Estimation and On-line Toxicity Monitoring. *Biosensor and Bioelectronics **8**, 49-58.*

16. Kong, Z., P.; Vanrolleghem, P.; and Verstraete, W. (1994) Automated Respiration Inhibition Kinetics Analysis (ARIKA) with a Respirographic Biosensor. *Wat. Sci. Tech., **30**(4), 275-284.*

17. Kong, Z., P. Vanrolleghem, P.; Willems, P.; and Verstraete, W. (1996) Simultaneous Determination of Inhibition Kinetics of Carbon Oxidation and Nitrification with Respirometer. *Wat. Res., **30**(4), 825-836.*

18. Kroiss, H.; Schweighofer, P.; Frey, W.; and Matsche, N. (1992) Nitrification Inhibition – A Source Identification Method for Combined Municipal and Industrial Wastewater Treatment Plants. *Wat Sci. Tech, **26**(5-6), 1135-1146.*

19. Love, N. G. and Bott, C. B. (2000) A Review and Needs Survey of Upset Early Warning Devices. *Project 99-WWF-2, Water Environment Research Foundation.*

20. Metcalf and Eddy, Inc. (1991) *Wastewater Engineering – Treatment, Disposal, and Resuse, 3rd edition.* McGraw-Hill, Inc., New York.

21. Nirmalakhandan, N.; Sun, B.; Arulgnanendran, V.J.; Mohsin, M.; Wang, X. H.; Prakash, J.; and Hall, N. (1994) Analyzing and Modeling Toxicity of Mixtures of Organic Chemicals to Microorganisms. *Wat. Sci. Tech. **30**(10), 87-96.*

22. Nirmalakhandan, N.; Peace, J.; Egemen, E.; Mohsin, M.; Sun, B.; and Hall, E. (1996) Estimating Toxicity of Mixtures of Organic Chemicals to Activated Sludge Using Surrogate Test Cultures. *Wat. Sci. Tech. **34**(7-8), 87-92.*

23. Owen, W.F.; Stuckey, D.C.; Healy, J.B.; Jr.; Young, L.Y.; and McCarty, P.L. (1979) Bioassay For Monitoring Biochemical Methane Potential and Anaerobic Toxicity. *Water Research, **13**, 485-492.*

24. Perle, M; Kinshie, S.; and Shelef, G. (1995) Some Biochemical Aspects of the Anaerobic Degradation of Dairy Wastewater. *Wat. Res., **29**, 1549-1554.*

25. Richardson, M. (1985) *Nitrification Inhibition in the Treatment of Sewage.* Burlington House, London.

26. Rinzema, A., van Lier, J.B., and Lettinga, G. (1988) Sodium Inhibition of Acetoclastic Methanogens in Granular Sludge from a UASB Reactor. *Enzyme and Micro. Technol., **10**, 24-32.*

27. Rogers, K.R., and Mascini, M. (2000) Biosensors for Analytical Monitoring. Accessed 2000. Web page available at *http://www.epa.gov/crdlvweb/chemistry/iochem/into.htm.*

28. Rozzi, A., M. C. Tomei, A. C. Di Pinto, and N. Limoni (1997) Monitoring Toxicity in Anaerobic Digesters by the RANTOX Biosensor: Theoretical Background. *Biotechnology and Bioengineering, **55**(10), 33-40.*

29. Santiago, I. and Grady, L. (1990) Simulation Studies of the Transient Response of Activated Sludge. *J. Sanit. Eng., **33**, 125-132.*

30. Smith, D.P., and McCarthy, P. L. (1989) Energetics and Rate Effects on Methanogenenesis of Ethanol and Propionate in Perturbed CSTRs. *Biotechnol. Bioeng., **34**, 39.*

31. Spanjers, H.; Vanrolleghem, P; Olsoon, G.; and Dold, P. (1996) Respirometry in Control of the Activated Sludge Process. *Wat. Sci. Tech, **34**(3-4), 117-126.*

32. Speece, R.E. (1996) *Anaerobic Biotechnology for Industrial Wastewaters.* Archae Press, Nashville, Tennessee.

33. Sun, B.; Nirmalakhandan; Hall, E.; Wang, X.H.; Prakash, J.; and Maynes, R. (1994) Estimating Toxicity of Organic Chemical to Activated-Sludge Microorganisms. *Journal of Environmental Engineering, **120**(6), 1459-1469.*

34. Tanlinli, Ilhan and Sertac Tokta (1994) Oxygen Uptake Rate Inhibition Test: A Modified Method for Priority Pollutants. *Environmental Technology, **15**, 979-98.*

35. Temmink, H.; Vanrolleghem, P.; Klapwijk, A.; and Verstraete, W. (1993) Biological Early Warning Systems For Toxicity Based On Activated Sludge Respirometry. *Wat. Sci. Tech.*, ***28****(11-12), 415-425.*

36. Vanrolleghem, P.; Kong, Z.; and Coen, F. (1996) Full-scale On-line Assessment of Toxic Wastewaters Causing Change in Biodegradation Model Structure and Parameters. *Wat. Sci. Tech.*, ***33****(2), 163-175.*

37. Vanrolleghem, P.; Kong, Z.; Rombouts, G.; and Verstraete, W. (1994) An On-line Respirographic Biosensor for the Characterization of Load and Toxicity of Wastewaters. *J. Chem. Tech. Biotechnol.*, ***59****, 321-333.*

38. Volskay, V. T. and Grady, Leslie C. P., Jr. (1988) Toxicity of Selected RCRA Compounds to Activated Microorganisms. *J. of Wat. Pollution Control Fed.*, ***60****(10), 1850-1856.*

39. Volskay, V.T and Grady, Leslie C. P., Jr. (1990) Respiration Inhibition Kinetic Analysis. *Wat. Res.*, ***24****, 863-874.*

40. Young, J.C. (1996) Fundamentals of Respirometry: Instrument Types and Basis of Operation. *51st Purdue Industrial Waste Conference Proceedings.* Ann Arbor Press, Inc., Chelsea, Michigan.

41. Young, J.C. and Tabak, H. (1993) Multilevel Protocol for Assessing the Fate and Effect of Toxic Organic Chemicals in Anaerobic Treatment Processes. *Wat. Env. Research,* ***65****(1), 34-45.*

42. Young, J.C. and Khandaker, N. R. (1995) Anaerobic Protocol For Assessing Industrial Waste Treatability. *50th Purdue Industrial Waste Conference Proceedings.* Ann Arbor Pres, Inc., Chelsea, Michigan.

43. Young, J.C.; Kuss, M.L.; and Nelson, M.A. (1991) Use of Anaerobic Respirometers for Measuring Gas Production in Toxicity and Treatability Tests. *84th Annual Meeting & Exhibition.* Vancouver, British Columbia.

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