EVALUATING THE SOURCE-EFFECT RELATIONSHIP OF INDUSTRIAL TOXINS IN WASTEWATER TREATMENT

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ABSTRACT

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A modified respirometric assay protocol was developed to allow the industrial WWTP to screen industrial wastewaters for inhibitory properties. All five industrial wastewaters tested revealed inhibitory properties. Large day-to-day variations were found, illustrating the need for a large database of results for comparison over time. Additionally, a small volume contributor, that was thought by the utility to be an unlikely source of problems, contributed significantly to the wastewater oxygen demand and demonstrated inhibitory properties. The modified respirometric procedure enabled the WWTP to identify possible industrial sources that could cause an upset event.

Lab-scale sequencing-batch reactors were used to determine the effects of cyanide and pH shock on activated sludge. Three reactors were shocked with increasing weak-acid complexed zinc cyanide or pHs of 5, 9, and 11. The resulting effects were compared to an un-shocked control reactor. It was found that respiration and nitrification were affected by the zinc cyanide complex, while COD removal, effluent TSS and dewaterability were not. Recovery was seen in less than 2 X solids residence time (SRT) for the nitrifying biomass and within 3 X SRT for the non-nitrifying biomass. The results of the pH experiment showed that the pH 11 shock affected the settleability, nitrification, COD removal, and effluent TSS levels of the reactors, while pH 5 and pH 9 shocks had no effect. Recovery was seen within 3 X SRT for both the nitrifying and non-nitrifying systems.

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1. INTRODUCTION

WWTP vulnerability

Despite being well designed, a wastewater treatment plant may be affected by sudden changes in influent wastewater characteristics such as the introduction of toxic or inhibitory chemicals, which can cause a treatment plant upset event. The source of these problematic chemicals is often industrial wastewater streams, which tend to be highly variable in composition, concentration, and flow (Geenens and Thoeye, 1998; Pardos and Blaise, 1999). The potential for wastewater treatment to deteriorate is exacerbated when the unpredictable nature of the flow is coupled with an event such as a discharge from an industrial start-up, a cleanout, or an accidental spill (O'Brien and Teather, 1995). Furthermore, as technology and industrialization advances, industrial discharge characteristics and toxicity become more complicated and more difficult to treat (Sponza, 2000). Often the toxicity in these waste streams arises from heavily concentrated wastes, which may consist of poorly degradable synthetic organic compounds, volatile organic compounds and heavy metals (Reemtsma et al., 1999). Moreover, the fraction of toxic or inhibitory organics that are soluble in industrial wastewaters often cannot be removed by coagulation and will flow to the bioreactor where the potential for plant impairment is greatest (Eckenfelder and Musterman, 1994). Furthermore, the upset event may not be the consequence of one particular toxicant, but a mixture of two or more. As a result, it is important to recognize the possibility of joint-toxic effects that low concentrations of chemicals in mixtures may cause (Hall et al., 1996).

Exposing the source

Most wastewater treatment plants have the means to detect changes in dissolved oxygen (DO) levels of the bioreactors to determine the status of plant operation (Cadena, 1995). The relative ease of measuring changes in DO levels at a treatment plant has lead to the use of respirometry as an indicator of biomass health or inhibition. Unfortunately, normal respirometry tests are a gross parameter, constrained by the detection of biomass inhibition and not the inhibition source.

It is often difficult for a wastewater treatment plant to expose the logical source or culprit of an upset event, due to the transient nature of toxic shock and influent organic

loads (Kelly et al., 1999). Furthermore, once toxic or inhibitory substances reach the bioreactor, operators are faced with a reactive, rather than proactive response (Cadena, 1995; Love and Bott, 2000). Ultimately, upset events can result in effluent violations and associated governmental fines, not to mention extended plant recovery. So, exposing the source of the event becomes paramount, not only for possible deferment of fines, but to prevent continued or future plant deterioration. Since respirometry is unable to identify sources that can result in significant effects, there is a need for another means to identify toxic sources.

Exposing the effects

Little is known about how different chemical sources upset process performance (Love and Bott, 2000). An upset event can vary from a temporary deterioration in performance to a prolonged effect, depending on the length of the influent disturbance (Love and Bott, 2000). Many process deterioration effects are believed to result from upset events caused by toxic or inhibitory substances. Love and Bott (2000) surveyed Water Environment Research Federation (WERF) subscribers to identify the most common treatment effects of upset events. It was reported that ineffective chemical and biological oxygen demand (COD/BOD) removal, ineffective nitrification, deflocculation, non-filamentous foaming, non-filamentous sludge bulking, and ineffective denitrification were the most common effects. Respiration inhibition and loss of biomass are also known to occur (Eckenfelder and Musterman, 1994).

By determining common treatment effects associated with certain chemical sources, it will be possible to develop a system that allows operators to know what treatment effects to expect based on the source. This will give operators an opportunity to limit treatment deterioration by applying the necessary corrective action.

Defining the experiment

This thesis is a collection of results from three different experiments that are related primarily by the issue of toxicity. One study component considered the source effect relationship for known toxins. A second study component evaluated a protocol for detecting respirometric toxicity in industrial wastewaters.

Realizing the need for an in-depth look at the source-effect relationship for different potential toxicants, WERF provided funding to investigate this relationship for seven different toxic or inhibitory substances. Two of these source-effect studies, cyanide and pH, are presented herein. Common treatment effects for both cyanide and pH shock were determined.

  While examining the effects of pH and cyanide on activated sludge, an opportunity to investigate the source of a severe upset event at a regional industrial wastewater treatment plant presented itself. Assistance was requested to help pinpoint the source problem industry. No upset events occurred during this testing, so a culprit was impossible to determine. However, through testing, potential inhibitory industrial sources were identified. The results from this experiment are also presented herein

2. MATERIALS AND METHODS

2.1. HOPEWELL SAMPLING

2.1.1. Study Site

Hopewell Regional Wastewater Treatment Facility (HRWTF) handles wastewater from a primary wastewater treatment plant and all the surrounding industries. The primary plant is a 6.5 million gallons per day (mgd) domestic wastewater treatment facility with flows received from Hopewell, parts of Prince George County, The Federal Correction Institute, Riverside Regional Jail, and Fort Lee. The domestic wastewater undergoes primary treatment and chlorination. The effluent is then pumped to HRWTF. All industrial wastewater, which is 85% of the total flow and the majority of the total COD load, feeds directly into the HWRTF headworks. There are five major contributing industries: 1) Honeywell; 2) Hercules; 3) Goldschmidt; 4) Smurfit-Stone (formally Stone Container); and 5) Stone-HAP (the hazardous air particles division of Smurfit-Stone. The majority of these industries produce a variety of different chemicals, with some industries continually changing the chemicals produced. HRWTF is designed to treat 50 mgd of combined domestic and industrial wastewaters to meet all Federal and State water quality standards. Currently, HRWTF handles about 35 mgd of combined domestic and industrial wastewater.

The integrated domestic and industrial waste stream enters the headworks where it flows through three bar screens into eight primary clarifiers. The waste stream then flows into the UNOX covered aeration tanks for secondary treatment. The high-purity oxygen, which is produced in an on-site cryogenic plant, is pumped into these four sealed reactors. The wastewater then flows into the final clarifiers and the treated effluent is discharged to the James River. The biosolids removed from the waste stream are pumped into gravity thickeners and then sludge holding tanks. In the final stage the biosolids are sent to the dry solids centrifuges where up to 65 percent of the water is removed. The "dry" biosolids are then destroyed in an on-site incinerator and the residual ash is disposed of in an approved landfill.

2.1.2. Current Hopewell Sampling Procedures

Hopewell operators collect approximately 3L of mixed liquor from the basin

before flowing to the clarifier, and 3L of final effluent from the secondary clarifier. These samples are placed together in a bucket for a 1:1 dilution. Samples from each industrial wastewater are also collected. An operator then adds a certain volume of an industrial wastewater to a 300ml biological oxygen demand (BOD) bottle and fills the remaining volume with the 1:1 mixed liquor solution. The operator then measures with a stopwatch and tabulates the time it takes for the DO level to decrease by 50%. This procedure is only implemented when loss of treatment is suspected.

2.1.3. Collection and Shipment

Twenty-four hour composite samples of the final effluent from HRWTF (3 L) and five industrial effluents (300 ml each) were collected in polyethylene plastic bottles at HRWTF every Monday and Thursday from June 19 to August 16, 2002, excluding a two week period between June 26 and August 13. A grab sample of mixed liquor (3 L) was collected from the basin before flowing to the secondary clarifier, and shipped at the same time. All samples were packaged in ice and shipped overnight to the Virginia Tech environmental engineering laboratories. Goldschmidt samples were not sent on Mondays, because the plant is closed over the weekend and no composite sample could be obtained.

2.1.4. Resuscitation and Dilution

Coolers were received late Tuesday and Friday mornings. The mixed liquor sample was removed from the ice and allowed to settle. One liter of the supernatant was removed and replaced with one liter of Blacksburg/Virginia Tech Wastewater Treatment Plant raw influent. The supplemented sample was poured into a twenty-gallon bucket. The three liters of mixed liquor were aerated and mixed on a stir plate for one hour at room temperature to revive the bacteria and restore aerobic conditions. After one hour, three liters of HRWTF final effluent were added to the bucket to create a 1:1 dilution. An additional six liters of Virginia Tech Pilot Plant 10 day solids retention time (SRT) effluent was added to decrease the mixed liquor concentration and to enable duplicate assays to be run for all conditions. Aeration and mixing were continued for one more hour before sampling began, and continuously from that point on.

2.1.5. Biogenic Substrate Solution

A readily biodegradable biogenic substrate was added at a rate of 2 ml per minute, Beginning with the second hour of aeration. The biogenic substrate solution is the same as that described in paragraph 2.3.1. A solution with a total COD of 31,250 mg/L was fed at 1 ml/min for 4 to 6 hours during testing. The goal of this addition was to revive the biomass to a physiological state that remained relatively constant during the experiment and to minimize errors due to changes in the mixed liquor concentration during the testing procedure. Mixed liquor suspended solid measurements were taken from the mixed liquor stock bucket at the start of sampling, periodically throughout, and at the conclusion of sampling. The average MLSS concentration obtained from these samples was used to normalize SOURs, and the percent change averaged –8% to +9% (negative denoting decrease, positive denoting increase).

2.1.6. Specific Oxygen Uptake Rate (SOUR) Testing Procedure

Experimentation started after the two-hour resuscitation period. There were two phases to the SOUR sampling. SOUR assays were conducted by adding a known volume of a chemical effluent to a 300 ml BOD bottle and filling the remainder with diluted mixed liquor. The first stage of sampling involved SOURs performed on all five industrial wastewaters at volume additions of 3 ml, 5 ml and 10 ml. The mixed liquor/final effluent SOUR contained no industrial effluent, so it was used as the control, or endogenous SOUR, for the experiments on that particular day. This control was maintained in a semi-endogenous state through the addition of the biogenic soluble substrate. No biogenic substrates were added during the SOUR experiments. The second stage of sampling involved SOURS performed on all five industrial wastewaters at volume additions similar to the dilutions of the particular wastewater seen at the headworks of HRWTF. These dilution volumes are discusses in more detail in section 3.2.

2.2.   PILOT PLANT

The biomass used for the both the pH and cyanide laboratory source-effect experiments was taken from 10 day and 2 day SRT pilot plant systems. The experiments were conducted according to the schedule provided in Table 2.1.

Table 2.1   Dates when source-effect experiments occurred.

Experiment	Dates
10 day pH	3/22/02-4/20/02
2 day pH	11/22/02-11/28/02
10 day Cyanide	2/19/03-3/4/03
2 day Cyanide	3/11/03-3/17/03

2.2.1. Design and Setup

The pilot plant facility received wastewater from the Virginia Tech campus and was designed to serve as a consistent source of mixed liquor for laboratory stress experiments. The facility consisted of two sequencing batch reactors (SBRs) maintained at two different solids retention times (SRTs) to discourage nitrification in one reactor but to allow it to occur in the other. The reactor configurations and operating parameters are summarized in Table 2.2 below. The pilot plant began operation on March 23, 2001.

Table 2.2   Configurations and operating parameters for pilot plant systems.

	System A	System B
Reactor Working Volume	180 L	180 L
Target Temperature	20°C	20°C
Target SRT	2 days	10 days
Target Nominal Hydraulic Retention Time (HRT)	1 day	1 day
Cycles per Day	4	4
Feed Time per Cycle (with mixing and aeration on)	Step feed: 7 min every 1.5 hours	~ 6 min
React Time per Cycle	5 hours	5 hours
Settling Time per Cycle	1 hour	1 hour

2.2.2. Daily Maintenance and Operation

Wastewater was pumped from an adjacent manhole to two 200 L tanks. Starting September 2001, a supplementation program was initiated to boost the COD load to the

pilot plant reactors and, therefore, achieve higher mixed liquor suspended solids concentrations. The supplement added 400 mg/L COD and contained equal COD quantities of acetate and glucose as well as a 15:30 fertilizer (K-mart GrowBest All Purpose Plant Food). The amount of plant fertilizer to be added was determined based on an average value of influent ammonia of 18 mg/L N (before supplement addition), an average total COD value of 768 mg/L (including supplement COD) in the influent and a growth yield of 0.5 mg COD biomass/mg COD. It was determined that 20 g of fertilizer/day should be added to each reactor, which is equivalent to 15 mg/L N. In addition to nitrogen, the fertilizer also added 13.1 mg/L P, 12.5 mg/L K, 0.15 mg/L Fe, 0.07 mg/L Cu, 0.06 mg/L Zn, 0.05 mg/L Mn, 0.02 mg/L B and 0.0005 mg/L Mo. Calcium sulfate was also added to the supplement at 20 g $CaSO_4 \cdot \frac{1}{2} H_2O$/reactor-day in order to maintain the monovalent:divalent cation ratio in the influent below 4 (on a milliequivalent basis) to avoid causing deflocculation through an imbalanced cation ratio (Higgens and Novak, 1997a); the amount to be added was based on average cation concentrations measured in the influent with ion chromatography prior to supplement addition.

Due to difficulties blocking nitrification in the 2 day SRT reactor, a new supplement program was implemented for the 2 day SRT reactor. The COD load was doubled (400 mg/L COD as glucose + 400 mg/L COD as sodium acetate) and the amount of fertilizer was increased by a factor of 3. Moreover, the influent was pumped into the 2 day SRT reactor on a step-feed mode, by which 1/3 of the total influent volume is pumped at the beginning of a cycle, 1/3 one and a half hours later and the last 1/3 of the volume 3 hours after the beginning of the cycle. This new feed design, which was started August 2002, allowed for higher COD concentrations to be maintained in the reactor, thereby favoring the growth of heterotrophic organisms and limiting the development of a significant nitrifying population. The higher COD loading also allowed for higher biomass concentrations to be achieved. Due to the increase in COD loading, the amount of calcium sulfate added was increased accordingly; this increase resulted in the development of high concentrations of sulfides forming in the sewage holding tank, and subsequently being pumped into the reactor. This ultimately led to an uncontrolled growth of filamentous organisms. The reactor was reseeded and calcium carbonate was

used instead to avoid formation of sulfide-dependant filaments. Therefore, a total of 36 g $CaCO_3$ was added to the 2 day SRT influent tank each day.

Although there were few problems achieving nitrification in the 10 day SRT reactor system, a change was made to this system in August 2002 as well to establish a higher level of nitrifying bacteria in the 10 day SRT biomass. The ammonia supplement to the sewage was increased to yield a total average influent ammonia-N concentration around 45 mg/L, which is 50% higher than during the previous supplementation period. The source of additional divalent cations was also changed to calcium carbonate, to a total of 14 g $CaCO_3$ per day.

In addition to supplement addition, the sewage was settled for 30 minutes prior to being pumped into the reactors at the beginning of a cycle, and was mechanically mixed for ½ hour before the settling period and aerated at all other times to prevent the development of septic conditions. Wastage was collected and measured for each of the two reactors and corrected weekly to maintain the proper solids retention time. Any attached biomass was scraped daily from the reactor walls back into the suspension. The ambient temperature in the shed housing the pilot plant was monitored to ensure that it remains between 20 and 25ºC in the winter for proper operation of the nitrifying and non-nitrifying reactors. Similarly, the shed was air-conditioned during the summer to maintain an ambient temperature of 20ºC. Controlling the ambient temperature ensured that the temperature of the mixed liquor in the reactors stayed at around 20ºC

2.3. LABORATORY-SCALE SOURCE EFFECT EXPERIMENT REACTORS

2.3.1. Inhibitory Concentrations (IC_{XX})

Inhibitory concentrations (IC_{XX}) for the cyanide source-effect expiriment were determined one day prior to initiating each source-effect experiment to ensure that mixed liquor composition was representative of the biomass that will be used to inoculate the source-effect reactors. The procedure was based on inhibition of respiration. Tests were using the SOUR procedure outlined in section 2.4.9. Mixed liquor from the 2 and 10-day SRT pilot plant reactors was used in the IC_{XX} assays. The mixed liquor was aerated for 4 hours prior to testing to remove any biodegradable materials, and both MLSS and MLVSS were determined. Undiluted mixed liquor was then added to 300 ml BOD bottles and spiked with varying masses of contaminant. Soluble COD was composed of

$^1/_3$ protein (beef extract, phytone, bacto-casitone, yeast extract), $^1/_3$ carbohydrate (fructose, galactose, glucose) and $^1/_3$ organic acids/alcohols (glacial acetic acid and glycerol) on a COD basis (see Appendix F). The soluble COD was added to the mixed liquor in the BOD bottle to achieve a starting concentration of 100 mg/L as COD, to ensure that unrestricted respiration would occur throughout the 10 minute test. Oxygen consumption rate tests were conducted to determine the oxygen uptake rate (OUR) of the mixed liquor exposed to the contaminant, and were performed according to section 2710 B of Standard Methods (APHA, 1998). Specific OURs (SOURs) were calculated by dividing the OUR by the MLVSS concentration, and the inhibitory concentrations were determined by plotting SOUR versus contaminant concentration or % inhibition versus contaminant concentration and fitting a second order polynomial or exponential function through the data (best fit determined by least squares analysis, Excel 2000 or Sigma Plot). By looking at the inhibition relative to the control reactor, the IC_{15}, IC_{25}, and IC_{50} concentrations were determined for the laboratory source-effect experiments.

Cyanide was added in a weak acid form to prevent problems with volatilization of hydrogen cyanide (discussed later in section 4.2). The MINEQL+ software program (Environmental Research Software®, 1998) was used to calculate the amount of un-complexed zinc, as zinc sulfate, added to sodium cyanide to form a zinc-cyanide complex. MINEQL+ uses solubility constants and operator-inputted values of pH and elements/compounds present to calculate concentrations. For the simulation, it was assumed that pH was 7.6 and no elements/compounds were present in the reactor other than zinc, cyanide, and water.

Icxx assays were not determined for the pH source-effect experiment. Instead, the pH maintained in the control reactor for previous experiments averaged between 7 and 8 was used as a basis for selecting shock pHs. The pH levels chosen for this study illustrate the effect of pH over a wide range. An acidic pH of 5 and alkaline pHs of 9 and 11 were chosen, while the control reactor pHs averaged 7.67 ± 15 and 7.58 ± 16 for the 10 and 2 day systems, respectively. A solution containing equal concentrations (on a milliequivalent basis) of NaOH and $Ca(OH)_2$ was used for the base pH adjustment experiments to avoid causing deflocculation through an imbalanced monovalent:divalent ratio. For the acid shocked reactor, 1N sulfuric acid was used instead of HCl to prevent chloride interference during the COD and ion chromatography procedures.

2.3.2. Reactor Configuration

The laboratory-scale reactors were designed to mimic the pilot-scale sequencing batch reactors. The laboratory system consisted of four beakers maintained at the 10-day target SRT and four maintained at the 2-day target SRT. Within each SRT, one reactor were used as a control reactor and the other three were shocked with varying concentrations of contaminants. Reactor configurations and operating parameters are summarized in Table 2.3 below. Times for SBR phases were chosen to closely coincide with pilot plant values. The only exception to this was that step-feeding was not used in any of the 2 day SRT experiments. Temperature control, in the form of a 13 degree water bath, was used instead to prevent nitrification from returning.

Table 2.3   Configurations and operating conditions for laboratory-scale reactors.

Reactor Working Volume	3.5 L
Target Nominal Hydraulic Retention Time (HRT)	1 day
Cycles per Day	4
Feed Time per Cycle (with mixing and aeration on)	~ 2-3 min
React Time per Cycle	5 hours
Settling Time per Cycle	1 hour

2.3.3. Daily Maintenance and Operation

To perform toxic shock experiments, soluble contaminants were manually added with the influent during the first cycle of operation. For all subsequent cycles, influent was pumped over a period of approximately 2 to 3 minutes. The influent pump flow rates were checked daily. Influent used to feed the laboratory reactors was obtained daily from the same manhole that supplied the pilot plant. It was settled for 30 minutes to mimic primary clarification before being decanted to a 20 L bucket. The settled influent received the same supplemental feed as the pilot plant influent. The influent for the

laboratory reactor experiments was mixed and refrigerated throughout the toxic shock experiments.

During one cycle each day but before wastage, attached growth biomass was scraped off of the reactor walls back into suspension to maintain a suspended growth system. Mixed liquor was wasted manually once daily from the 10-day reactors due to the low wastage volumes required. Wastage was automatically controlled for the 2 day reactors and was removed toward the end of each cycle. Wastage volumes were corrected to account for sampling, but were not corrected to account for suspended solids in the effluent (rather, the true SRT was calculated based on measured effluent TSS). Timers used to control the mixers, air compressors and influent, effluent and wastage pumps were checked daily to ensure proper operation. J-shaped tubes were used to waste biomass from the 2-day reactors and to decant the treated effluent from all reactors. The height of these tubes was inspected daily to ensure the proper volumes are maintained.

2.3.4. Monitoring Schedule

Samples were collected to monitor influent, effluent and mixed liquor quality characteristics. Tables 2.4 and 2.5 list the frequency and name of tests performed during operation of the reactors for both the 10 and 2 day SRT systems, respectively. For all influent tests, samples were pulled from the influent bucket during mixing to ensure uniform sampling. For all effluent tests, the total effluent volume for a cycle was collected and mixed before testing to maintain more uniform samples and to eliminate any changes in effluent quality over the decant time. Mixed liquor samples were pulled at the beginning of the cycles under mixed and aerated conditions to ensure that solids were properly mixed. The reactors were operated for a minimum of one SRT and no more than 3 SRTs. If recovery (defined as a return of the quality parameters to control reactor levels for a considerable number of cycles) was noted within this time, reactor operation was discontinued.

2.4. ANALYTICAL PROCEDURES

Constituents were analyzed as described below. In all cases, soluble is defined as that which passes through a 0.45 µm filter. For the pH and cyanide complex source-effect

studies, samples were analyzed according to Tables 2.4 and 2.5 for the 10 and 2 day SRT experiments, respectively.

2.4.1. Alkalinity

Samples for alkalinity were run in duplicate due to the high degree of reproducibility. Alkalinity was performed using a potentiometric titration to a pH of 4.5 as outlined in section 2320 B of Standard Methods (APHA, 1998).

2.4.2. Ammonia

Ammonia-nitrogen was analyzed either by distillation/titration (methods 4500-NH_3 B and 4500-NH_3 C) as described in Standard Methods (APHA, 1998), or by ion chromatography. For the latter, 5 ml of soluble sample was injected into a Dionex DX120 suppressed conductivity ion chromatograph using an AS40 autosampler. The DX120 used a CS12 cation separation column with a CG12 guard column for cation analysis of NH_3 and the sample was carried in an eluent of 20 mM methane sulfonic acid. All samples were filtered and stored at -20°C for no more than 28 days prior to ion chromatography analysis.

Table 2.4   10-day SRT laboratory-scale reactor monitoring schedule.

	Test	1st day	2nd and 3rd days	4th to 10th day	11th to 30th day
Effluent	Soluble Contaminant	every cycle	every other cycle	every day	every other day
	COD	every cycle	every other cycle	every day	every other day
	PH	every other cycle	every day	every other day	every 4 days
	TSS	every cycle	every other cycle	every day	every other day
	Ammonia	every cycle	every other cycle	every day	every other day
	Nitrite/Nitrate	every cycle	every other cycle	every day	every other day
	Alkalinity	every other cycle	every day	every other day	every 4 days
Influent	COD	every day	every day	every day	every day
	Ammonia	every day	every day	every day	every day
	TSS/VSS	every day	every day	every day	every day
	PH	every other day	every other day	every other day	every other day
	Alkalinity	every other day	every other day	every other day	every other day
	TKN	every 3 days	every 3 days	every 3 days	every 3 days
Mixed Liquor	SVI	1st cycle	every day	every day	every other day
	Carbonaceous SOUR	1st cycle	every day	every other day	every 4 days
	NGR	1st cycle	every day	every other day	every 4 days
	MLSS/MLVSS	1st cycle	every day	every day	every other day
	CST	1st cycle	-	-	last cycle
	Microscopic Evaluation	1st cycle	every day	every other day	every other day
	Total Contaminant	every cycle	every day	every day	every other day

Table 2.5   2-day SRT laboratory-scale reactor monitoring schedule.

	Test	1st day	2nd, 3rd and 4th days	5th to 7th day
Effluent	Soluble Contaminant	every cycle	every other cycle	every day
	COD	every cycle	every other cycle	every day
	pH	every other cycle	every day	every other day
	TSS	every cycle	every other cycle	every day
	Ammonia	every cycle	every other cycle	every day
	Nitrite/Nitrate	every cycle	every other cycle	every day
	Alkalinity	every other cycle	every day	every other day
Influent	COD	every day	every day	every day
	Ammonia	every day	every day	every day
	TSS/VSS	every day	every day	every day
	pH	every other day	every other day	every other day
	Alkalinity	every other day	every other day	every other day
	TKN	every 3 days	every 3 days	every 3 days
Mixed Liquor	SVI	1st cycle	every day	every day
	Carbonaceous SOUR	1st cycle	every day	every other day
	NGR	1st cycle	every day	every other day
	MLSS/MLVSS	1st cycle	every day	every day
	CST	1st cycle		last cycle
	Microscopic Evaluation	1st cycle	every day	every other day
	Total Contaminant	every cycle	every day	every day

2.4.3. Capillary Suction Time (CST)

Capillary suction time (CST) is an indicator of mixed liquor dewaterability. CST was performed according to section 2710 G of Standard Methods (APHA, 1998).

2.4.4. Chemical Oxygen Demand (COD)

COD tests were performed on total and soluble fractions of the influent and on soluble fractions of the effluent for each reactor. The test was performed following the procedure outlined in section 5220 C of Standard Methods (APHA, 1998), with minor modifications. The ferrous ammonium sulfate titrant concentration was changed to 0.05 M to increase sensitivity, and the mercuric sulfate concentration was decreased to 1.33 g/L because the samples contained low levels of chloride. This action was implemented to reduce the volume of hazardous waste generated.

2.4.5. Microscopic Observations

Microscopic evaluations were performed with approximately 0.4 ml of mixed liquor from each reactor using wet mounts with a cover slip. Floc morphology was characterized as per Jenkins *et al.* (1993) and floc diameter was also measured. The following parameters were measured qualitatively on a 0-4 scale: shelled amoebae, small flagellates, large flagellates, free-swimming ciliates, carnivorous ciliates, crawling ciliates, wide-mouthed stalked ciliates and narrow-mouthed stalked ciliates. Metazoa (rotifers, nematodes, etc.) were monitored for general abundance. Filament abundance as measured using the subjective scoring scale given by Jenkins *et al.* (1993). The microscope used for general evaluations was a brightfield Olympus model CH-2 with 10X eyepieces. Filament evaluations were performed using a phase contrast brightfield Olympus model BH-2 with 15X eyepieces. All evaluations were performed using the 10X objective lenses located on each microscope. Microscopic observations for the cyanide and pH source-effect experiments are found in Appendix E.

2.4.6. Nitrate/Nitrite

Samples for nitrate and nitrite were analyzed in duplicate using ion chromatography (method 4110 C) as described in Standard Methods (APHA, 1998). All samples were filtered and stored at $-20^{o}C$ for no more than 28 days. Upon thawing the samples, 5 ml of each was loaded into a vial and placed into an AS40 autosampler. Samples were injected into a Dionex DX120 suppressed conductivity ion chromatograph containing an AS14 anion separation column with an AG14 guard column for anion analysis. Samples were carried in an eluent of 3.5 mM Na_3CO_3 and 1.0 mM $NaHCO_2$.

2.4.7. Nitrate Generation Rate (NGR)

For the NGR tests, 300 ml samples were pulled from the reactors and placed in well aerated, 500 ml wide mouth Erlenmeyer flasks. These samples were spiked with 100 mg/L soluble COD (same as that described in section 2.3.1) and 20 mg/L $(NH_4)_2CO_3$ as N to achieve the maximum nitrate generation rate. Starting at time 0, 10 ml samples were collected every 10 minutes. Samples were centrifuged for 3 minutes on a Fisher Scientific Centrific centrifuge at 3400xg to pellet the solids. The supernatant was removed and stored at $-20^{o}C$ for no more than 28 days. For analysis, these samples were

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thawed and filtered through a 0.45 μm filter, and analyzed for nitrate by ion chromatography as described in section 2.3.7. The NGR (mg/L-min as oxygen demand) was determined from the nitrate profile by calculation, as described for the IC_{xx} determination procedure. The residual mixed liquor used during the NGR assay was re-introduced to the reactors as only a small quantity of readily degradable COD and ammonia were added, and no toxin was added. Therefore, it was possible to use as much biomass as was needed to achieve reliable results.

2.4.8. Sludge Volume Index (SVI)

SVI analysis was conducted on mixed liquor samples as per section 2710 D in Standard Methods (APHA, 1998). A 250 mL graduated cylinder was used for the test so that more accurate volume readings could be taken.

2.4.9. Specific Oxygen Uptake Rate (SOUR) for Source-Effect Experiments

Mixed liquor samples were pulled and analyzed by the SOUR assay protocol in duplicate for each reactor to determine the SOUR. The samples were aerated for 5 minutes before being placed in a 300 ml BOD bottle and spiked with 100 mg/L COD (defined in section 2.2.1) to ensure that respiration was not substrate limited. Oxygen uptake rate tests followed the procedure outlined for oxygen consumption rate tests given by section 2710 B of Standard Methods. These samples were re-introduced to the reactors after SOURs were completed as only a small quantity of readily degradable COD was added.

An automatic data acquisition system was used to run the oxygen consumption rate tests. This system consisted of two Orion oxygen electrodes, model 97-08, attached to an Acumet dual channel pH/Ion meter, model AR25. Dissolved oxygen readings were recorded every 6 seconds using LabView 6i software operating on a Dell Dimension XPS P100c personal computer.

2.4.10. pH

pH measurements were taken according to Section 4500-H^+ B in Standard Methods. pH measurements were made using an Acumet pH meter model 910 with an Acumet liquid-filled polymer body combination electrode and a Ag/AgCl reference element.

2.4.11. Potassium

Samples for soluble potassium were run in triplicate using a Perkin-Elmer 5100 PC Atomic Absorption (AA) Spectrophotometer. Samples were drawn by vacuum through a capillary tube and carried through a gas mixtures of acetylene and air. The gas stream carried the sample through a flame, which ionizes metals. Light is then passed through the flame and the potassium ions absorb an amount of light (at a wavelength of 766.5 nm) proportional to the amount of ions present. The decrease in light detection corresponds to the amount of potassium ions present, which is converted by the AA to a concentration of potassium in mg/L. The AA has a detection limit of 2.5mg/L potassium, so 1:20 dilutions were necessary. 1 ml of Cesium chloride ($CsCl_2$) was added to the diluted sample volume of 10 ml (1 ml of diluted sample was extracted to allow for $CsCl_2$ addition) to eliminate sodium interference.

2.4.12. Suspended Solids

Total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed using the method outlined in sections 2540 D and 2540 E of Standard Methods (APHA, 1998). TSS and VSS wereperformed on mixed liquor samples, whereas TSS only was analyzed for effluent samples due to the low concentration of suspended solids in the samples and limited sample volumes available. Volumes to be filtered were determined by ease of filtration, with volumes requiring less than two minutes to filter at 20 psi vacuum.

2.5.   DATA PRESENTATION AND STATISTICAL ANALYSIS

For most of the quality parameters monitored during this study, duplicate or triplicate measurements were conducted so that statistical analysis could be performed (Table 2.6). Actual values are given in Appendices B through D.

Table 2.6   Number of replicates for each of the quality parameters monitored during the source-effect experiments.

Effluent quality parameter	# replicates	Mixed liquor quality parameter	# replicates	Influent quality parameter	# replicates
Ammonia	2	MLSS/MLVSS	3	COD	3
Nitrite/Nitrate	2	SOUR	2	TSS	3
Potassium	2	NGR	1	pH	2
Soluble COD	3	SVI	1	Alkalinity	2
TSS	3	CST	3	TKN	2
pH	2	Microscopic Evaluation	1	Ammonia	2
Alkalinity	2				

In the cyanide and pH source-effect experiments, data are presented as the average of the triplicate measurements with error bars representing one standard deviation. When duplicates were measured, as in all Hopewell SOUR measurements, error bars are used to represent the higher and lower values, while the bar graph represents the average measurement.

To assess recovery of the shocked reactors to control levels, statistics were performed on the duplicate and triplicate sample tests using Dunnett's method for multiple comparisons with a control. In all cases, the significance level tested was $\alpha = 0.05$. When an effect was significantly different, the probability was less than 0.05. In the text, this is designated by stating that $\alpha = 0.05$. Dunnett's critical t-values used in the calculation were provided by the Virginia Tech Statistics Department, Blacksburg, VA . Microsoft Excel was used to carry out the calculations. An example calculation is given in Appendix A.

3. HOPEWELL REGIONAL WASTEWATER TREATMENT FACILITY (HRWTF) RESPIROMETRIC AND INHIBITION EXPERIMENTS

3.1. LITERATURE REVIEW

Methods for detecting activated sludge metabolic health

Owing to the complexity of industrial effluents, there is a need for a screening method that can accurately detect the metabolic health of the activated sludge and the toxicity of influent wastewaters (Reemtsma et al., 1999). Conventional testing parameters of COD and suspended solids used in many plants are often unable to detect the presence of inhibitory or toxic chemical in the waste stream (Hao et al., 1996). Several new biological assays and systems have been developed that are better able to identify and quantify toxicity or inhibition problems in wastewater treatment plants than conventional parameter testing (Tzoris et al., 2002). The ideal assessment method of activated sludge health and wastewater toxicity is inexpensive, on-line, sensitive, requires minimum operator attention, and has a rapid response time (Dorwood and Barisas, 1984; Massone et al., 1998; Kelly et al., 1999; Reemtsma et al., 1999; Love and Bott, 2000; Gu and Choi, 2001). The majority of the newer and more widely used biological assays involve 1) bioluminescence; 2) bacterial electrodes; and/or 3) respiration to determine toxicity or inhibition. Ultimately, the goal of these bioassays is to alert operators to the presence of toxic or inhibitory chemicals by being more indicative of potential impact on activated sludge, which may enable the operators to prevent process upset problems (Cadena, 1995; Dutka et al., 1983; Guerra, 2001).

Bioluminescence assays involve either the use of naturally luminescent bacteria or bacteria genetically engineered to bioluminesce (Kelly et al., 1999). The Microtox[®] system, which utilizes the naturally luminescent bacterium *Photobacterium phosphoreum,* now known as *Vibrio fischerii,* is widely adopted as a toxicity screening procedure in many countries (Brown et al., 1996; Hao et al., 1996; Bundy et al., 1997). Engineered bioluminescent bacteria often include *lux* marked bioreporters for different metal toxicities and other waste stream components (Brown et al., 1996; Kelly et al., 1999; Gu and Choi; 2001). The principle of either test is based on the ability of the

bacteria to emit light. The light output of the bacteria is measured before and after exposure to an influent sample. The reduction of light intensity is proportional to the degree of toxicity present in the sample (Hao et al., 1996). Though, some bioluminescence assays measure increases in light intensity rather than decreases, depending on the bacteria used. Bioluminescence assays are known to be rapid, sensitive, and inexpensive as well as an *in vivo* real-time measurement (Hao et al., 1996; Gu and Choi, 2001). However, intrinsic difficulties have been found with these systems due to pH and saline buffering requirements, issues with turbidity, problems with reproduciblity, and the bacteria not being representative of activated sludge communities (Dutka et al., 1983, Kelly et al., 1999).

Bacterial electrodes have the ability to adeptly measure toxicity levels in wastewater samples by using intact, living bacteria as an alternative for enzymes at the surface of a membrane electrode (Dorward and Barisas, 1984). Production of carbon dioxide (CO_2) by bacterial cells, such as *Escherichia coli*, is measured potentiometrically. The production of CO_2 by *E.coli* is a result of the complex respiration of the cells. An inhibition of bacterial respiration by a pollutant is measured by a decrease in CO_2 production. Overall, the bacterial electrode is inexpensive and easy to implement. Disadvantages include a lack of sensitivity for slow-acting pollutants at low concentrations (Dorward and Barisas, 1984).

Respirometric inhibition is one of the most common methods for assessing wastewater toxicity to activated sludge (Kim et al., 1994; Kong et al., 1994; Kong et al., 1996; Kelly et al., 1999). This assay involves measuring the respiration of activated sludge microorganisms in contact with a wastewater sample in a closed respirometric chamber of defined volume. Very sensitive pressure sensors monitor the change in pressure, or change in dissolved oxygen in the sample, as a result of oxygen consumption from bacterial activity. In this method, the oxygen uptake rate (OUR) of activated sludge with a wastewater sample is compared to a nontoxic control. A decrease in uptake of dissolved oxygen, or a decrease in pressure change, indicates respiration inhibition (ASTM D5120-90; Tzoris et al., 2002). Respirometric assays offer reproducible and sensitive toxicity measurements, while being simple to implement and unaffected by turbidity. Unfortunately, online respirometric systems tend to be expensive and slow to respond in emergency situations (Brown et al., 1996; Tzoris et al., 2002).

Modified respirometric assay for Hopewell Regional Wastewater Treatment Facility

HRWTF handles about 35 mgd of combined domestic and industrial wastewater from five main industries and the surrounding area. Beginning the last week of December 2001, HRWTF was hit with what appear to be multiple influent shock loads that caused the biological process to malfunction. At least three separate sudden increases of influent COD seem to be associated with the incidents. As influent COD increased, effluent total suspended solids (TSS) started to increase significantly (73 mg/L on January 1), peaked at 608 mg/L on January 5, and did not decrease below 30 mg/L until January 19. Deterioration in effluent COD followed effluent TSS by a few days and peaked at 1,244 mg/L on January 9. A significant volume of solids was lost from the system early in the event and the ability of the process to degrade organic compounds deteriorated. The wastewater treatment process was re-seeded with new biomass on January 23 to assist in recovery.

Due to the potential for significant inhibitory events at HRWTF, the utility was interested in developing an approach for detecting toxicity in it is influent. With the aim to extend the benefits of a respirometric assay, a modified protocol has been developed. The protocol includes a sample management strategy, an assay, and a data analysis approach. Each of these components are discussed next.

A sample management strategy is proposed as recommended by Jenkins (cited in Love and Bott (2000)). The wastewater treatment plant collects daily influent samples from the major contributing industries, stabilizes these samples as appropriate, and refrigerates for seven days. On Day eight, if no upset event occurs, Day 1 samples are discarded and sample management continues in this manner each subsequent day. If an upset event occurs, the previous week's samples can be analyzed to determine the toxicant, which may help identify the discharger.

The modified respirometric assay utilizes the specific oxygen uptake rate (SOUR) assay to assess the potential respiration inhibition of activated sludge by five of the six main industrial wastewaters that discharge to Hopewell Regional Wastewater Treatment Facility (HRWTF). A specific volume of one industrial wastewater is added to a 300 ml BOD bottle. The remaining bottle volume is filled with a grab sample of diluted mixed liquor shipped from the treatment plant and revived in the laboratory before performing

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the assay. An oxygen uptake rate is measured, then normalized to the measured mixed liquor concentration to obtain the SOUR value. During stage one, SOURs were performed on all five industrial effluents at volume additions of 3 ml, 5 ml and 10 ml, to obtain a range of concentrations. A SOUR of the diluted mixed liquor sample was used as the control for the experiments conducted on each measurement. The mixed liquor was maintained in a semi-endogenous state, where it was fed soluble COD, but was not fed any soluble COD during the SOUR experiments. In later tests, stage two, the volumes of industrial wastewater added to the BOD bottle were changed from 3, 5 and 10 milliliters to a range of three volumes that were representative of full-scale dilution for each industrial wastewater received at the plant headworks.

Multiple kinetic models are used to describe different growth responses to substrates. Monod kinetics are typically used to describe microbial growth in the presence of a limiting growth substrate that is not inhibitory. The value of the specific growth rate coefficient, μ, is dependent on the concentration of the limiting growth nutrient. The relationship between μ and the growth rate limiting substrate is shown in Figure 3.1. This is typically for an un-inhibited bacterial culture. For microbial populations exposed to inhibitory substrates, Andrews kinetics are often observed (Grady et al., 1999). In this instance the specific growth rate reaches a maximum then declines as the inhibitory substrate concentration increases (Figure 3.2). Figure 3.3 illustrates the different trending patterns expected as a result of this experiment. Here, the graphical SOUR results depend on where the concentrations of growth rate limiting substrate, reflected by the 3, 5, and 10ml wastewater volume addition, fall on the Monod or Andrews curves. Option 1 (Figure 3.3) reflects no change in SOUR with increasing industrial wastewater volume. Option 2 (Figure 3.3) shows an increase in SOUR with increasing wastewater volume. Option 3 (Figure 3.3) shows a decrease in SOUR with increasing wastewater volume, suggesting inhibition. Option 4 (Figure 3.3) shows an initial increase than a decrease with increasing wastewater volume, suggesting possible inhibition.

In this study, two inhibition scenarios were examined. The first scenario involves the observation of inhibition trends, discussed previously as Options 1-4. The second inhibition scenario involves the SOUR/$SOUR_0$ ratio. When this ratio is less than one, that

particular day is determined to be inhibitory, whether or not there is an inhibition trend. A SOUR/$SOUR_0$ ratio less than one signifies that the SOUR of the sample with industrial wastewater added is respiring at a much lower rate than the endogenous $SOUR_0$, which implies inhibition. These two scenario conditions were used to determine an inhibitory sample day.

μ ∝ SOUR

Growth Rate Limiting Substrate Concentration

Figure 3.1   Monod Curve.

μ ∝ SOUR

Growth Rate Limiting Substrate Concentration
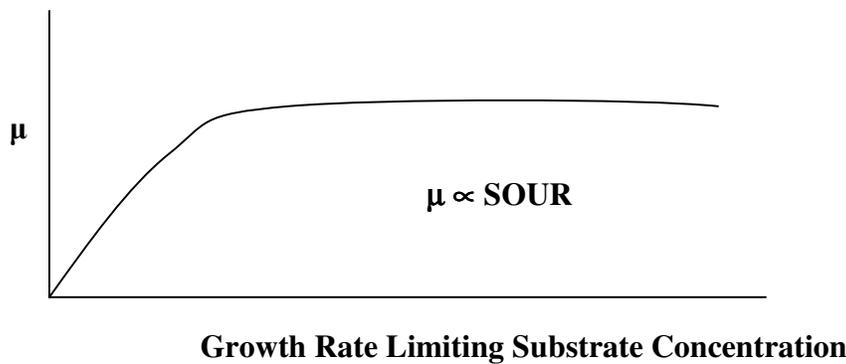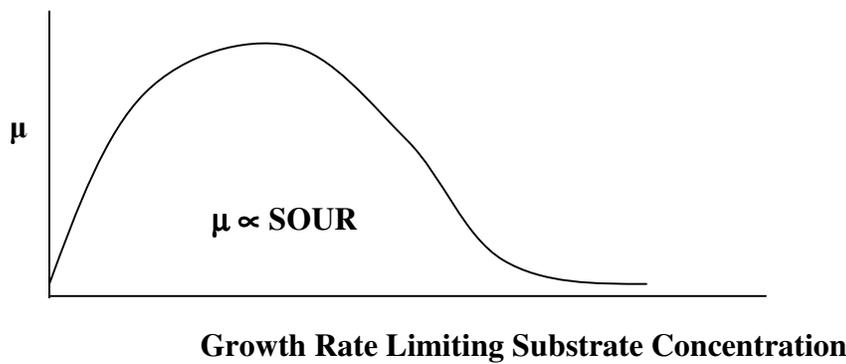
Figure 3.2.   Andrews Curve.

Option 1
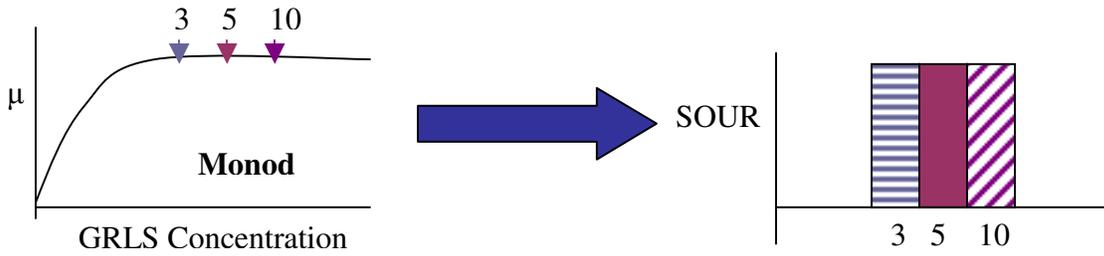
Option 2
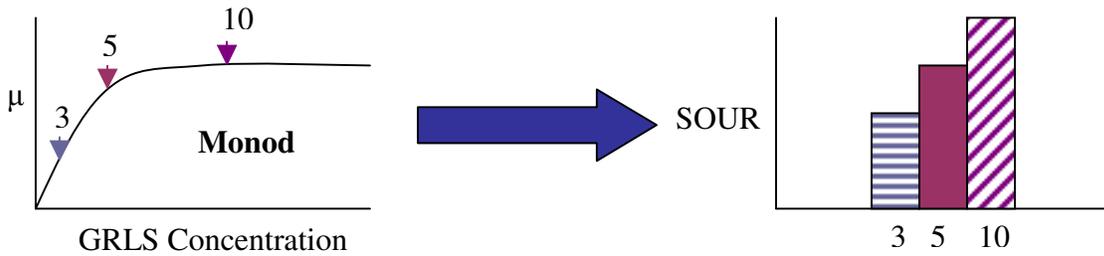
Option 3
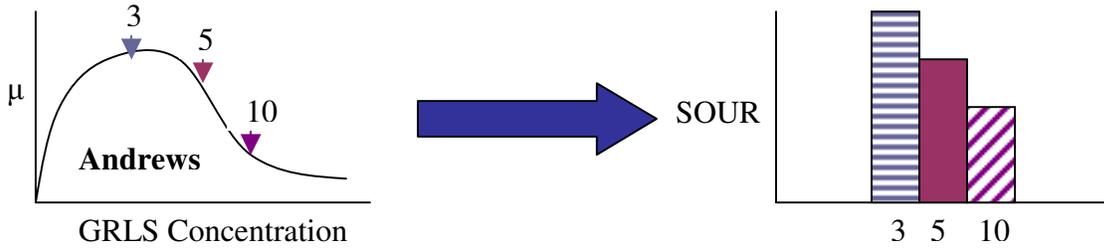
Option 4
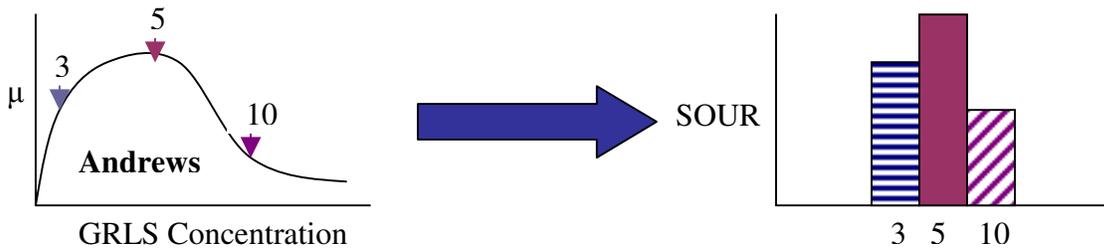
Figure 3.3.   Kinetics-SOUR graphic relationship.
GRLS = growth limiting substrate. Numbers represent ml of sample added per 300 ml bottle. Option 1 represents no inhibition with increasing volume additions. Option 2 represents increasing activity with increasing volume additions. Option 3 represents increasing inhibition with increasing volume additions. Option 4 represents possibly inhibition with increasing volume additions.

Objectives

The goal of this investigation was to use the afore mentioned modified respirometric assay to identify: 1) problematic industrial wastewaters that contribute to HRWTF influent; and 2) trends with certain wastewaters that indicate inhibition. This study tested five major industrial wastewaters that discharge to HRWTF, at concentrations equivalent to, less than and greater than those seen by the treatment plant. In addition, the assay and analytical protocols were modified so that results could be compared among wastewaters on a given day and over time for a given wastewater. The knowledge gained may help identify problematic sources and suggest a course of action for future protection from upset shock. Possible deferment of fines from HRWTF to the implicated culprit may also be possible.

3.2.   HRWTF FLOW DILUTION CONSIDERATIONS

After preliminary results, it was realized that the dilution effect of the industrial wastewaters flowing into the treatment facility needed to be accounted for. Two more days of sampling tests (stage two), September 25 and September 26, were performed using appropriate new dilution volumes of each industrial wastewater. Honeywell, Hercules, and Goldschmidt were the only industrial wastewaters tested on September 25, because the other effluents were not included in the shipment. All industrial wastewaters were tested on September 26. The new dilution volumes were comparable to dilutions encountered by each industrial wastewater due to full-scale flows, in terms of COD (mg) contribution. The new volumes for each wastewater are presented in Table 3.1. Volume two in Table 3.1 is the average volume that represents actual COD loading. Volumes one and three were chosen to be less than and greater than, respectively, the average to achieve a range for comparison and to illustrate inhibition trends.

Table 3.1.   Adjusted volumes to be used in stage-two SOUR experiments.

| | (ml) | | |
	Volume 1	Volume 2	Volume 3
Honeywell	40	80	120
Hercules	5	16	30
Goldschmidt	0.1	0.4	1
Smurfit-Stone	70	120	160
Stone-HAP	1	5	10

3.3.   DATA PRESENTATION

Error bars are provided on duplicate data to represent the higher and lower measurements that contribute to the average bar graph or scatter plot calculation.

3.4.   RESULTS AND DISCUSSION

3.4.1. Mixed Liquor Characteristics

Suspended solid measurements were taken throughout every experiment and the mixed liquor suspended solid concentrations were calculated to determine the specific oxygen uptake rate. Figure 3.4 shows the MLVSS concentrations over one sampling day when fed the biogenic soluble substrate solution. By adding biogenic soluble substrate, the MLVSS concentration was maintained within 3.5% of the starting concentration over the time period (up to six hours) of testing. Figure 3.5 shows the average measured mixed liquor concentrations over the sampling periods for stage one and two.
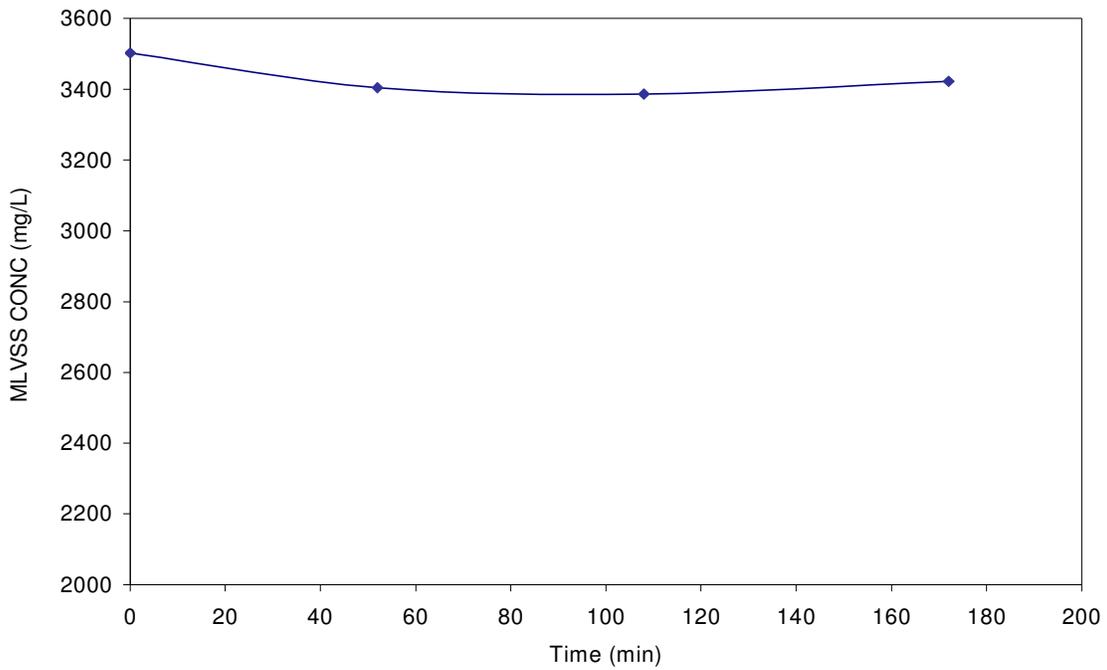
Figure 3.4   Mixed liquor trends maintained with addition of biogenic substrate from July 26, 2002.
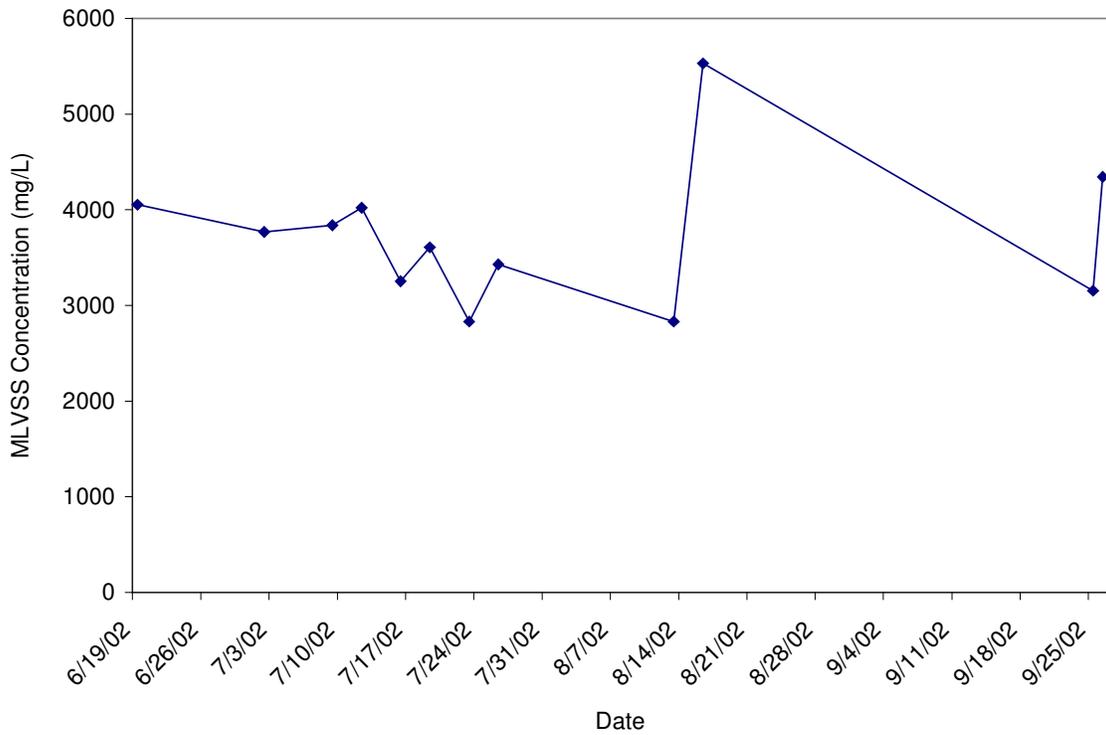
Figure 3.5   Average mixed liquor concentration variations over time for stage one and two SOUR assays.

3.4.2. Industrial Wastewater Characteristics

Table 3.2 shows the average soluble CODs for the five tested industrial wastewaters. Goldschmidt was shown to have the highest average COD, followed by Stone-HAP and Hercules. The COD concentrations for Hercules, Goldschmidt, and Stone-HAP were calculated to be almost twice the averages reported by HRWTF for the month of July, but remained below the maximum reported. Figures 3.6 and 3.7 compare the measured average flow and average total COD for each industrial wastewater, as measured by HRWTF. It is important to note, that wastewaters with very low flow can have a very high COD concentration and vice versa. Figure 3.7 shows the percentage of total COD contributed to the wastewater treatment facility by each of the different industries. These values were obtained from measurements taken by HRWTF during the month of July, 2002.

Table 3.2.   Soluble COD averages and ranges over sampling period.

(mg/L)	Honeywell	Hercules	Goldschmidt	Smurfit-Stone	Stone-HAP
Avg ± Stdev	749±705	15,393 ± 4834	29,926 ± 18,269	1,830 ± 3,771	25,524 ± 3,545
Max	2,400	21,883	47,671	11,593	33,451
Min	262	4,755	1,548	68	4,853
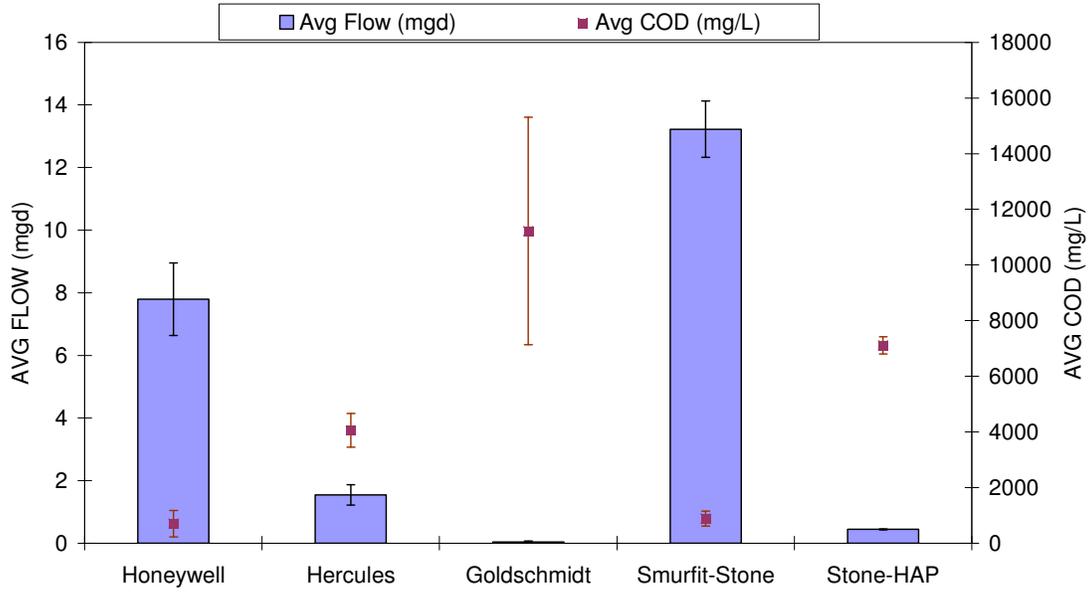
Figure 3.6.   Industrial effluent characteristics of July 2002. Error bars represent higher and lower values contributing to the bar graph average.
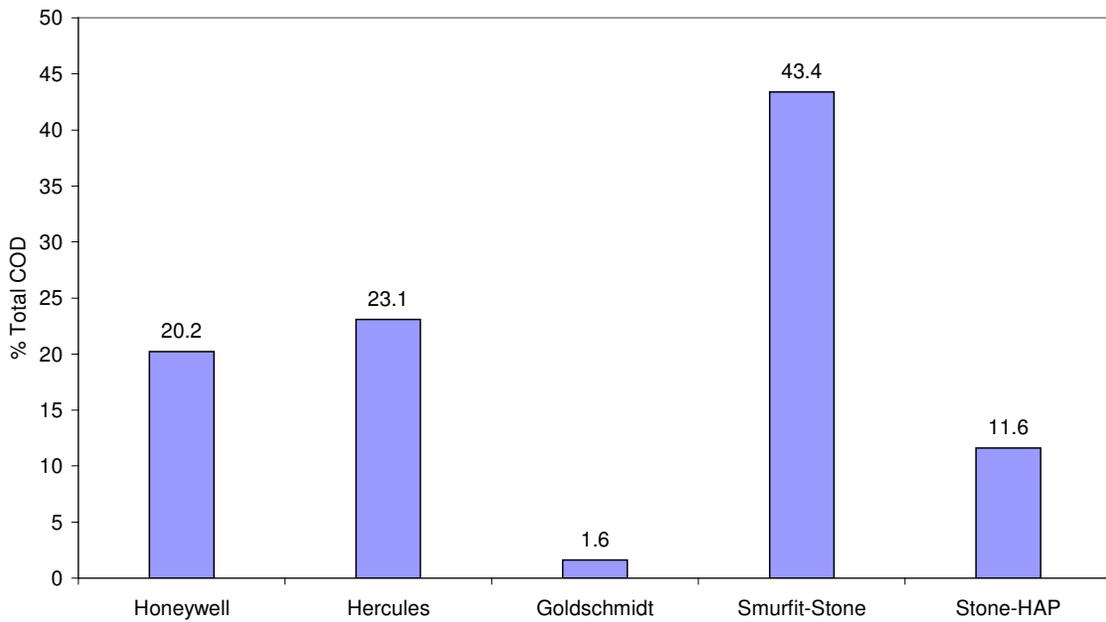
Figure 3.7.   Effluent COD percentages contributing to HRWTF in July 2002. Data provided by HRWTF.

3.4.3. Stage One Sampling Results

An example of the impact of varying industrial wastewater volumes on the specific

oxygen uptake rate (SOUR) is shown in Figure 3.8. The smaller the SOUR measurement, the lower the oxygen demand. Some trends of inhibition are visible with increasing volume (e.g. Goldschmidt), but individual SOURs are difficult to compare due to differences in COD concentrations and differences in each MLVSS physiology. The results were difficult to compare from day-to-day due to these same issues.
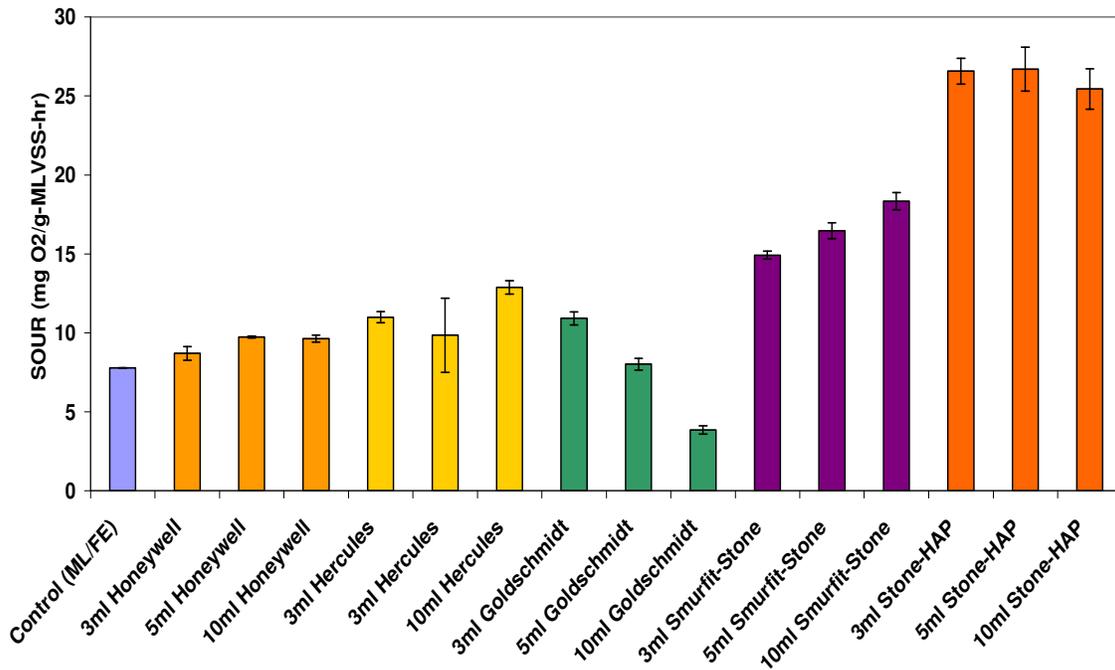
Figure 3.8.   SOUR results from 7/26/02. Error bars represent higher and lower values contributing to the bar graph average.

The varying endogenous mixed liquor concentrations over the summer sampling period are illustrated in Figure 3.9. The term endogenous means the biomass is respiring for survival, but not for growth. Here, the endogenous sample is equivalent to a control. This graph is evidence that the physiology of the endogenous mixed liquor fluctuates greatly over the sampling period. The mixed liquor fluctuated in composition and settleability on each sample day, indicating potential physiological differences in the biomass. Figure 3.10 shows an example of COD variability in one specific industrial wastewater (Hercules). The fluctuations apparent in this wastewater are characteristic of the majority of the wastewaters tested.
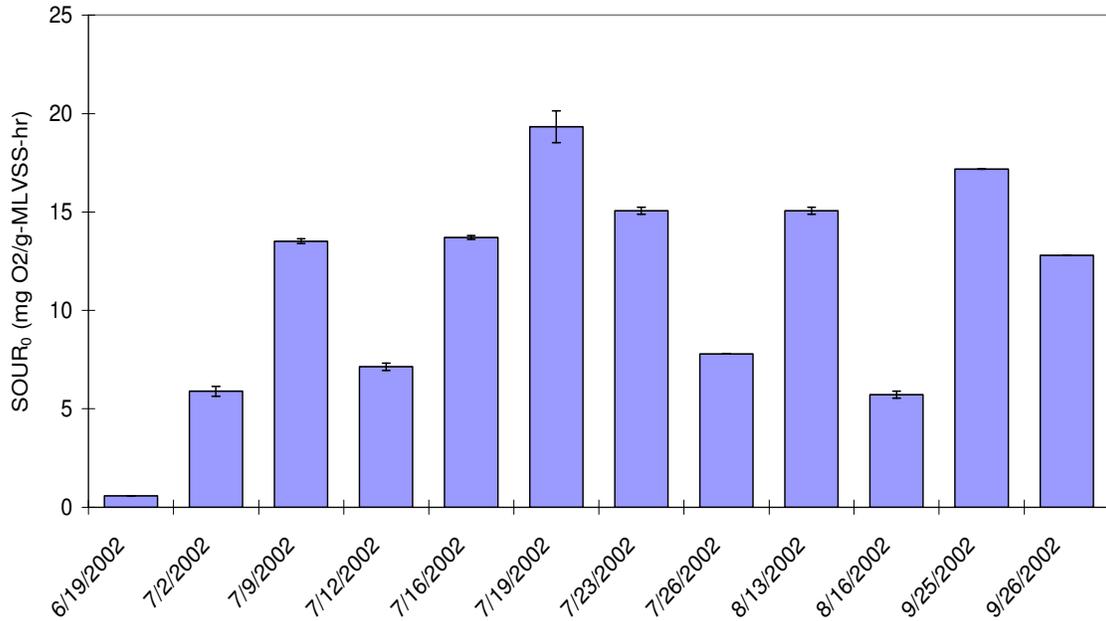
Figure 3.9.   Endogenous SOUR (SOUR$_0$) variations over the sampling period. Error bars represent higher and lower values contributing to the bar graph average.
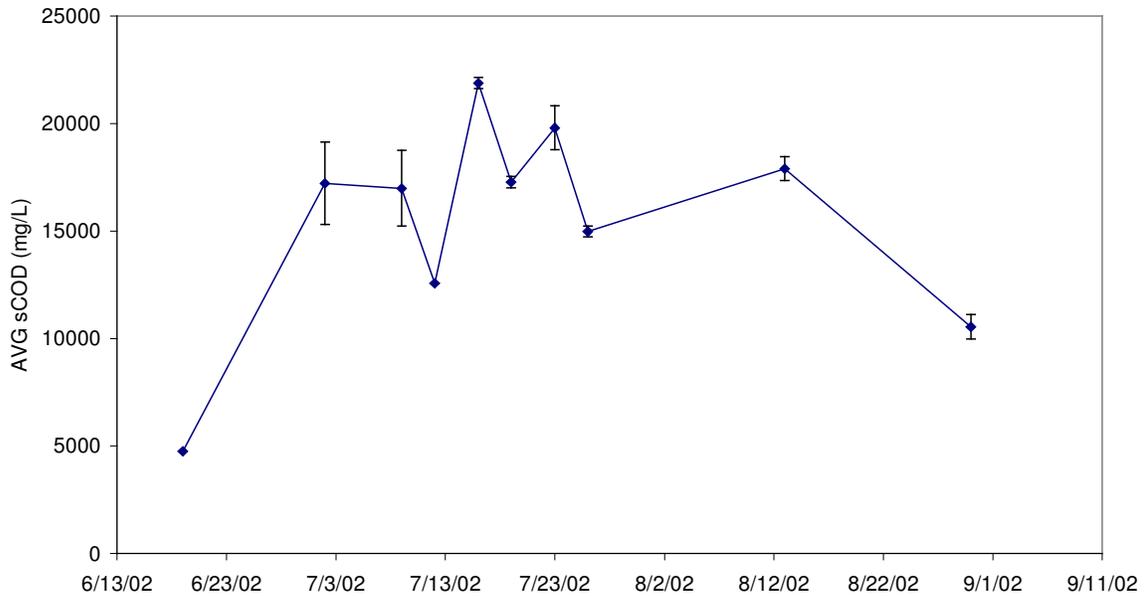
Figure 3.10.   Soluble COD variations in the Hercules wastewater over the sampling period. Error bars represent higher and lower values contributing to the bar graph average.

Due to the differences in wastewater COD and endogenous mixed liquor SOUR, day-to-day comparisons were impractical. The need for normalization of SOUR results became apparent. Since COD can change significantly over time, COD normalization of the SOURs was attempted first. The SOUR for each date was standardized to the amount of sCOD (mg) found in the volume of wastewater added and results are shown in Figure 3.11 for Honeywell. This normalization resulted in all of the industrial wastewaters samples possessing inhibition trends (SOUR/sCOD decreased with increasing wastewater volume). It is believed that a bias exists in this normalization approach. It is suspected that not all the COD was bioavailable during the SOUR assay time period (approximately ten minutes). This effect would become more pronounced with larger volume additions. Therefore, all the industrial wastewaters may have shown inhibitory trends with this normalization approach due to dividing by the total COD when only part contributes to the SOUR. Of course, it is also possible that all wastewaters are inhibitory; however, this option was considered to be unlikely. Therefore, an alternate normalization was attempted. As discussed previously, mixed liquor physiology varied greatly from day-to-day. To eliminate this effect, wastewater SOUR measurements were normalized to the endogenous control ($SOUR_0$) measured on that specific day. The low substrate/biomass ratio used in this specific oxygen uptake rate method limits the growth of microorganisms and maintains the initial physiological state of the original biomass during the test. Therefore, it is assumed that the $SOUR_0$ reflects the actual microbial composition. The results from this manipulation for Honeywell are shown in Figure 3.12.
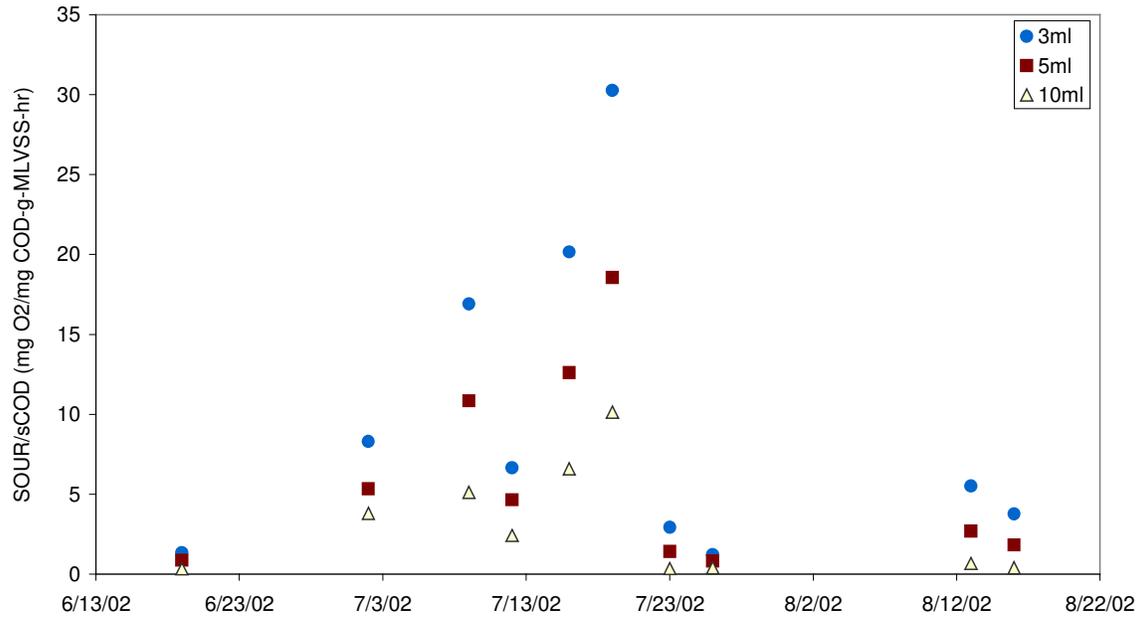
Figure 3.11.   COD normalized Honeywell SOUR experiments over sampling period.

Figure 3.12.   Endogenous normalized Honeywell wastewater over sampling period.
Solid arrows indicate inhibitory trends as defined in Figure 3.3 (Option 3).
Striped arrows indicate potential inhibitory trends as defined in Figure 3.3
(Option 4). Error bars represent higher and lower values contributing to
the bar graph average.

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From Figure 3.12 it is possible to see that there are three days with clear inhibition trends, denoted by the blue arrows. This suggests that over the ten minute assay, Honeywell wastewater was inhibitory at the doses applied on these dates. Overall, there is a large variation in SOUR measurements over the sampling period, but inhibition trends can be seen with this normalization. The major disadvantage is that relative comparison of the intensity of inhibition between wastewaters on a given day is not possible due to the differences in COD concentrations.

Goldschmidt Results

Figure 3.13 shows the results from Goldschmidt's normalized SOUR tests from once weekly samples. Like Honeywell, Goldschmidt had three days of clear inhibition trends, denoted by the blue arrows, suggesting that over the ten-minute assay that Goldschmidt wastewater was inhibitory at the doses applied. There was one day that inhibition was suggested due only to the extremely low $SOUR/SOUR_o$, less than one, measurement on that day (red arrow). Variation over the sampling period was also found. If Goldschmidt wastewater had been tested as often as the other industries, more inhibitory days may have resulted.
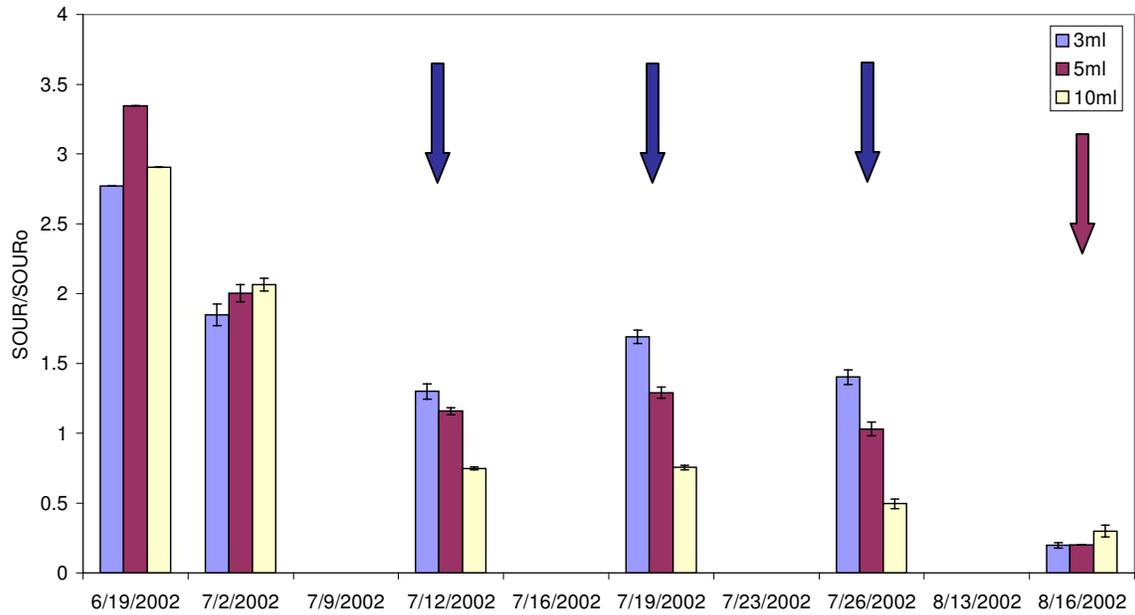
Figure 3.13.　Endogenous normalized Goldschmidt wastewater over sampling period. Solid arrows indicate inhibitory trends as defined by Figure 3.3 (Option 3). Red arrow indicates possible inhibition based on extremely low SOUR/SOUR$_o$ at all volumes on that day. Error bars represent higher and lower values contributing to the bar graph average.

Stone-HAP Results

Figure 3.14 shows the results from Stone-HAPs normalized SOUR tests. There was only one day, denoted by the blue arrow, where clear inhibition trends exist. This suggests that over the ten-minute assay, Stone-HAP wastewater was inhibitory at the doses applied. The striped arrows suggests days with less-clear inhibition trends. There were four days that inhibition was suggested due only to the extremely low SOUR/SOUR$_o$, less than one, measurement on those days (red arrows). It is obvious that there is a distinct variation of results over the sampling period.

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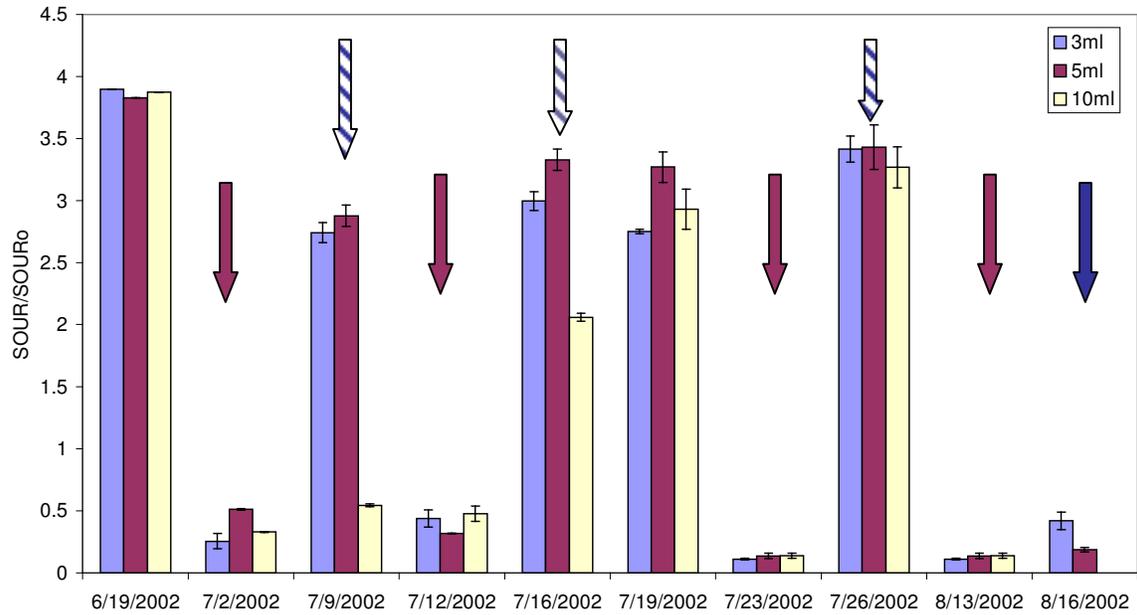
Figure 3.14. Endogenous normalized Stone-HAP wastewater over sampling period. Solid arrows indicate inhibitory trends as defined by Figure 3.3 (Option 3). Striped arrows indicate potential inhibitory trends as defined in Figure 3.3 (Option 4). Red arrows indicates possible inhibition based on extremely low $SOUR/SOUR_o$ at all volumes on that day. Error bars represent higher and lower values contributing to the bar graph average.

Hercules Results

Figure 3.15 shows the results from Hercules' normalized SOUR tests. There were three days with clear inhibition trends found over the sampling period (blue arrows), suggesting that over the ten minutes assay that Hercules wastewater was inhibitory at the doses applied. There was also one day with a less-clear inhibition trend (striped arrow). A large variation of SOUR results were also present in Hercules wastewater.
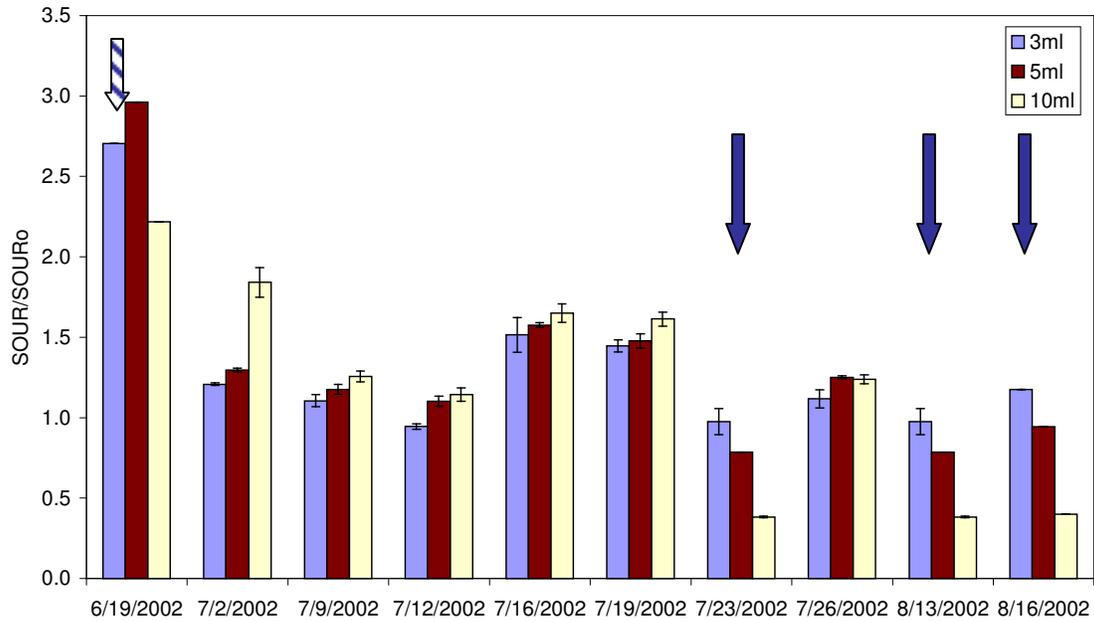
37
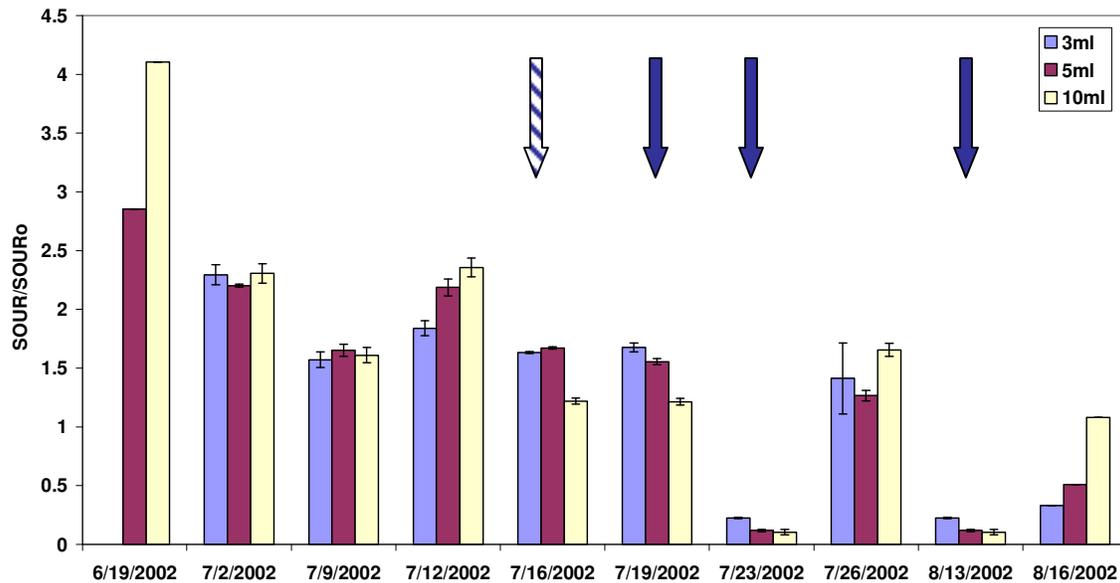
Figure 3.15. Endogenous normalized Hercules wastewater over sampling period. Solid arrows indicate inhibitory trends as defined by Figure 3.3 (Option 3). Striped arrows indicate potential inhibitory trends as defined in Figure 3.3 (Option 4). Error bars represent higher and lower values contributing to the bar graph average.

Smurfit-Stone Results

Figure 3.16 shows the results from Smurfit-Stone's normalized SOUR tests. There were no clear inhibition trends found in Smurfit-Stone's wastewater. In fact, respiration rate typically increased with increasing volume. There were four days that inhibition was suggested due only to the extremely low $SOUR/SOUR_o$ less than one, measurements of that day (red arrows).

Interesting to note was that Smurfit-Stone believed their wastewater composition to be constant because their day-to-day COD concentrations were constant. But, from Figure 3.16, it is shown that even though COD may be constant over time, it does not imply that their wastewater has no potential for inhibition.
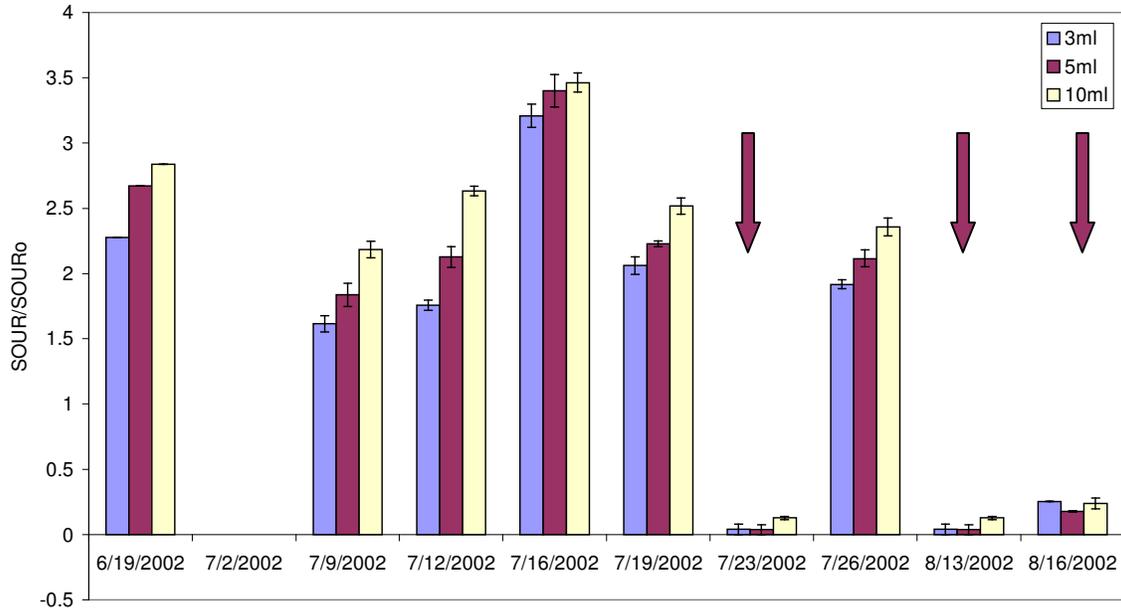
Figure 3.16.   Endogenous normalized Smurfit-Stone wastewater over sampling period. Red arrows indicates possible inhibition based on extremely low SOUR/SOUR$_o$ at all volumes on that day. Error bars represent higher and lower values contributing to the bar graph average.

3.5.   STAGE TWO SAMPLING RESULTS

From Table 3.1, it can be seen that the SOUR measurements collected in stage one were made using volumes that, in all but one case, represented dilutions that were very different from those experienced by HRWTF. New dilution volumes were calculated to achieve the same COD mass loading during the assay as is experienced at the treatment plant. The average value was calculated from average full-scale mass loadings, then lower and higher values were chosen to complete the volume range. These calculations are shown in Tables B.15 and B.16 in Appendix B. Stage two sampling occurred over a two-day time period and results are given in Figures 3.17 and 3.18. Figure 3.17 shows that Hercules and Goldschmidt have clear trends toward inhibition, while Honeywell does not. Smurfit-Stone and Stone-HAP wastewaters were not received that day. On the second day of sampling all industrial wastewaters were tested. Figure 3.18 shows no inhibition trends for any of the wastewaters. This illustrates further the day-to-day variability inherent in these industrial wastewaters. Though there were no inhibition trends, both Goldschmidt and Stone-HAP had SOUR/SOUR$_0$ values less than one indicating inhibition. It is also important to note that Goldschmidt, an industrial

wastewater with a very small flow but considerable COD contribution, can have a similar SOUR contribution relative to those wastewaters with much larger flows, like Stone-HAP.
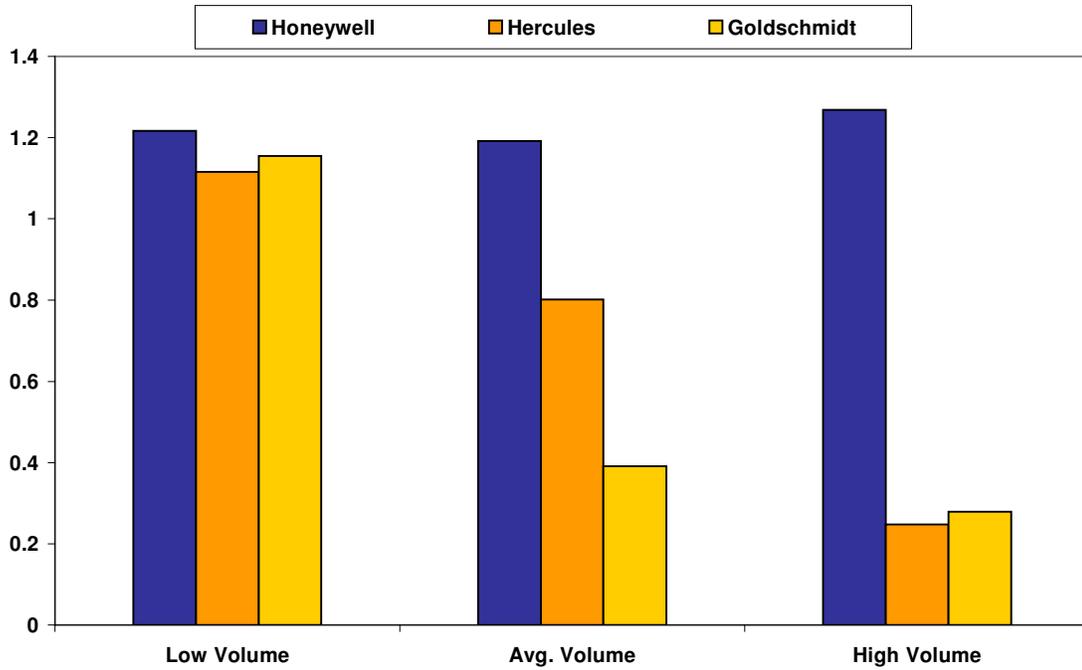
Figure 3.17.   Stage-two results for 9/25/02.
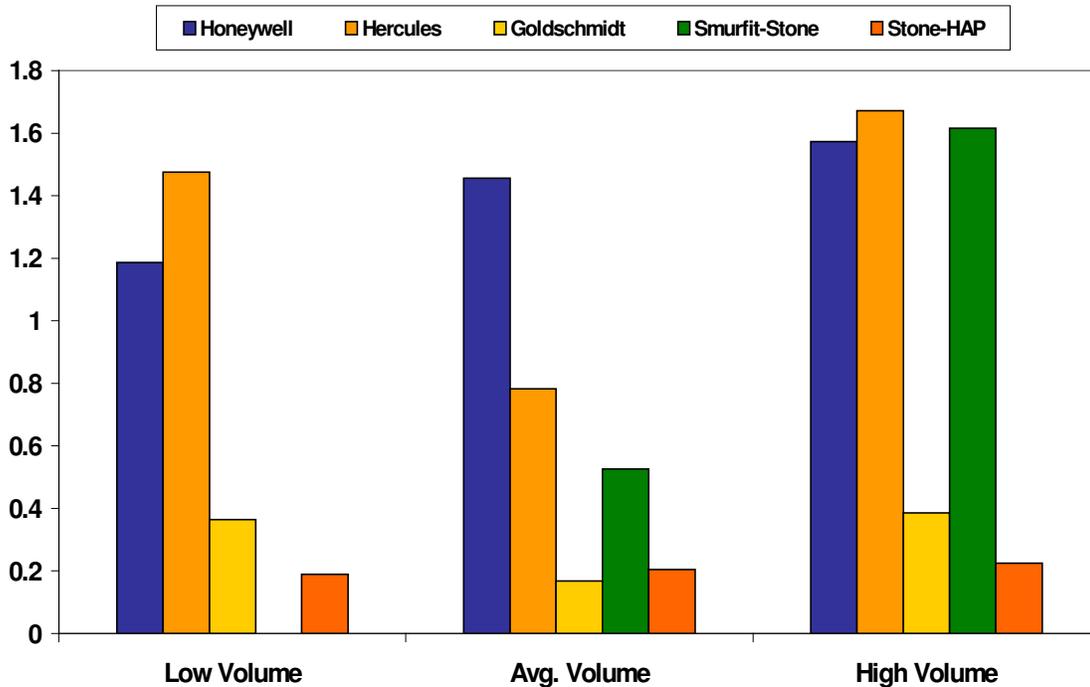
Figure 3.18.　　Stage-two results for 9/26/02.

3.6.　　CONCLUSIONS

　　Overall, it has been shown that there is great potential for inhibition inherent in the influent stream entering the Hopewell Regional Wastewater Treatment Facility. Honeywell, Hercules, Goldschmidt, and Stone-HAP showed inhibition trends or the possibility of inhibition when volumes of the wastewaters were increased. These trends varied over the period of sampling for each wastewater, indicating the potential for significant daily variations in the COD content. All five industrial wastewaters were also found to have $SOUR/SOUR_0$ measurements less than one on at least one sampling day, indicating inhibition. Interestingly, it was revealed that industrial wastewaters known to have very consistent characteristics in terms of COD and flow contributions, like Smurfit-Stone and Stone-HAP, did show variations in inhibitive properties from day-to-day. Furthermore, it was shown that industrial wastewaters with very small flows, which may not have been previously suspect can have a large inhibitory effect on the biomass of the plant, like Goldschmidt. This illustrates that a wastewater with an appreciably lower flow, compared to other wastewaters, can still play a major role in process effects and possible inhibition when all characteristics of the wastewater are taken into account.

It is important to note that the SOUR assays were conducted over ten minute periods. This approach is much more sensitive to detecting inhibition than a long-term assay. A treatment plant provides more adaptation time, which may prevent onset of process performance problems in the presence of an inhibitor that is revealed in a 10 minute assay. Therefore, an effect that is seen during a ten-minute assay may be easily stifled within an hour or two in a large reactor. It is recommended that additional studies be conducted to compare the short-term and long-term assay results.

Complete dissolved oxygen (DO) uptake sampling is conducted at HRWTF only when loss of treatment due to inhibitory industrial loading or BOD overloading is suspected. When performed, the operator determines the dilution volume needed to achieve a fifty percent loss of DO. The results from these tests do not seem to convey enough information to characterize trends or to determine the problem source. From this report it is evident that it is important to normalize respiration values to the mixed liquor characteristics of that day to fully interpret results. HRWTF already collects mixed liquor suspended solid measurements, along with flow rates and COD contributions. Therefore, no new measurements would need to be taken by HRWTF. The only substantial change necessary would be to manipulate the data using similar approaches to those used in this report, to take into account the differences in physiology of the mixed liquor. Manipulation of data can give more information on what is actually occurring in the treatment plant and possibly implicate the source of inhibition. To provide more consistency and easier interpretation with results, it is advisable to feed a biogenic substrate solution to the source biomass if the respiration assays will take longer than thirty minutes. This is to maintain the physiology and MLSS concentration of the biomass. These parameters are important if the SOUR measurements are to be compared, because the mixed liquor concentration needs to remain relatively constant.
It is also important to conduct the respiration assays at industrial wastewater dilutions representative of those found at the headworks. A minimum of two dilutions, one on either side of the average, should also be tested to allow for trends to be observed.

It is recommended that these respirometric assays be performed at regular intervals throughout the year, not only when inhibition is suspected. This will create a database of results for comparison when an upset event does occur and allow trend correlation with

full-scale events. The investment in a computerized DO acquisition system is also recommended. An acquisition system can evolve the understanding of the $SOUR/SOUR_0$ versus full-scale effect relationship, while saving valuable operator time.

Overall, this investigation was able to identify potentially inhibitory industrial wastewaters. It also demonstrated a way to modify the SOUR assay protocol and the data analysis approach so that the inhibition potential of different wastewaters could be assessed on a relative basis.

CYANIDE SOURCE-EFFECT EXPERIMENT

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4. CYANIDE SOURCE-EFFECT EXPERIMENT

4.1. LITERATURE REVIEW

There are three main sources of cyanide that can contribute to wastewater streams: 1) atmospheric deposition; 2) domestic; and 3) commercial/industrial, with industrial being the largest contributor (Wild et al., 1994). Cyanide is produced on an industrial scale of 2 to3 million tons per year and is found in many different industrial wastewaters (Gijzen et al., 2000). It is prevalent in the wastewaters of manufacturing industries involved in metal plating, pharmaceuticals, iron and steel production, synthetic fibers, plastics, coal gasification, coal coking and re-leaching, petroleum refining, photofinishing, herbicides and insecticides (Lordi, 1980; Luthy, 1981; Gaudy et al., 1982; Wild et al., 1994; Hung and Pavlostathis, 1997; White et al., 1998;). The metal plating industry typically produces small volumes of highly concentrated cyanide wastes that originate in playing vats and rinse waters (Lordi et al., 1980). Conversely, the iron and steel industry generally produces large volumes of dilute cyanide wastes (Lordi et al., 1980). Therefore, each industry provides a separate challenge in treating cyanide waste streams. The toxicity and prevalence of cyanide in waste streams make cyanide an important chemical to investigate in relation to biological wastewater treatment systems.

Many different forms of cyanide can exist, both organic and inorganic. Analytically, the term "cyanide" refers to the group of simple or complexed chemical compounds that contain the cyanide ion, CN^-, also known as free cyanide. Cyanides are categorized into two forms, simple and complexed. Simple, or free cyanide includes the cyanide anion, CN^-, and hydrogen cyanide, HCN. Complexed cyanides are those compounds where the cyanide anion complexes with metals in water. The relationship between simple and complex cyanides in water is dependent on pH and heavy metal concentrations. Under alkaline conditions free cyanide is completely ionized and forms stable metal complexes. At neutral and acidic conditions, free cyanide is weakly ionized and formation of volatile hydrogen cyanide is favored (Wild et al., 1994). Due to the volatility of HCN, it has been experimentally shown that up to 66% of cyanide can be stripped from solution by aeration in both water and activated sludge, creating health hazards for people (Raef et al., 1977b; Gaudy et al., 1982).

Cyanide can form both weak and strong metal complexes, depending on the metal present. Strong complexes occur in the presence of iron and cobalt metals and are generally less toxic to microorganisms (Fallon et al., 1991; Zheng and Dzombak, 2003). Typically, cyanide weakly complexes with sodium, potassium, calcium, cadmium, lead, nickel and zinc metals (Wild et al., 1994; Torrens, 2000; Zheng and Dzombak, 2003). Weak cyano-metal complexes, especially zinc (Zn^{2+}) and cadmium (Cd^{2+}) dissociate extensively to the metal ion and CN^- (HCN), creating a greater availability of cyanide for the biomass and greater potential for biomass and aquatic toxicity (Torrens, 2000). With cyanide compounds the toxic effects are usually attributed to the concentration of hydrogen cyanide in the solution. The toxic effects of the corresponding heavy metals are deemed less significant, but can have additive properties (Blaha, 1976a). In wastewater streams, it has been found that most cyanide will exist in the complexed form, but high concentrations of simple cyanide can occur (Blaha, 1976a; Lordi et al; 1980; Gijzen et al; 2000). Absorption to biomass has also been documented, but is considered less important (Raef et al., 1977a).

A chemical's toxicity is a key consideration in evaluating its impact on organisms in biological wastewater treatment systems and in the environment (Blum and Speece, 1991). Hydrogen cyanide is considered the most toxic form of cyanide. In humans, the average fatal single dose of cyanide is 50 to 60 mg, while low doses of cyanide can cause headache, nausea and vomiting (Wild et al., 1994). Concentrations in the range of 0.01 to 0.1 mg cyanide have been found to kill some aquatic species, especially those in embryonic or early juvenile stages (Blaha, 1976a). Sub-lethal concentrations of cyanide also have the potential for negative effects on aquatic species, especially when coupled with the toxicity of dissociated heavy metals from complexed cyanides (Blaha, 1976a). In general, cyanide complexes with metal species are less toxic than free cyanide. However, those weakly complexed cyanides, such as zinc and cadmium, remain extremely toxic due to the easy dissociation of the compounds in water (Blaha, 1976a; Wild et al., 1994). It is these more toxic species and their molecular effects that remain central to the investigation of cyanide in wastewater treatment streams.

The molecular effects of cyanide to both prokaryotic and eukaryotic cells have been extensively studied. Cyanide is a non-specific inhibitor capable of interfering with

many different enzymes (Lewandowski, 1984). Specifically, there are three major mechanisms by which cyanide inhibits growth and causes toxicity to cells (Knowles, 1988). The first mechanism is chelation to di- and tri-valent metals in metallic enzymes. Cytochrome oxidases, the terminal enzymes in the electron transport chain, are the most commonly studied metallic enzymes affected by cyanide (Solomonson, 1981; Knowles 1988; Yoshikawa and Caughey, 1990; Patel et al., 1992; Arden et al., 1998). Cytochrome oxidase is the cellular component responsible for reducing dimolecular oxygen during respiration, so inhibition of this enzyme leads to inactivation of respiration, or cell suffocation (Solomonson, 1981; Patel et al., 1992) Specifically, cyanide prevents the re-oxidation of cytochrome oxidase when the CN^- iron interferes with the heme (iron-containing group) of this enzyme (Solomonson, 1981; Arden et al., 1998). This rapid inhibition of respiration is responsible for the well renowned reputation of cyanide as a deadly poison.

The second mechanism by which cyanide inhibits growth and causes toxicity to cells is by reacting with keto compounds (Knowles, 1988). Reaction of cyanide with these compounds, primarily the α-keto acids pyruvate and α-ketoglutarate, results in the formation of cyanohydrins. This reaction is found to exert a protective effect against CN^- toxicity, by converting the toxic form into the less toxic cyanohydrin form that is easier for bacteria to degrade (Solomonson, 1981). Although some protection is provided through these reactions, the cyanohydrin products are derivatives of enzyme substrates that can also inhibit those enzymes (Knowles, 1988).

The third mechanism by which cyanide inhibits growth and causes toxicity to cells is the reaction of Schiff-base intermediates to form inhibitory nitrile derivatives (Knowles, 1988). All enzymatic reactions of Schiff base intermediates are susceptible to cyanide inhibition (Solomonson, 1981). Susceptible reactions include: 1) inhibition of the enzyme that catalyzes the last step in catabolism of hydroxyproline, an important amino acid found in cartilage, by mammals; and 2) inhibition of clostridial acetoacetate decarboxylase, an enzyme that catalyzes the decarboxylation of acetoacetate in certain anaerobic bacteria (Solomonson, 1981).

Treatment of cyanide containing wastewaters using biological processes is well documented. Industrial wastewaters generally contain levels between 0.01 mg/L and 10

mg/L of total cyanide, but have been known to exceed 10,000 mg/L (Wild, 1994). Since cyanide is a known aquatic pollutant discharge limits have been imposed on wastewater treatment plants to limit the toxic effects of cyanide on natural waters. In general, cyanide-containing wastewaters arrive at treatment plants predominantly in dissolved, metal-complexed forms (Lordi et al., 1980). If levels of cyanide are not inhibitory, there are four main removal fates by which cyanide can be removed: 1) stripping to atmosphere as HCN; 2) adsorption to particulates and removal by clarifiers; 3) conversion to another chemical species; and 4) biological degradation (Raef et al., 1977a). Cyanide stripping has been found to account for approximately 15% of total cyanide removed in aerated systems, while adsorption is relatively insignificant because most cyanide is found in the dissociated form (Raef et al., 1977a; Wild et al., 1994). As a result, most remains in the wastewater as it flows into the bioreactor.

Once in the bioreactor, cyanide can undergo the aforementioned chemical conversion or biological degradation. If the levels of cyanide are too high there is the potential for inhibitory effects and disruption of the biological treatment process (Ludzack and Schaffer, 1962; Raef et al., 1977b; Lewandowski, 1984; Wild et al., 1994; Kholdebarin et al., 1998; Lee and Park, 1998; Gijzen et al., 2000; Torrens, 2000). The commonly accepted mechanism of cyanide toxicity, which is blocking of the respiratory electron transport chain, indicates that mixed aerobic cultures should be more sensitive to cyanide effects than anaerobic cultures (Zintgraff et al., 1969). Within mixed aerobic cultures, the inhibition of nitrification and denitrification are the most commonly observed effects of cyanide toxicity (Zintgraff et al., 1969; Lewandowski, 1984; Daigger and Sadick, 1997; Kholdebarin et al., 1998). This is explained by the fact that concentrations inhibiting autotrophs are often an order of magnitude less than those that inhibit heterotrophs, making it possible for a wastewater treatment plant to remove organic matter while being unable to nitrify (Daigger and Sadick, 1997). Specifically, cyanide is believed to inhibit the ammonia oxidizing bacteria, preventing ammonia oxidation to nitrite by interfering with the cytochrome oxidase of the respiratory electron transport system (Kholdebarin et al., 1998). Inhibition of denitrification is caused by reduced or inactivation of nitrate and nitrite reductase by cyanide (Solomonson, 1981). Though nitrification is the major inhibition effect, low growth yields have been found in

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the presence of high cyanide levels as well (Zintgraff et al., 1969). Severe inhibition of bacterial respiration, oxygen uptake rates decreasing with increasing cyanide concentrations, and COD removal have also been documented (Ludzack and Schaffer, 1962; Zintgraff et al., 1969)

Anaerobic wastewater treatment, specifically methanogenesis, is also sensitive to the presence of cyanide. High levels of cyanide were found to inhibit the production of methane as well as to affect chemical oxygen demand (COD) removal (Gijzen et al., 2000). However, acclimated anaerobic systems were not inhibited at moderately high levels of cyanide, but were also able to degrade the cyanide to ammonia and formate, eventually to bicarbonate, at over 90% efficiency (Fedorak et al., 1986; Gijzen et al., 2000).

4.2.   PREVIOUS CN⁻ SOURCE-EFFECT EXPERIMENT

A previous cyanide source-effect experiment was completed with the addition of sodium cyanide (NaCN) as the surrogate form of cyanide. Cyanide concentrations added to the reactors were determined during IC_{XX} assays using un-aerated samples of mixed liquor and cyanide to minimize the volatilization of HCN. As the experimental SBR reactors are aerobic cultures, volatilization of cyanide at the beginning of each experiment is thought to have been significant because diffused aeration was used. A strong odor of bitter almonds, characteristic of cyanide, was observed (even though the reactors were located in a fume hood). The odor disappeared after approximately one hour of operation, suggesting that volatilization as HCN did occur. It is believed the concentration of cyanide in the reactors was rapidly reduced below the targeted inhibition percentages. Analysis of the data supports this possibility, as few effects were seen even though significant inhibition of respiration and nitrification were expected based on literature reports. As a result, this source-effect experiment was repeated using zinc cyanide ($Zn(CN)_2$), a common weakly complexed cyanide found in many industrial waste streams, formed when free cyanide is in the presence of zinc metal or zinc cation. The results from this experiment are presented herein. These source-effect experiments are normally run for 3 X SRT of the biomass. In the case of the 10 day SRT system, recovery

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was seen for all tested parameters, so the reactors were shut down after 53 cycles (13.25 days).

4.3.   RESULTS AND DISCUSSION

4.3.1. Inhibitory Concentrations (IC$_{XX}$)

Different zinc cyanide concentrations were tested for impact on the SOUR of the 2 and 10 day SRT biomasses (Figures 4.1 and 4.2). From these two graphs, best-fit curves using a minimized sum of squared errors were developed. Using these curves, the inhibitory concentrations were determined based on a percentage inhibition of the control tests (zero contaminant added). The results of these calculations are listed in Table 4.1 below, and were the final concentrations added to each of the reactors at the beginning of the first cycle of the source-effect experiment. To negate the effects that the dissociated zinc may have on each reactor, an amount equivalent to the uncomplexed zinc in the IC$_{50}$ was added to the control reactor, as computed using the MINEQL+ software program (Environmental Research Software®, 1998). These amounts are listed on Table 4.2.

The equation shown on the figure:

$$y = .0678x^2 - 2.97x + 42.2$$
$$R^2 = .984$$
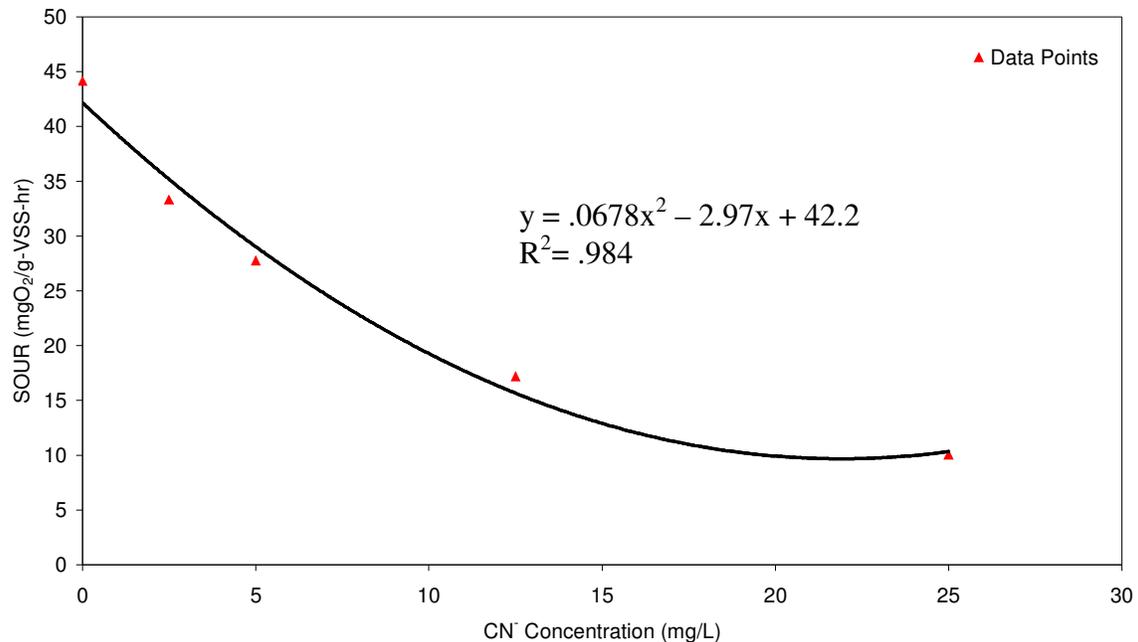
Figure 4.1   IC$_{XX}$ determination for 10 day SRT biomass exposed to zinc-cyanide complex.

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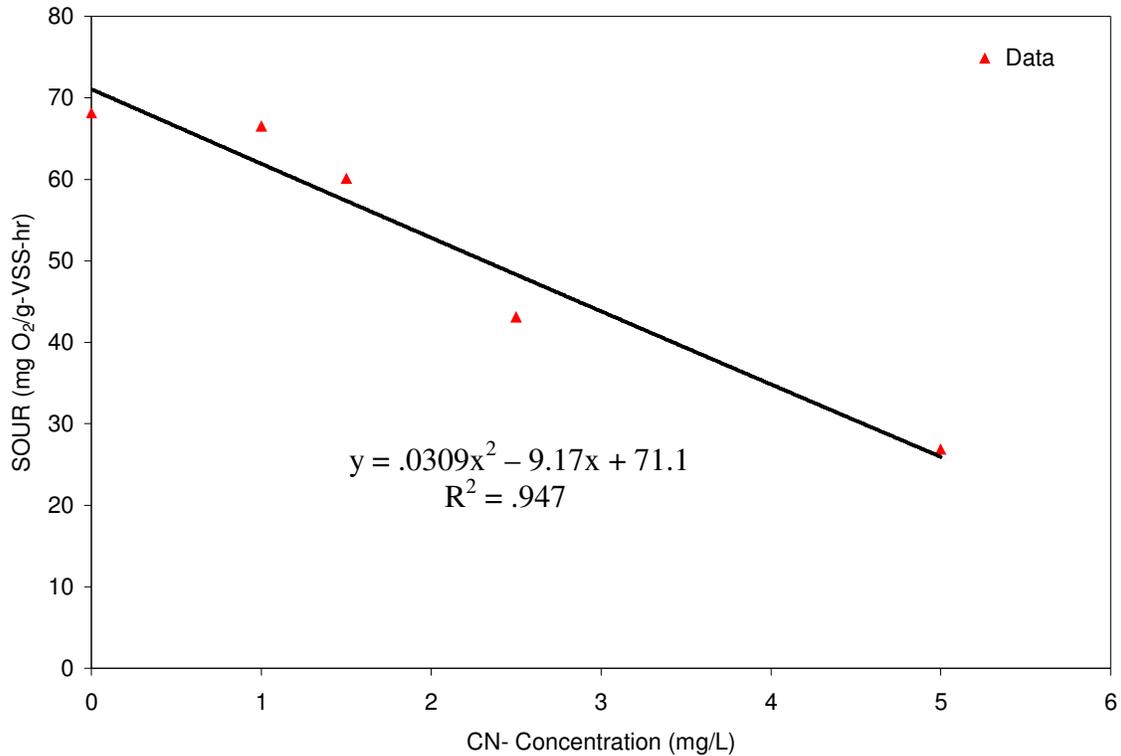
Figure 4.2 IC_{XX} determination for 2 day SRT biomass exposed to zinc-cyanide complex.

Table 4.1 IC_{15}, IC_{25} and IC_{50} values for zinc-cyanide complex in 10 and 2 day SRT mixed liquors.

	10 day SRT		2 day SRT	
	mg/L	mg CN^-/g MLVSS	mg/L	mg CN^-/g MLVSS
IC_{15}	1.7	0.53	1.4	1.4
IC_{25}	3.4	1.1	2.3	2.3
IC_{50}	8.4	2.6	5.5	5.5

Table 4.2   Mass of zinc added to control reactors containing 10 and 2 day SRT mixed liquors.

	10 day SRT		2 day SRT	
	mg as Zn^{2+}	g as $ZnSO_4.7H2O$	mg as Zn^{2+}	g as $ZnSO_4.7H2O$
Control	8.845	.0362	5.811	.0256

When looking only at the concentrations (mg/L) of cyanide added to the 10 and 2 day reactors, the ICxx concentrations added to the 10 day system were approximately 1.5

times the ICxx concentrations added to the 2 day system. This suggests that the 10 day SRT system is less sensitive than the 2 day. However, when concentrations are normalized to a mass contaminant per mass reactor solids basis (mg CN^-/g-MLVSS), the 10 day SRT system was found to have concentrations more than half those of the 2 day SRT system. This suggests that the 10 day SRT system is, in fact, more sensitive to the addition of cyanide than the 2 day SRT system. As previously discussed, cyanide is known to have a marked effect on the nitrification process. Because a significant portion of the oxygen demand in the 10 day SRT system comes from nitrifiers, this may explain why the IC_{XX} for the nitrifying 10 day SRT culture is lower on a mg/g basis than for the 2 day SRT biomass.

4.3.2. Influent Characteristics

Average influent characteristics over the course of the cyanide source-effect experiment are given in Table 4.3. The influent supplementation program was ongoing during this experiment and influent alkalinity was above the levels needed for steady nitrification in the 10 day SRT system, as 7.08 mg of $CaCO_3$ is required (or used) per mg NH_4^+-N removed, to convert ammonia to nitrate (Grady et al., 1999). The influent pH is within the normal range found in domestic wastewaters. Ammonia, TSS and VSS concentrations are typical of those found in medium strength domestic wastewaters (Metcalf and Eddy, 2003). The VSS/TSS ratio was stable and averaged 0.48 ± 0.11. The total and soluble COD concentrations are higher than are typically found in primary treated wastewaters (Metcalf and Eddy, 2003), but are in accordance with target influent CODs that are used to achieve typical mixed liquor concentrations in both pilot SBRs.

Table 4.3   Characteristics of wastewater used during cyanide source-effect experiment.

Characteristic	2 day average ± std dev	10 day average ± std dev
Alkalinity (mg/L as $CaCO_3$)	564 ± 22	356 ± 20
Ammonia (mg/L as N)	24 ± 5.3	11 ± 2.5
COD, soluble (mg/L)	874 ± 80	512 ± 90
COD, total (mg/L)	1,026 ± 158	726 ± 264
pH	7.4 ± 0.2	7.5 ± 0.2
TSS (mg/L)	255 ± 75	151 ± 53
VSS (mg/L)	89 ± 30	72 ± 21

4.3.3. Effluent Characteristics

Effluent sCOD data for the 10 day SRT system show that sCOD removal efficiency was unaffected by the zinc-cyanide complex in the IC_{15} or IC_{25} reactors and had only a small, brief effect in the IC_{50} reactor (recovery by cycle 6), as seen in Figure 4.3. In the 2 day SRT system sCOD removal efficiency in the IC_{15} and IC_{25} reactors was unaffected (Figure 4.4). The IC_{50} reactor experienced a severe loss of sCOD removal for the first two cycles as compared to the control, but recovered by cycle 3. This loss of COD removal capability in the IC_{50} reactor corresponds to a decreased SOUR relative to the control (data discussed later). These results suggest that cyanide only affected the 2 day IC_{50} reactor. On a mass cyanide loading to mass MLVSS basis, the 2 day SRT IC_{50} reactor received the highest shock load among all cyanide source-effect experiments, but not the highest concentration (see Table 4.1). This result suggests that using mass cyanide load per mass MLVSS may be a better predictor of impact on sCOD removal efficiency for weak acid cyanide shock loads.
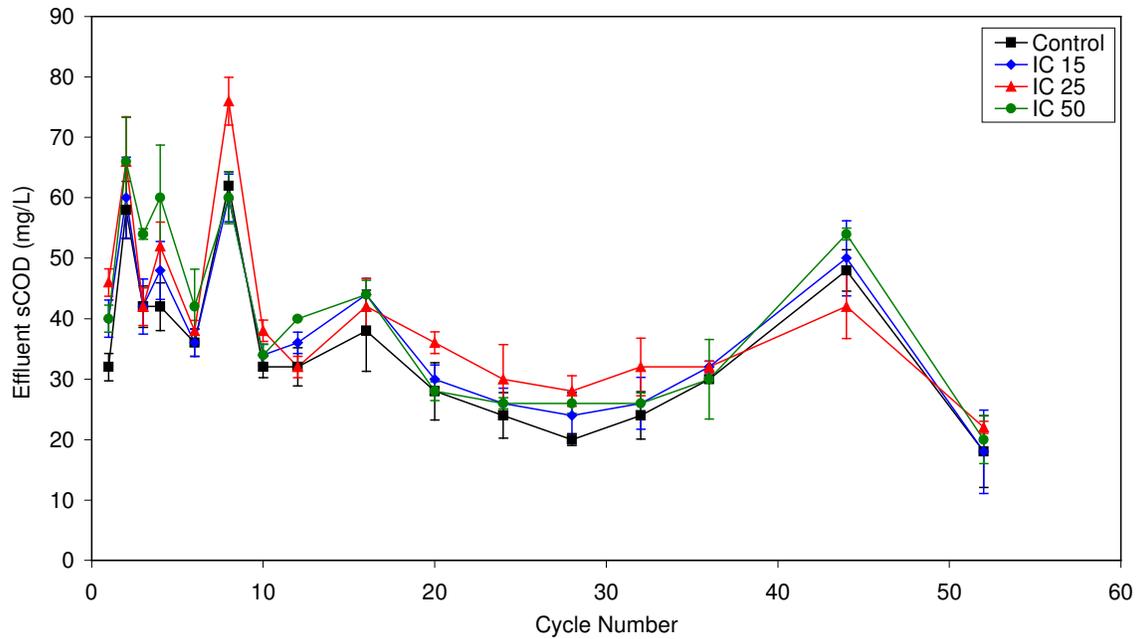
Figure 4.3   Soluble effluent COD for 10 day SRT reactors exposed to zinc-cyanide complex.
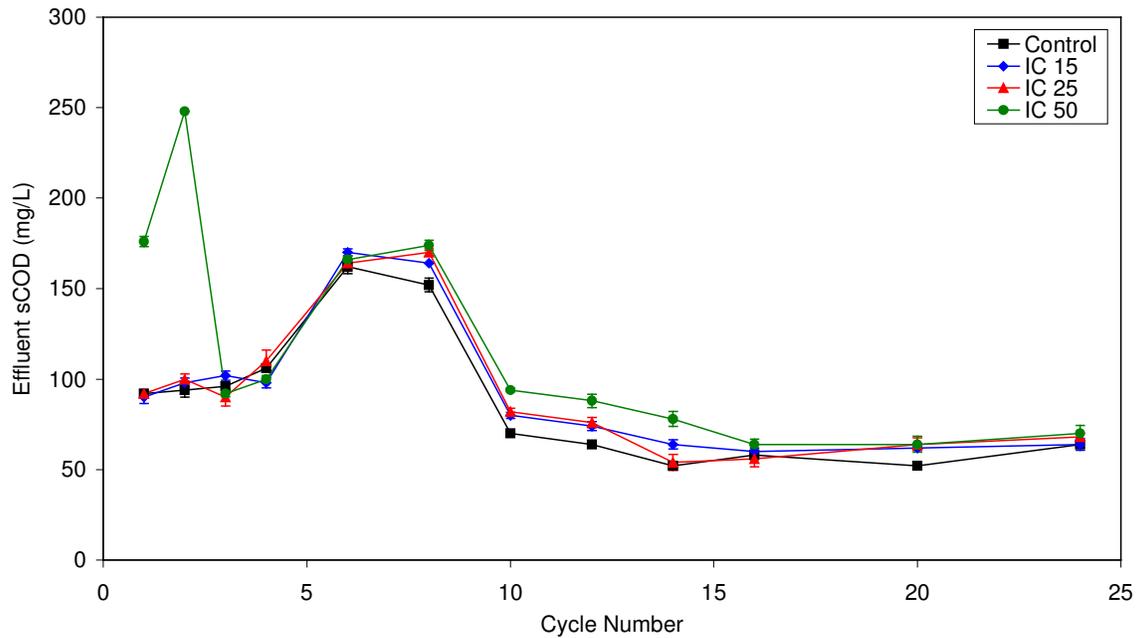

Figure 4.4   Soluble effluent COD for 2 day SRT reactors exposed to zinc-cyanide complex.

As with effluent sCOD removal efficiency, effluent TSS was largely unaffected by the addition of zinc-cyanide complex. For the 10 day SRT system the IC_{25} reactor

showed significantly ($\alpha = 0.05$) higher suspended solids relative to the control at cycles 2, 10, and 12, while the IC_{50} reactor showed higher suspended solids relative to the control at cycle 1 (Figure 4.5). These results cannot be attributed to cyanide as no trending effects were seen with increasing cyanide concentration. In the 2 day SRT system the IC_{15}, IC_{25}, and IC_{50} reactors all deviated from the control until cycle 8 (Figure 4.6). As with the 10-day SRT biomass, these results cannot be attributed to cyanide as not trending effects were seen with increasing cyanide concentration. Overall, effluent TSS seemed unaffected by the addition of zinc-cyanide complex to each reactor. These data also suggest that the addition of zinc, a thiol reactive heavy metal, was not present in its free and reactive form in sufficient quantity to activate a glutathione-gated potassium efflux event. This is validated by potassium data, presented later in Figure 4.18. The increase in effluent TSS for the IC_{25} and IC_{50} reactors at cycle 12 in the 2 day SRT system cannot be explained.
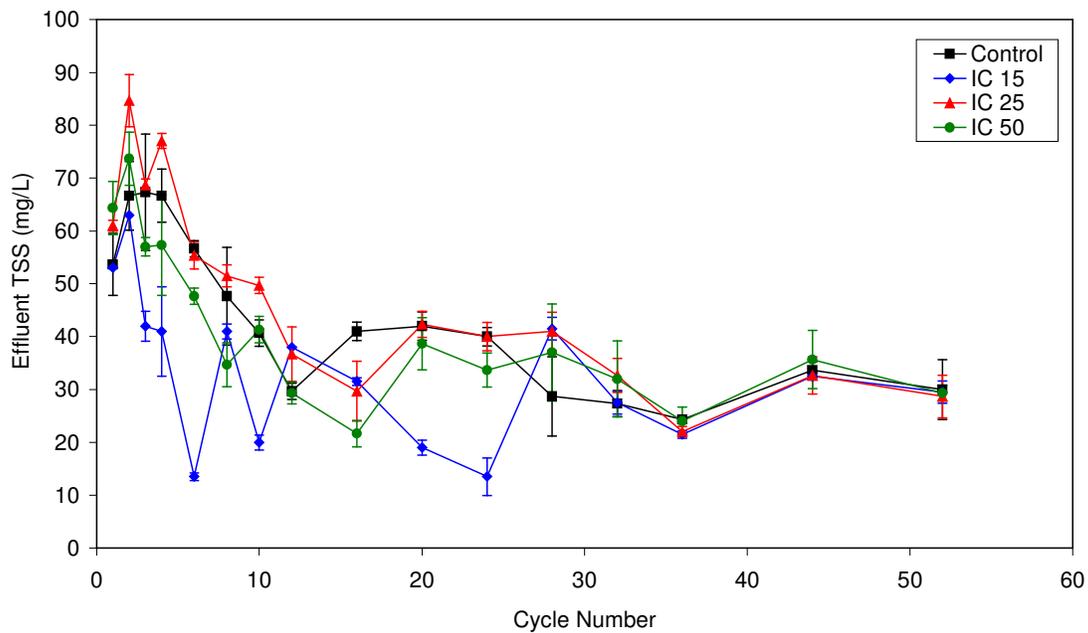

Figure 4.5   Effluent TSS for 10 day SRT reactors exposed to zinc-cyanide complex.
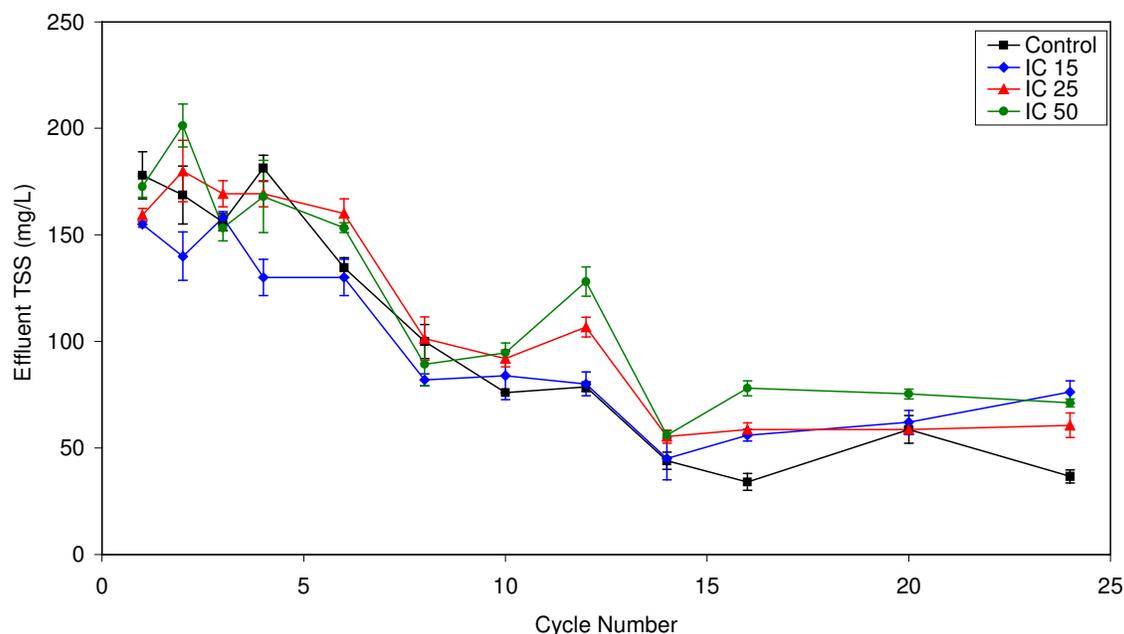
Figure 4.6   Effluent TSS for 2 day SRT reactors exposed to zinc-cyanide complex.

As previously discussed, cyanide is a known inhibitor of nitrification, and the results for the nitrifying 10 day SRT biomass shown in Figures 4.8, 4.9 and 4.10 are consistent with this effect. All 10 day SRT reactors were nitrifying prior to cyanide addition. After cyanide addition, a dramatic increase in effluent ammonia was observed in the IC_{50} reactor, illustrating an inhibition of ammonia oxidizing bacteria at high concentrations of cyanide (Figure 4.8). The ammonia oxidizing bacteria recovered by cycle 4, which corresponds with lower theoretical levels of free cyanide (based on a dilution curve assuming no reaction or absorption) (Figure 4.7). At cycle 4, levels of free cyanide in the IC_{50} reactor were approximately 3 mg/L as CN^- (Figure 4.7). The IC_{25} reactor was unaffected by similar levels, so it is believed that it was the decreased free cyanide concentration, and not AOB adaptation, that resulted in AOB recovery. AOB recovery resulted in a sharp increase in nitrite (Figure 4.9). This increase in nitrite is most likely due to the nitrite oxidizing bacteria being unable to deal with the large influx of nitrite, causing a delay in nitrite conversion; however, some inhibition of nitrite oxidizing bacteria may have occurred. There were moderate levels of nitrite found in the IC_{15} and IC_{25} reactors, suggesting slight inhibition of nitrite oxidation that corresponded with the

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level of zinc-cyanide complex introduced to each reactor. Figure 4.10 demonstrates lagging trends in nitrate conversion consistent with the increasing inhibitory concentrations in the shocked reactors. Overall, there was clear inhibition of nitrification with the addition of increasing concentrations of zinc-cyanide complex, but the systems all recovered to within control performance levels by cycle 10. This suggests that weak metal-complexed cyanide inhibits nitrification, but that this inhibition is reversible.
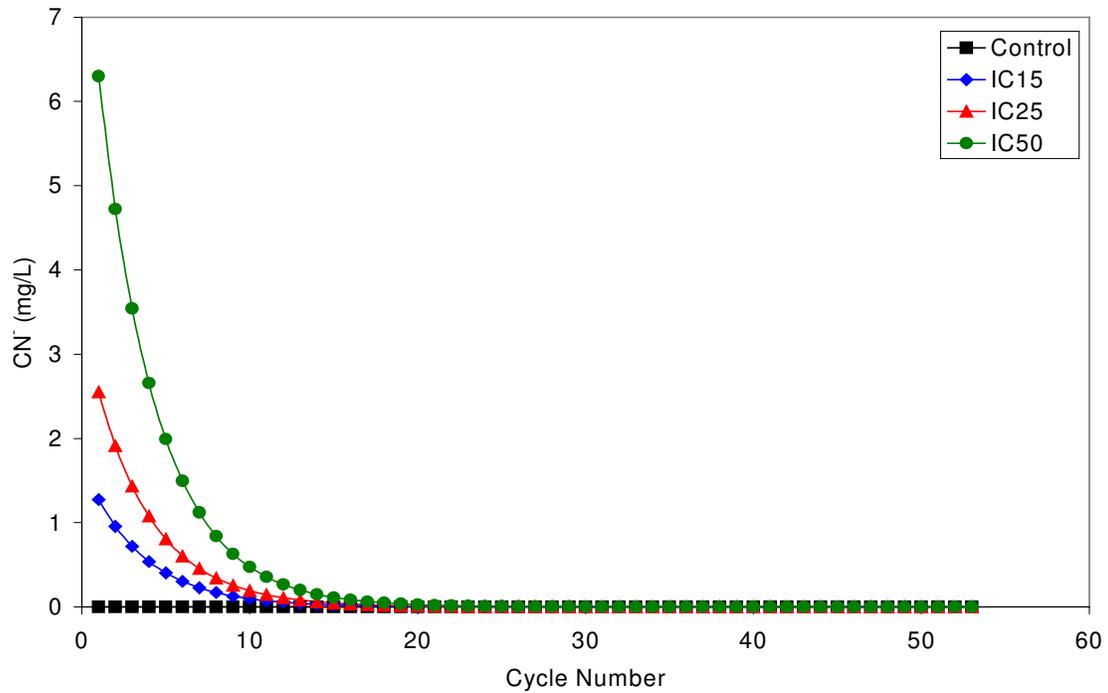

Figure 4.7   Theoretical dilution of free cyanide in the 10 day SRT system exposed to zinc-cyanide complex.

Figure 4.8   Effluent ammonia-N in the 10 day SRT reactors exposed to zinc-cyanide complex.

Figure 4.9   Effluent nitrite-N in the 10 day SRT reactors exposed to zinc-cyanide complex.

Figure 4.10   Effluent nitrate-N in the 10 day SRT reactors exposed to zinc-cyanide complex.

The 2 day pilot plant reactor was being operated to prevent nitrification and was not nitrifying to a significant degree the day before the experiment began. Figures 4.12 and 4.13 show a small amount of nitrite and nitrate, respectively, being produced, but these values are consistent with what was routinely observed with the 2 day SRT system biomass (minimal nitrification). Figure 4.11 shows the soluble effluent ammonia profile for the 2 day SRT system. There was a lot of variation in ammonia-N levels, but all the reactors held an average of about 11 mg/L. Overall, there were no clear trends that suggest an impact on the modest nitrification occurring in the 2 day SRT biomass. This is consistent with expectations, as nitrification was operationally prevented in the 2 day SRT system.
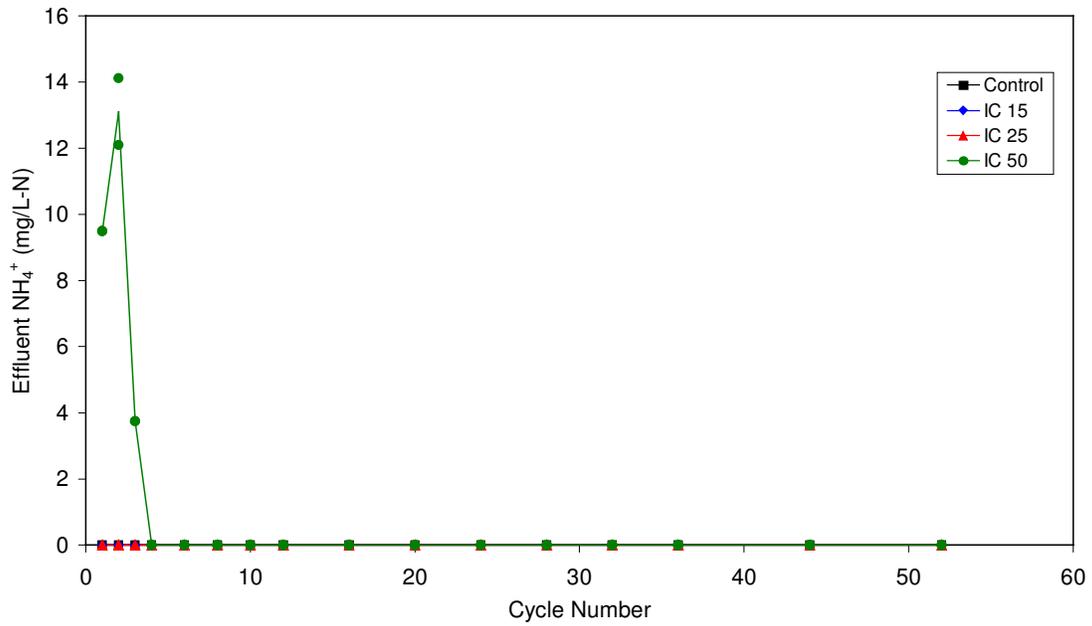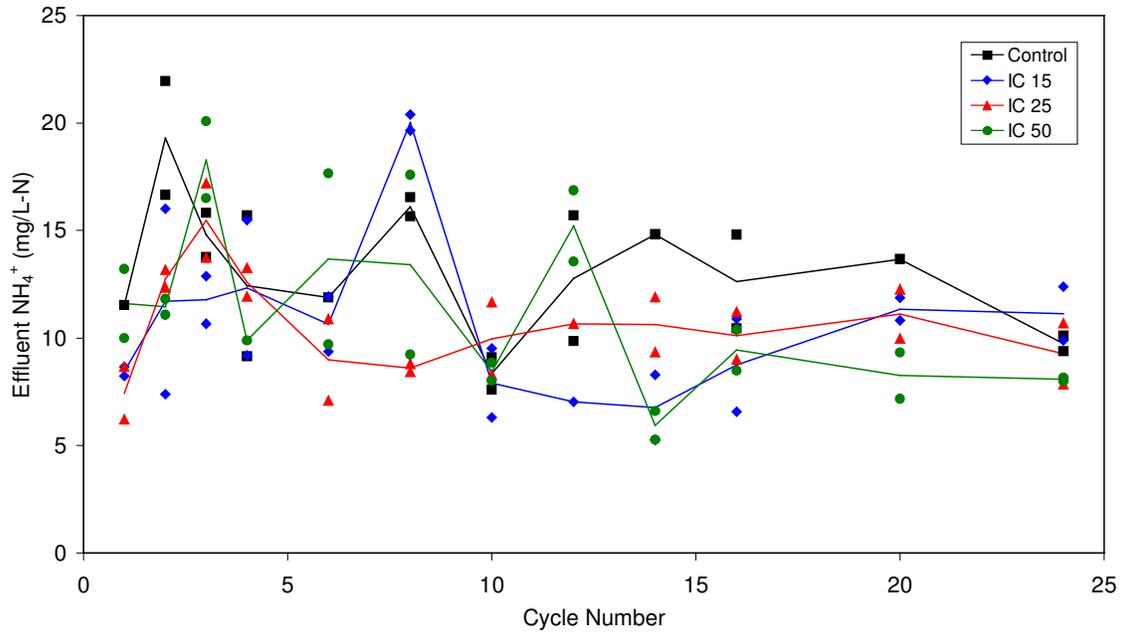
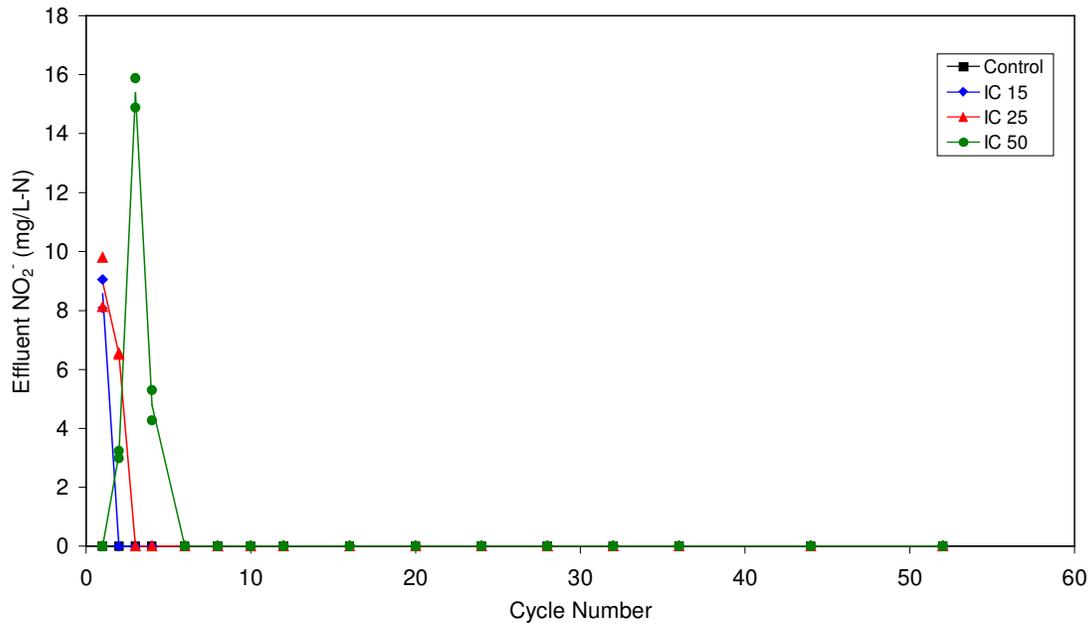Figure 4.11   Effluent ammonia-N in the 2 day SRT reactors exposed to zinc-cyanide complex.

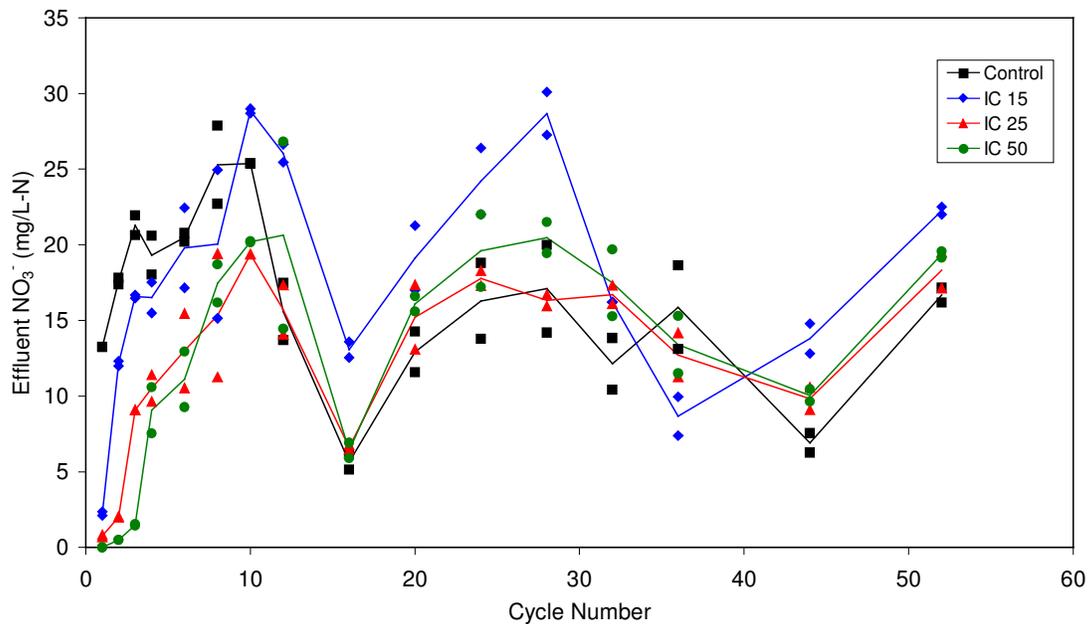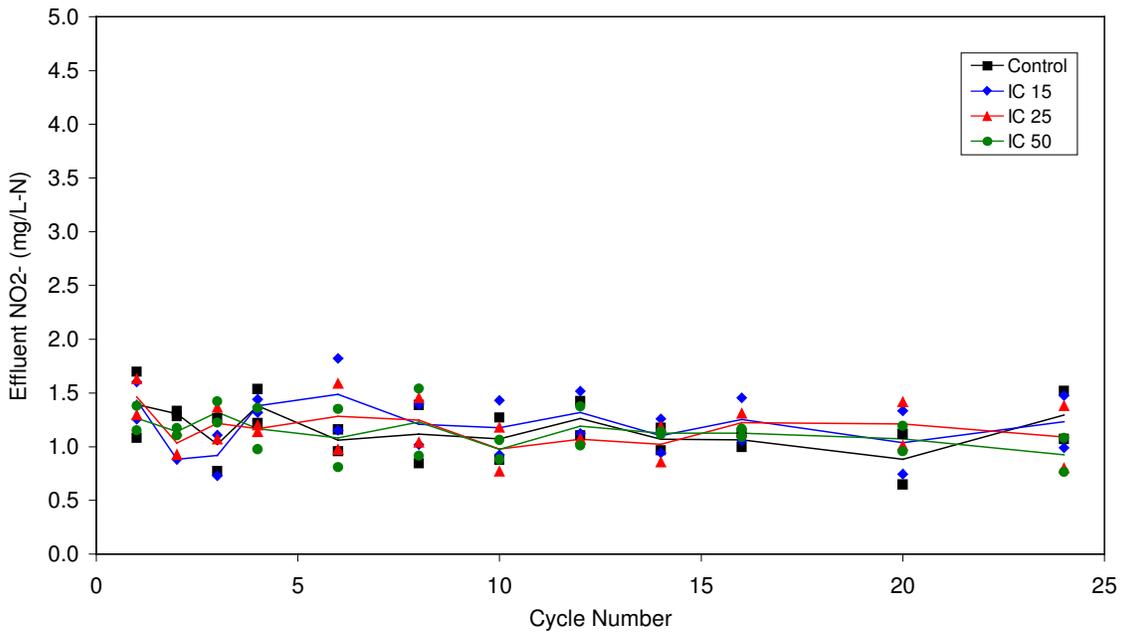Figure 4.12   Effluent nitrite-N in the 2 day SRT reactors exposed to zinc-cyanide complex.
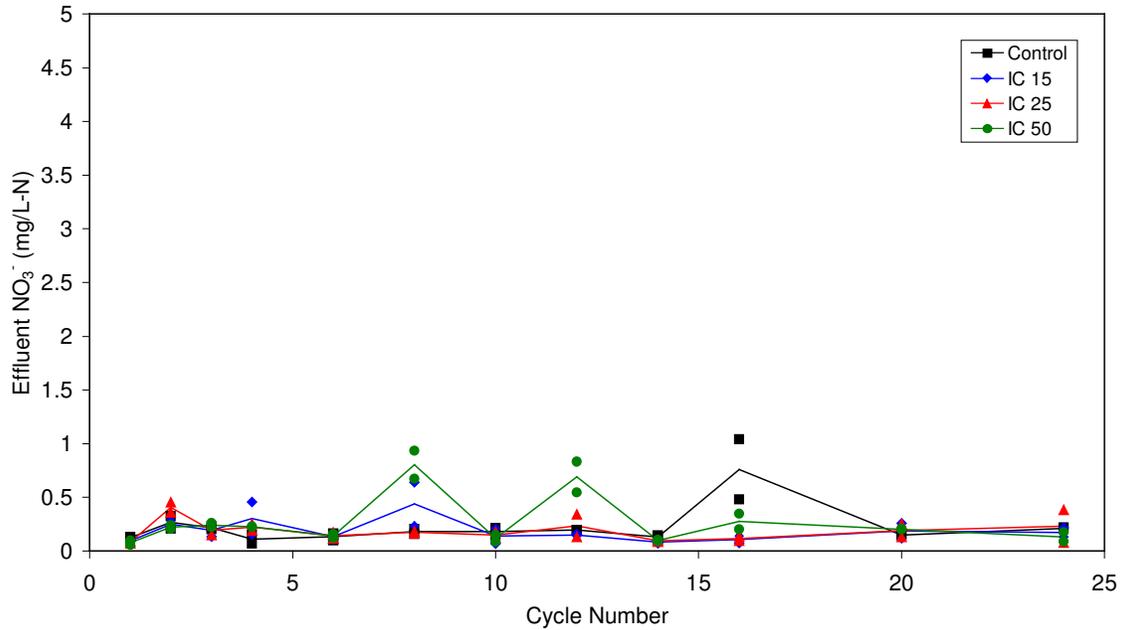
Figure 4.13   Effluent nitrate-N in the 2 day SRT reactors exposed to zinc-cyanide complex.

Alkalinity was monitored because it is consumed during nitrification and should provide an independent measure of correlation with the degree of nitrification. Effluent alkalinity concentrations for the 10 day and 2 day SRT reactors are shown in Figures 4.14 and 4.15, respectively. In the 10 day SRT system the control reactor has significantly lower alkalinity levels than the three shocked reactors (Figure 4.14). This was expected because nitrification was not interrupted in the control reactor, so more alkalinity should have been consumed. There are increasing levels of alkalinity corresponding to increasing inhibitory concentrations added to the reactors during the first five cycles. This correlates well to the brief inhibitory effects that were observed among the nitrifying 10 day SRT shocked reactors. Alkalinity levels in the 2 day system were uncharacteristic of any nitrification effects, because the 2 day SRT system was not nitrifying during this experiment.

Figure 4.14   Effluent alkalinity for the 10 day SRT reactors exposed to zinc-cyanide complex.
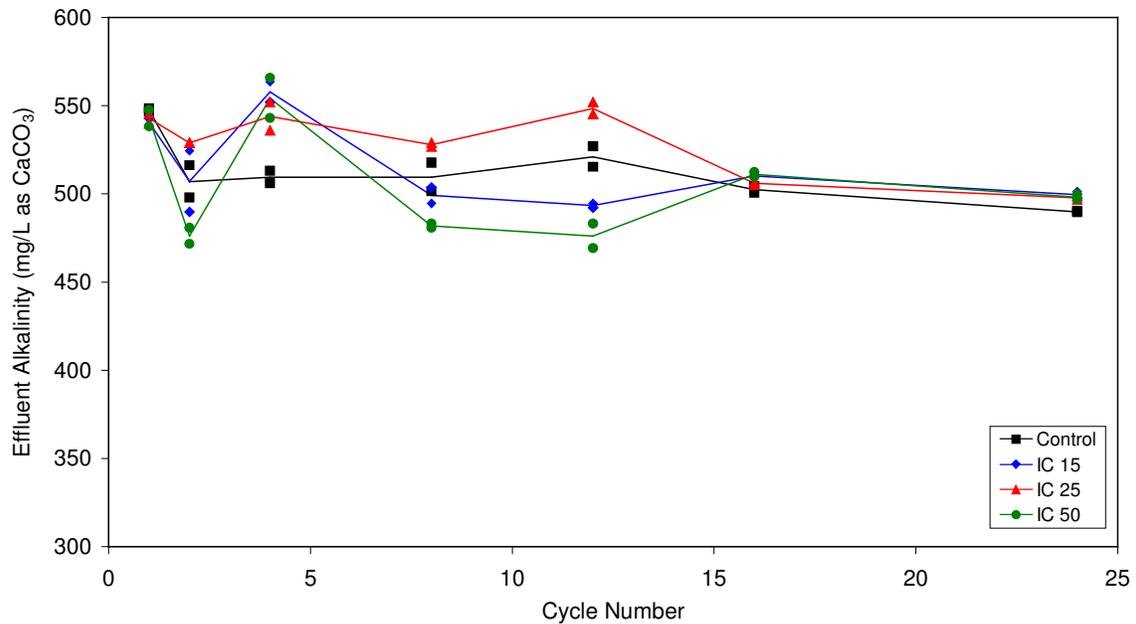
Figure 4.15   Effluent alkalinity for the 2 day SRT reactors exposed to zinc-cyanide complex.

Effluent pH values for the 10 day SRT system show a significant (α=0.05) increase from the control in the IC_{50} reactor during cycle 1 (Figure 4.16). This correlates

with the increase in alkalinity discussed previously. The IC_{15} and IC_{25} reactors did show an elevated pH at cycle 16, and the control showed a sharp decrease in pH at the last cycle; none of these outliers can be explained in terms of other measured water quality indicators. Effluent pH levels in the 2 day SRT system for the shocked reactors were elevated for most of the duration of the experiment; however, the deviation was small enough not to be significant (Figure 4.17).

Figure 4.16   Effluent pH for the 10 day SRT reactors exposed to zinc- cyanide complex.
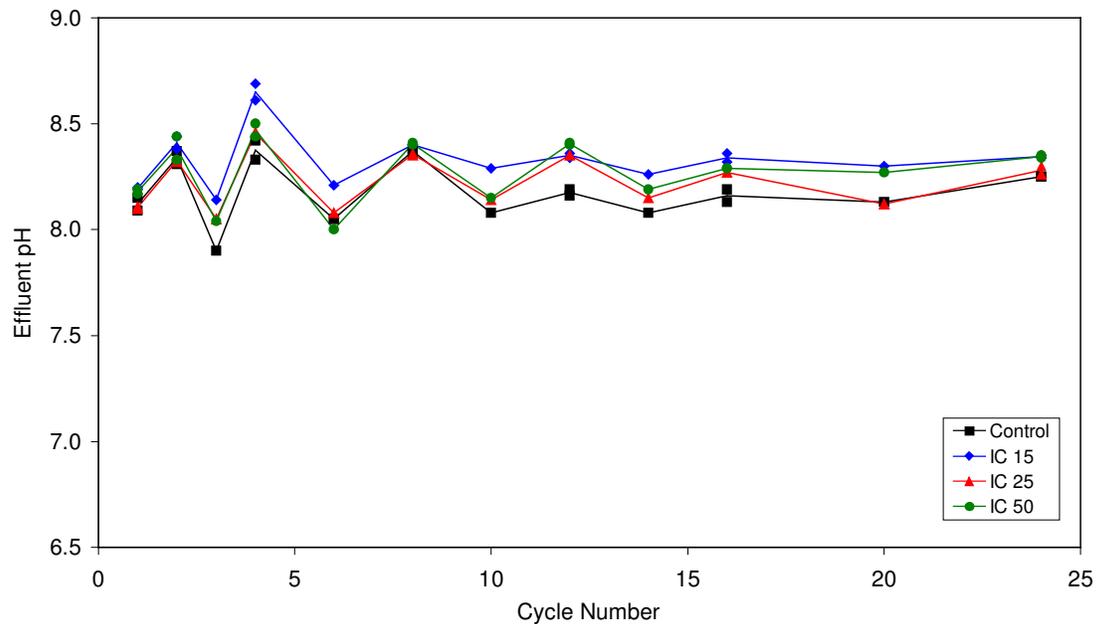
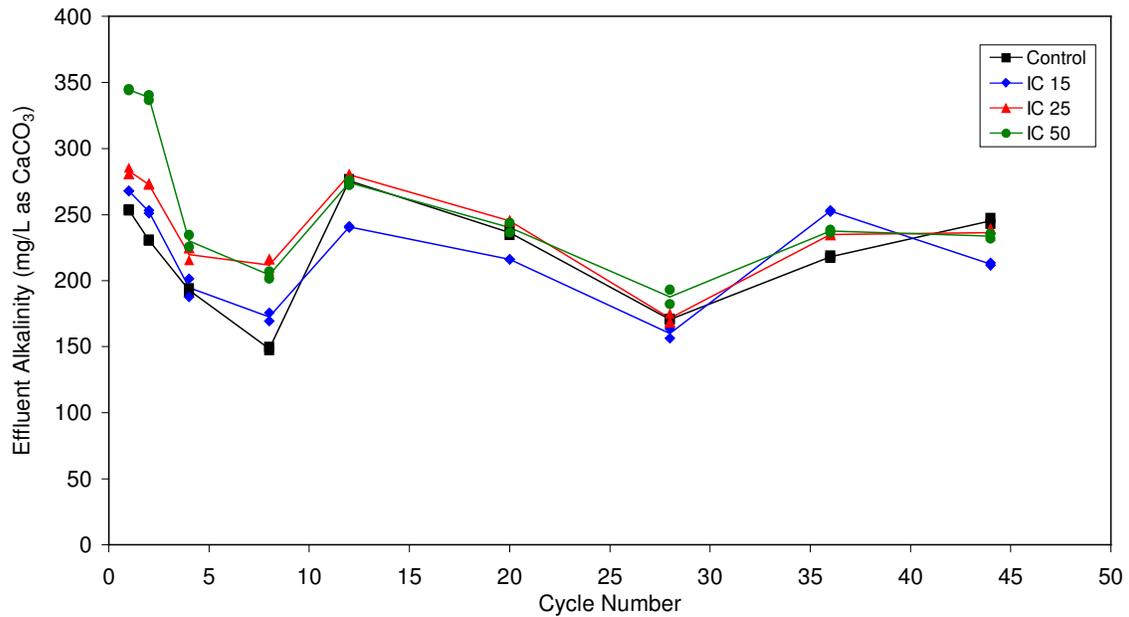Figure 4.17   Effluent pH levels for the 2 day SRT reactors exposed to zinc- cyanide complex.

Effluent soluble potassium levels were measured throughout both the 10 day and 2 day SRT experiments (Figures 4.18 and 4.19, respectively). No significant increases were seen in either the 10 day or 2 day SRT reactors for any concentration. The decreasing trend of the potassium levels in the 10 day reactors follows the decrease in MLSS and MLVSS in the same system. So, this overall decrease in potassium levels is probably due to the decreasing biomass concentrations. The K+ per MLVSS ratio was calculated at every cycle and was found to remain constant over the experiment: 0.014 ± 0.001, 0.014 ± 0.001, 0.013 ± 0.001, and 0.013 ± 0.001 for the Control, IC_{15}, $IC_{25,}$ and IC_{50} reactors, respectively (Table B.28 in Appendix B). This supports the supposition that the decrease in potassium levels was due to the decreasing biomass concentration.
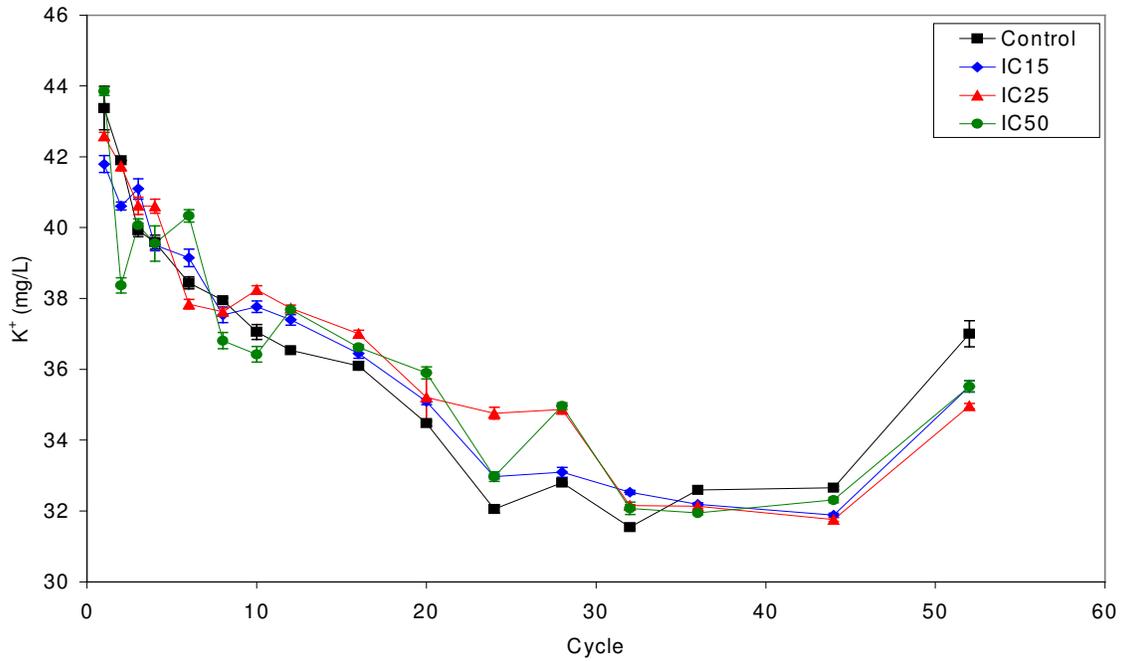
Figure 4.18   Effluent soluble potassium levels for the 10 day SRT reactors exposed to zinc-cyanide complex.
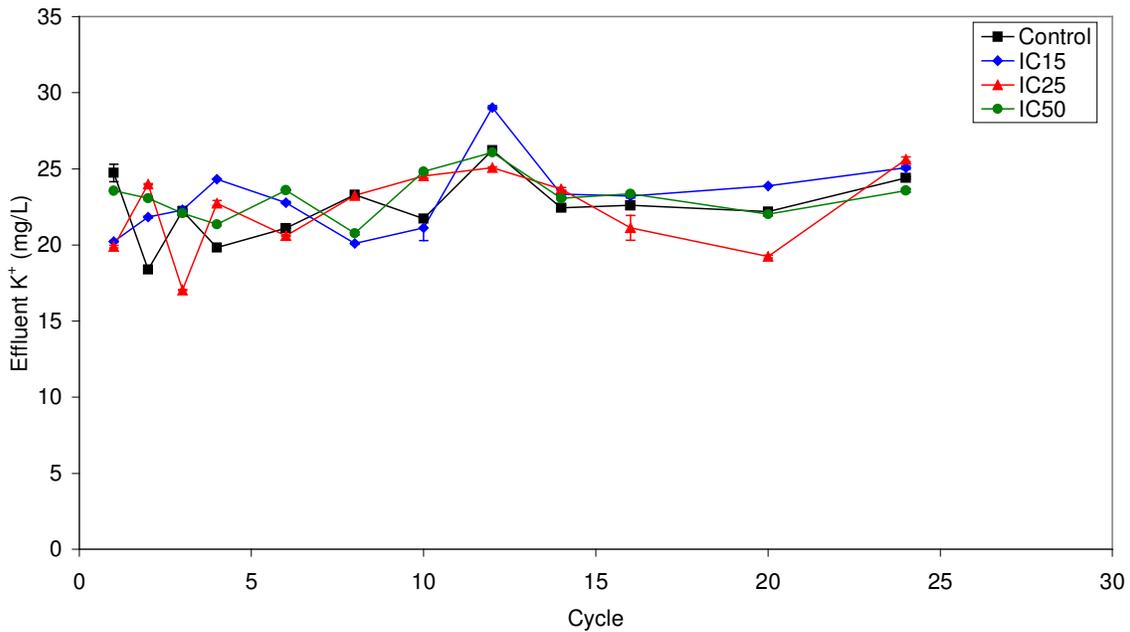
Figure 4.19   Effluent soluble potassium levels for the 2 day SRT reactors exposed to zinc-cyanide complex.

4.3.4. *Mixed Liquor Characteristics*

CST measurements were taken during the first seventeen cycles and last cycle of

the 10 day experiment and the first and last cycles of the 2 day experiment. The values were relatively unchanged. The 10 day SRT reactors averaged a CST of 16.5 ± 0.3 seconds, while the 2 day SRT reactors averaged a CST of 19.8 ± 0.4 seconds. These low values indicate that cyanide had little impact on the dewaterability of activated sludge.

The SVI data for the 10 and 2 day SRT reactors are shown in Figures 4.20 and 4.21, respectively. There was no significant increase in relation to the control for any of the experimental reactors at either SRT except for the 10 day SRT IC_{50} shocked reactor. The IC_{50} reactor had significantly higher SVI measurement at cycle 2, which was the first cycle when SVI was measured. The higher SVI remained below 120 mL/g, and this deviation recovered to that of the control by cycle 5. This observation correlates with the loss of ammonia oxidation in the IC_{50} reactor, and is consistent with Novak's hypothesis that the ammonium cation may cause weak floc structure (Novak, 2001). Beginning at cycle 14 (day 4) the 2 day SRT reactors began to trend upwards but the cause is unknown (Figure 4.21). Otherwise all 2 day shocked reactor SVI measurements were equivalent to, or below, the control.

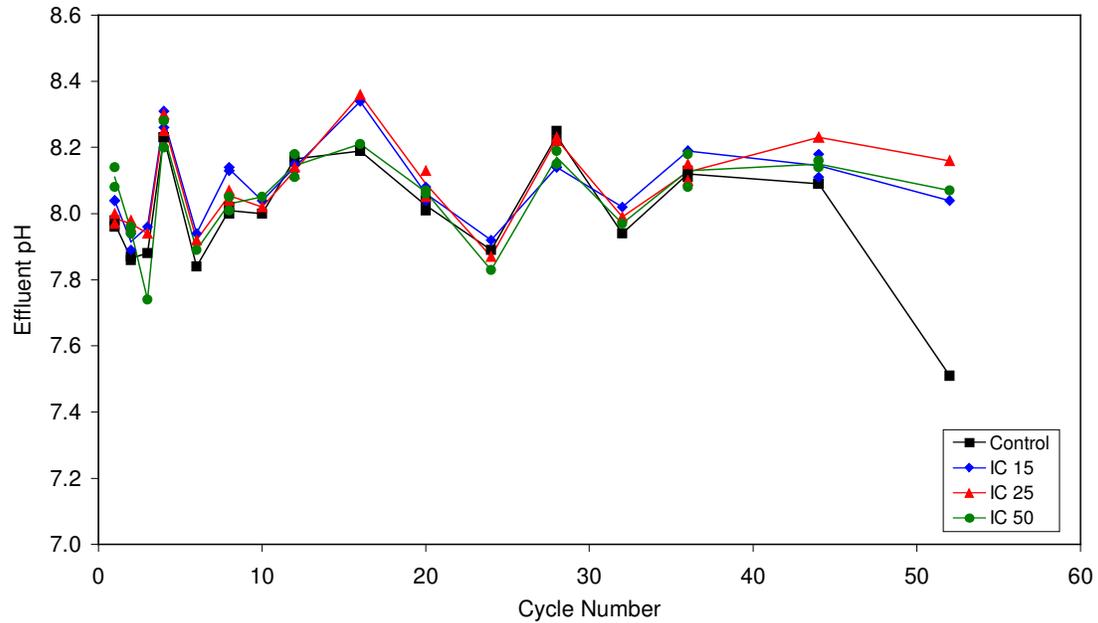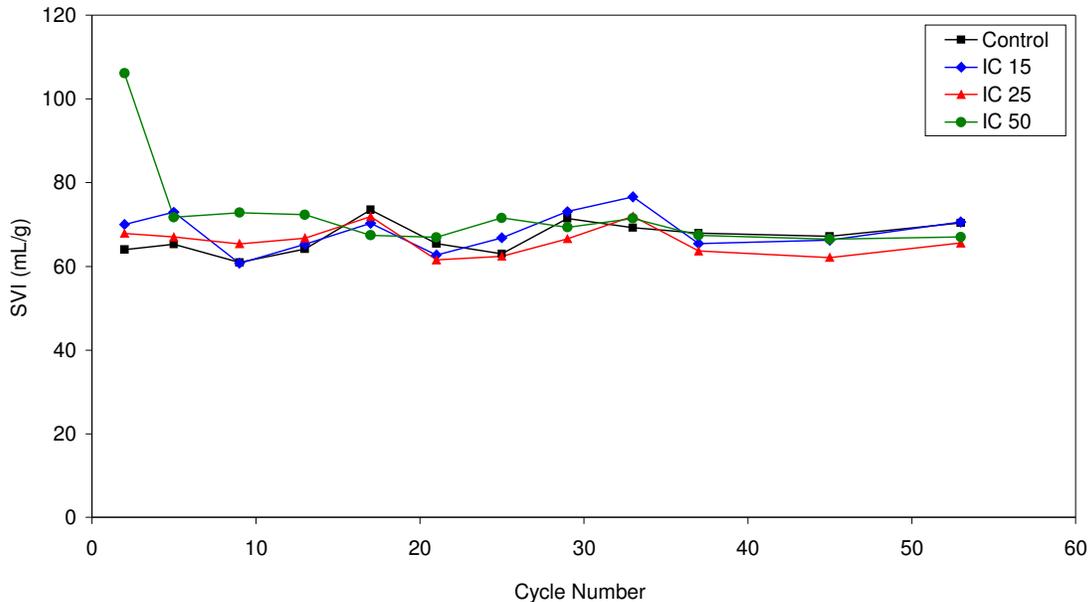Figure 4.20   SVI values for the 10 day SRT reactors exposed to zinc-cyanide complex.
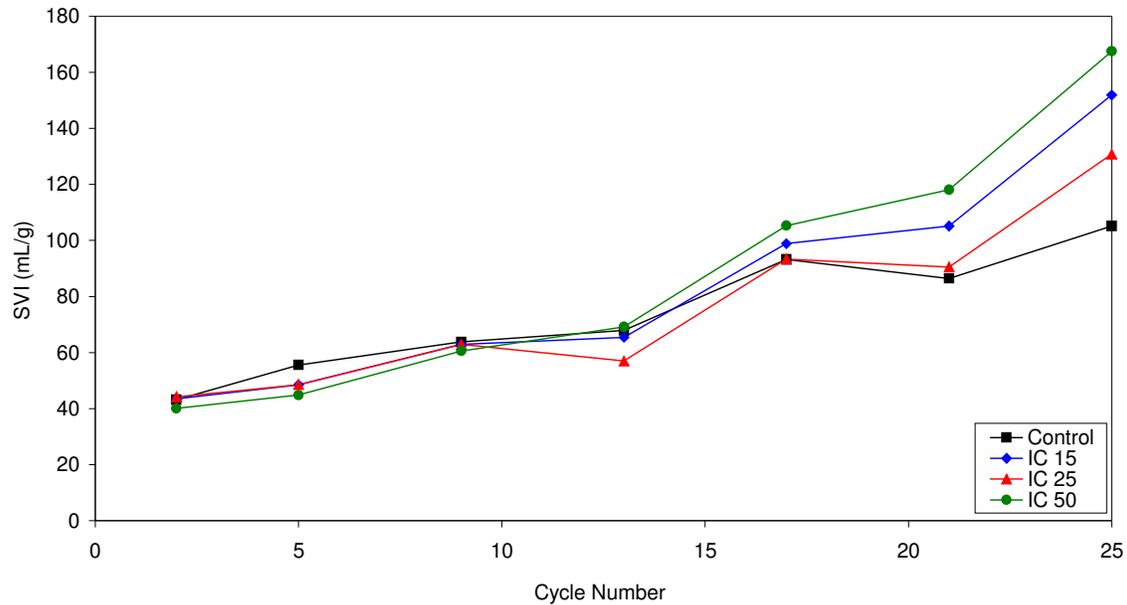
Figure 4.21   SVI values for the 2 day SRT reactors exposed to zinc-cyanide complex.

The mixed liquor suspended solids (MLSS) and volatile suspended solids (MLVSS) concentrations serve as indicators of system stability. Throughout the 10 day SRT cyanide source-effect experiment, the mixed liquor concentrations in all reactors decreased consistently over time (Figures 4.22 and 4.24). The decrease continued until a quasi -steady state was reached at around 3,000 mg/L in the 10 day SRT system. This effect has been seen before in other source-effect experiments (not shown) and is believed to be due to more suspended solids being present in pilot plant influent, increasing the MLSS concentration of the biomass that was brought into the laboratory at the start of the experiment, than in the influent fed to the laboratory reactors. This would explain the high MLSS concentration at the beginning of the experiment and the subsequent decrease throughout. The MLSS and MLVSS measurements in the 2 day SRT system remained relative constant around 1,075 mg/L and 758 mg/L (Figures 4.23 and 4.25, respectively). An overall summary of mixed liquor concentrations is given in Table 4.4 .

Table 4.4   Mixed liquor concentrations in 10 and 2 day SRT reactors exposed to zinc-
cyanide complex.

	MLSS (mg/L)	MLVSS (mg/L)	MLSS:MLVSS Ratio (%)
10 day SRT reactors			
Control	$3,966 \pm 669$	$2,612 \pm 423$	0.66 ± 0.02
IC$_{15}$	$3,964 \pm 809$	$2,607 \pm 512$	0.66 ± 0.02
IC$_{25}$	$4,071 \pm 763$	$2,666 \pm 468$	0.66 ± 0.01
IC$_{50}$	$4,140 \pm 812$	$2,708 \pm 503$	0.66 ± 0.01
2 day SRT reactors			
Control	$1,128 \pm 182$	792 ± 90	0.71 ± 0.05
IC$_{15}$	$1,084 \pm 180$	765 ± 90	0.71 ± 0.05
IC$_{25}$	$1,086 \pm 171$	771 ± 86	0.72 ± 0.05
IC$_{50}$	$1,000 \pm 255$	705 ± 168	0.71 ± 0.05

Figure 4.22   MLSS concentration in the 10 day SRT reactors exposed to zinc-cyanide
complex.
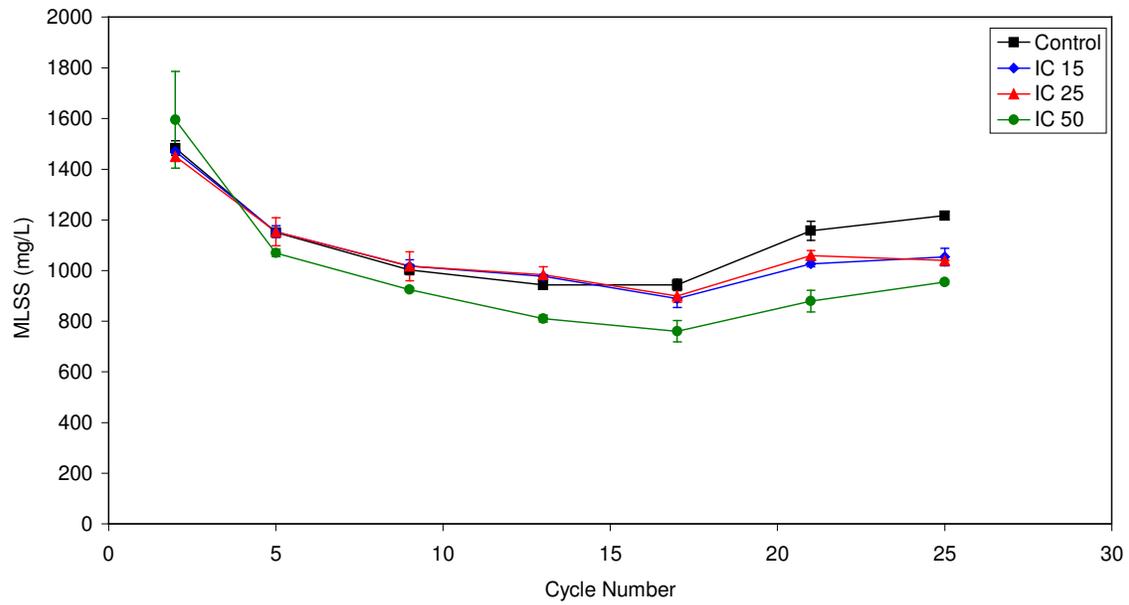
Figure 4.23   MLSS concentration in the 2 day SRT reactors exposed to zinc-cyanide complex.

Figure 4.24   MLVSS concentration in the 10 day SRT reactors exposed to zinc-cyanide complex.

Figure 4.25   MLVSS concentration in the 2 day SRT reactors exposed to zinc-cyanide complex.

The specific oxygen uptake rate (SOUR) is a test biomass viability. The higher the SOUR for a system, the more active the biomass in the system. SOUR results for the first thirty cycles of the 10 day cyanide experiment show cyanide significantly ($\alpha=0.05$) inhibited respiration (Figure 4.26). SOUR results also shows that the magnitude of respiration inhibition correlated with the dose of cyanide for all three shocked reactors. After cycle 30, all three shocked reactors had increased SOUR measurements compared to the control. There are a few explanations for why this overall SOUR trend may have occurred. The first possibility is that the cyanide killed the majority of the bacteria (more killed with higher concentrations) except a small number that may have been resistant. If this occurred, the bacteria would require an extended amount of time to grow to previous numbers and to regain substantial oxygen uptake (i.e. thirty cycles). The second possibility is that the cyanide bonded with the cytochrome oxidases of the bacteria in the shocked reactors and the various lag times in SOUR recovery represent the amount of time necessary for the bacteria to overcome the inhibition. The third possibility is that the zinc-cyanide complex addition primarily affected the nitrifiers in the biomass. The decrease in respiration correlates with the inhibition of nitrification (as seen by the nitrate

70

generation rate test (NGR)) discussed next. As nitrification recovered, according to NGR results, biomass respiration increased to normal levels. The differences between the NGR recovery and effluent nitrogen species recovery is due to the length of each assays. The NGR assay involves ten-minute samplings similar to the SOUR assay. So, the fact that these results correlate better than the results from effluent nitrogen species sampling, which occur every six hours, makes sense. In any case, there was a significant effect on cell respiration caused by zinc-cyanide complex addition.

In the 2 day SRT SOUR measurements only the IC_{50} reactor experienced significant (α=0.05) deviation from the control during the first eight cycles (Figure 4.27).. This could also be due to the reasons given above, where the zinc-cyanide complex added to the IC_{15} and IC_{25} reactors was insufficient to cause reduced SOUR effects. When referring to Table 4.1, it is shown that the 2-day IC_{50} reactor received a much larger dose (on a mg CN^-/g-MLVSS basis) than any other reactor in either the 10 or 2 day SRT systems. The fact that this dramatic results in the 2 day SRT IC_{50} reactor correlate with the largest added concentration, on a mass contaminant per mass reactor solids basis, illustrates the importance of using this normalization. It is also important to note that since the 2-day SRT system was not significantly nitrifying, yet still showed respiration effects, that the heterotrophic population was affected. At cycle 17, all the shocked reactors of the 2 day SRT system experienced a sharp decrease in SOUR activity as compared to the control, the cause of which is unknown. All reactors recovered near control values by the next measured cycle.
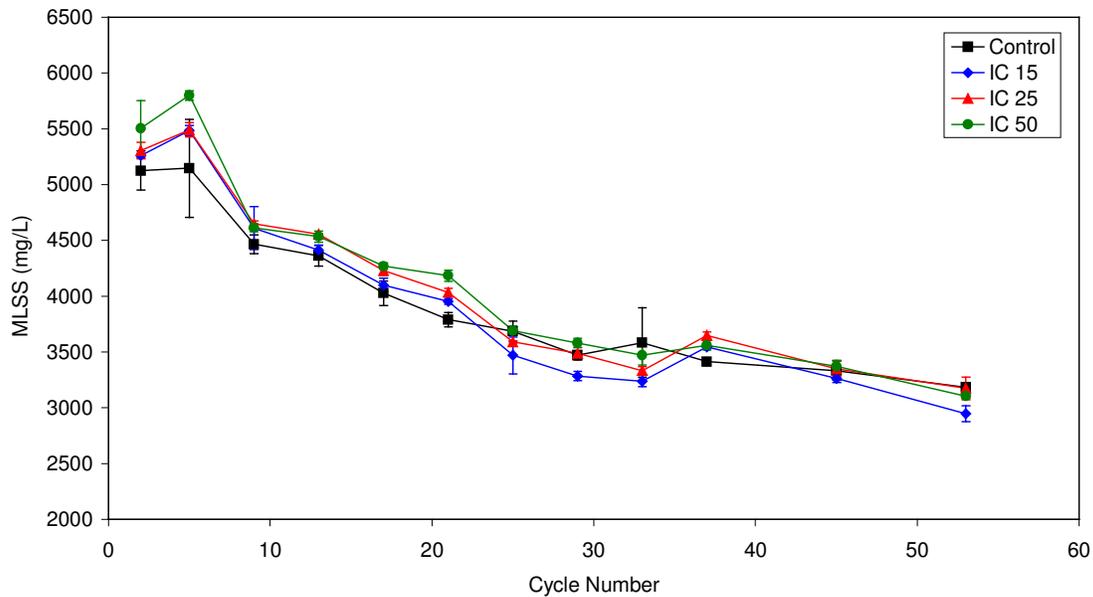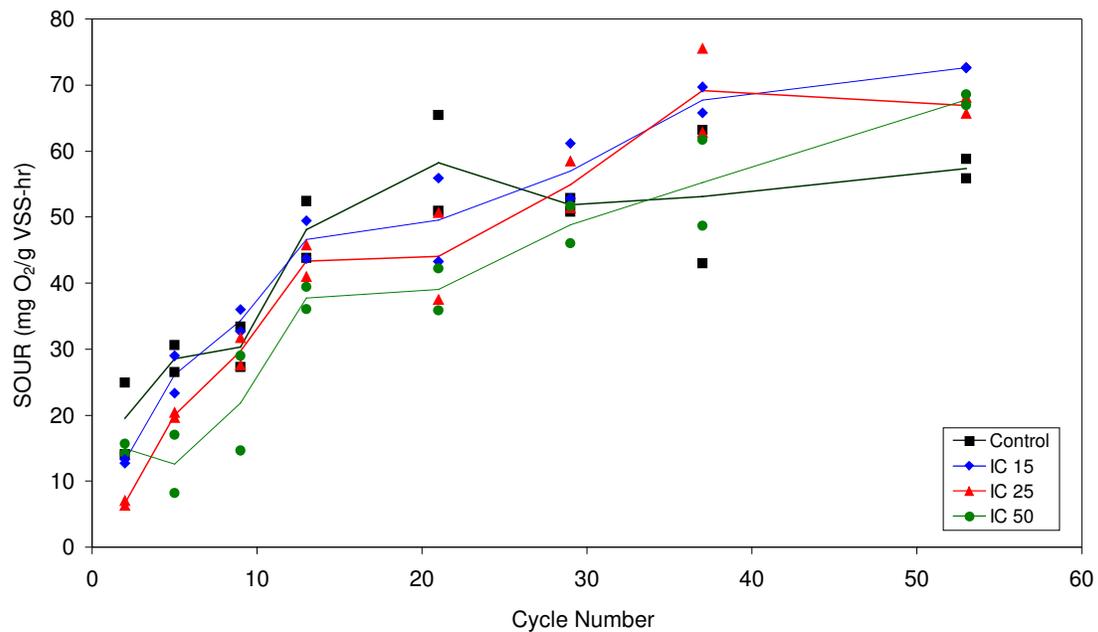
Figure 4.26   SOUR in the 10 day SRT reactors exposed to zinc-cyanide complex.

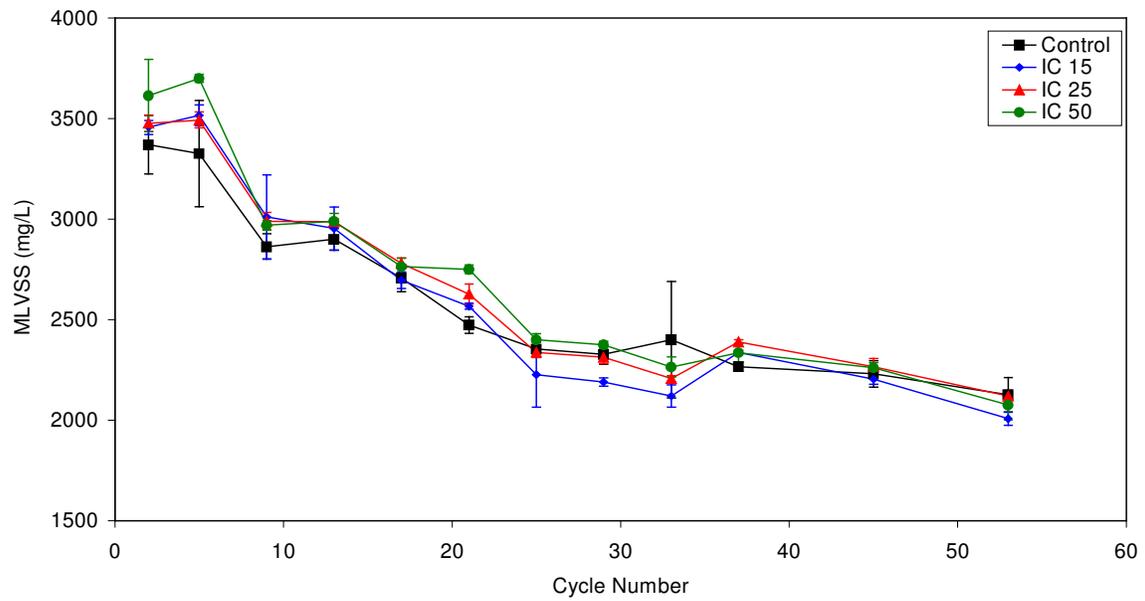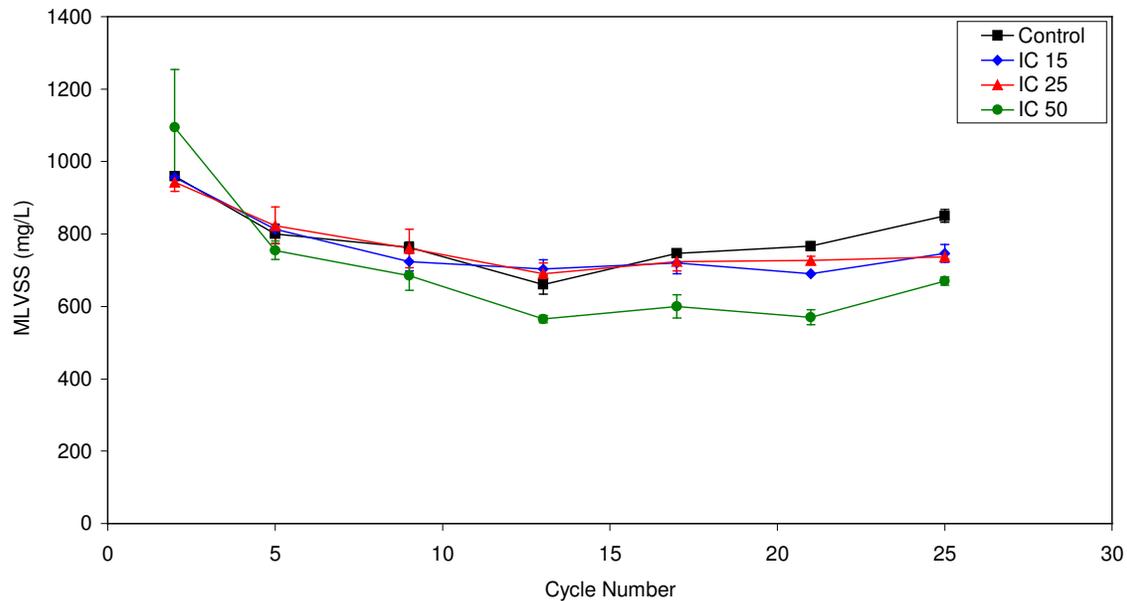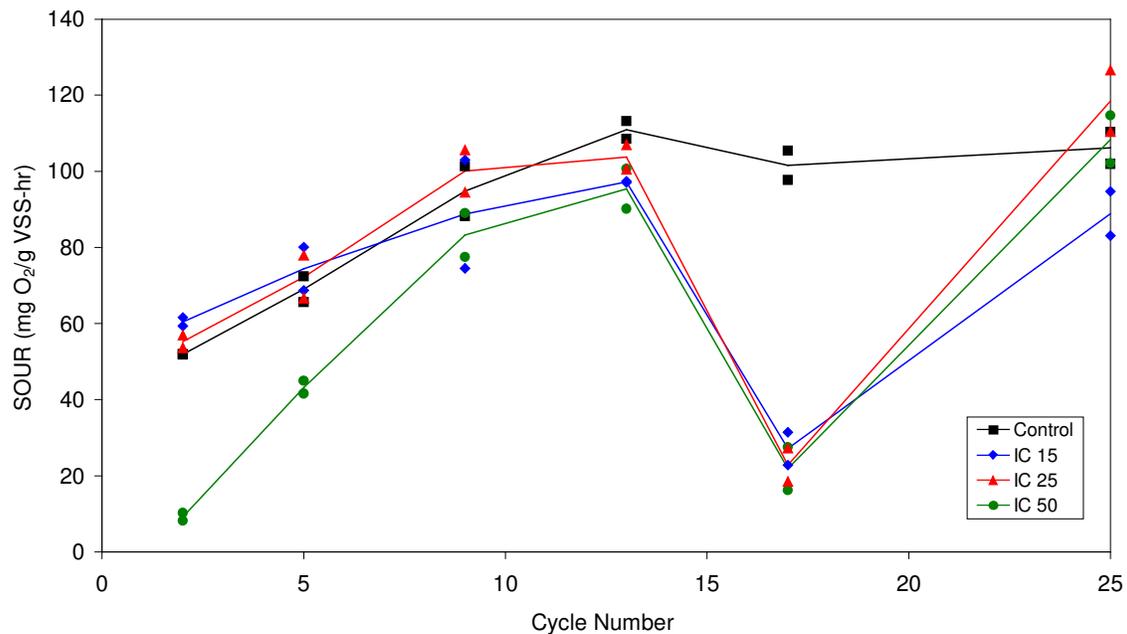Figure 4.27   SOUR in the 2 day SRT reactors exposed to zinc-cyanide complex.

The NGR test, as discussed previously, can be used as another measure of nitrification efficiency. Figure 4.28 shows that the NGR for the 10 day SRT shocked reactors was significantly (α=0.05) less than the control. It also shows that the NGR

levels correspond with the levels of cyanide addition. All shocked reactors recovered by cycle 30, whereas recovery of nitrification (according to nitrogen species measurements) for all shocked reactors occurred around cycle 10 (Figure 4.10). This discrepancy can possible be explained by time-span differences between the two measurements. The NGR assay measures the rate of nitrification every ten minutes over a one-hour period using MLSS at the beginning of a reactor cycle, whereas nitrogen species are measured after six hours in contact with the MLSS. Nitrification inhibition may be detected more readily when measured by the NGR assay. This is because there is a much shorter contact time between ammonia and the reactor biomass for complete nitrification to occur during the NGR assay. In this experiment, nitrogen species measurements suggest that with an additional 5 hours contact time shocked reactors may be able to fully nitrify ammonia contained in the influent, but at a slower nitrification rate. Overall, NRG assay results correspond with other water quality measurements that indicated nitrification inhibition, as discussed earlier.
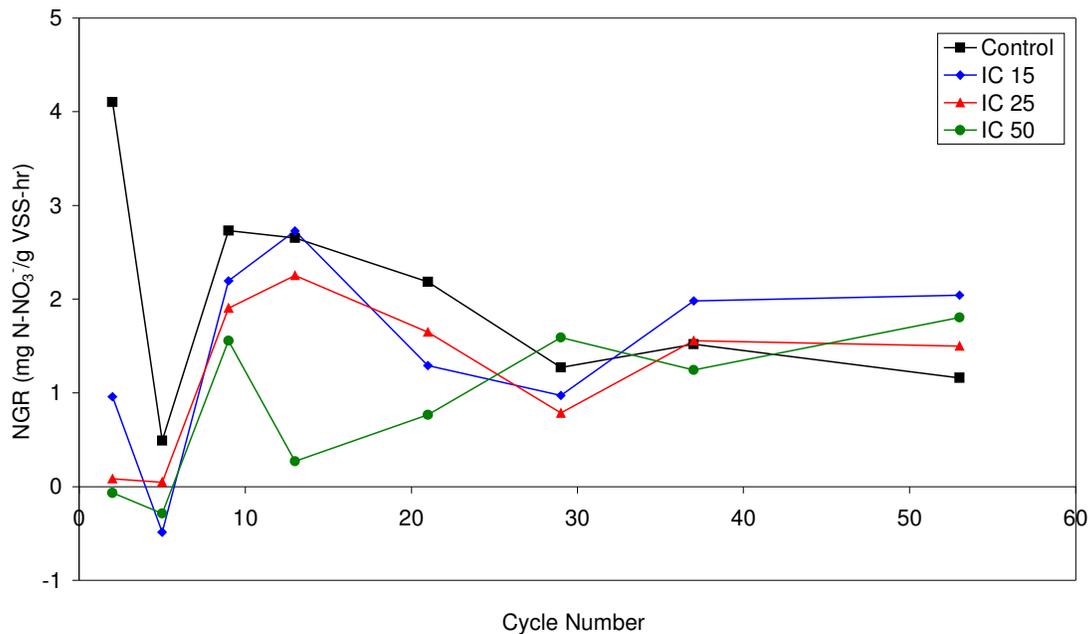
Figure 4.28   Nitrate generation rate for the 10 day SRT reactors exposed to zinc-cyanide complex.

4.4. SUMMARY/CONCLUSIONS

1) Zinc-cyanide complex had no significant effect on sCOD removal capability, effluent suspended solids concentrations, or dewaterability in any of the 10 day SRT shocked reactors. The 2 day SRT IC_{15} or IC_{25} systems were also unaffected. The 2 day SRT IC_{50} reactor had a small, but severe, loss of sCOD removal capability during the first 3 cycles.

2) Zinc-cyanide complex dramatically affected the respiration rate of the biomass in each of the shocked reactors. The level of inhibition correlated with the level of zinc-cyanide complex addition. This inhibition was expected, as cyanide is known to inhibit cytochrome oxidases and disrupt the electron transport chain.

3) That the respiration rate was affected, but COD removal was not suggests that the heterotrophic bacteria were inhibited by the zinc-cyanide complex addition but with a long enough cycle (six hours) they can overcome the inhibition or remove enough COD to decrease levels within control values. These results were found in both the 10 day and 2 day SRT systems.

4) From effluent nitrogen species data, it appears that zinc-cyanide complex briefly (over less than 1x SRT) affected nitrification in the 10 day SRT system. NGR results did not correlate with this quick recovery. The longer recovery observed in NGR testing suggest that the nitrification inhibition lasted longer than what effluent data indicated, though, over the cycle duration, the biomass was able to convert ammonia to nitrate. So, the nitrification inhibition may not have been extensive, but was still present. Overall, this inhibition was expected, as cyanide is a known inhibitor of nitrification.

pH SOURCE-EFFECT EXPERIMENT

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R. Kelly II
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5. pH SOURCE-EFFECT EXPERIMENT

5.1. LITERATURE REVIEW

Domestic wastewater contains a range of salts that provide buffering capacity, which keeps the pH near or slightly above neutral values (Metcalf and Eddy, 2003). In contrast industrial wastewaters can have variable or extreme pHs, depending upon the source for the wastewater, and often require buffering or pH adjustment (Eckenfelder, 2000). Industrial wastewaters that are treated alone or blended with domestic wastewater can undergo dramatic fluctuations in influent pH, thereby challenging the buffering capacity of the wastewater and causing pH changes in downstream bioreactors. Such changes in pH can hinder system performance by retarding the activity of both autotrophic and heterotrophic bacteria (Chong et al., 1997). The optimum pH range for most activated sludge treatment systems is usually between 6.5 and 8.0 (Grady et al., 1999). Mixed liquor organisms can die or experience retarded function if the pH drops below 4 or increases above 10 (Chong et al., 1997). Acidic pH levels can promote the growth of filamentous fungi and decrease the rate of organic stabilization in aerobic digestion, while alkaline pH levels can lead to death of the microorganisms (Grady et al.., 1999).

Changes in pH can have both direct and indirect effects on microorganisms. Extreme pH values can directly damage external cell structures outside the cytoplasmic membrane, such as flagella, chemoreceptors and cell walls (Dilworth and Glenn, 1999). There are few options for the cell to protect these structures from external chemical toxins, so the cell must produce structures that are resistant to adverse conditions, or the cell must dispense with the function altogether. This may mean that cells can become nonmotile and unable to relocate to more favorable environments in the presence of chemical stress (Dilworth and Glenn, 1999). At acid or alkaline pH, bacteria are challenged not just by excesses of H^+ or OH^-, but also by excesses or shortages of metal ions with solubilities that are affected by the pH shift. Under acidic conditions, it is necessary to consider the impact of the increased availability of aluminum, copper, zinc, cadmium, and manganese. At pH levels below five, cells may be unable to prevent the uptake of these metal ions, which can activate stress mechanisms (Dilworth and Glenn,

1999). To survive under low pH conditions, cells need to acquire detoxification mechanisms like those of acidophiles (Dilworth and Glenn, 1999). Under alkaline conditions, many of these same heavy metal ions are less bioavailable (Dilworth and Glenn, 1999). Since some are micronutrients (e.g., Na, Ca, Mg, Fe, Mn, Zn, Ni, Mo, Cu), limitations can result in limited cell growth and possible changes in biomass physiology.

Intracellular components are affected by changes in pH in a variety of ways. It is important for cells to hold intracellular pH fairly constant. They are able to achieve a relatively stable pH, even with significant changes to extracellular pH, through a range of mechanisms. The first defense against unfavorable pH is to minimize membrane permeability and maintain intracellular pH homeostasis, which is the ability to sustain intracellular pH within a narrow range of values despite variations in the pH of the environment (Booth, 1999; Dilworth and Glenn, 1999). It is generally accepted that this is achieved by controlling the activity of ion transport systems that facilitate proton entry and loss (Booth, 1985). A second defense option involves energy-requiring pumping systems. In bacteria, protons are translocated outwards across the cytoplasmic membrane by means of primary proton pumps linked to electron transport and ATP hydrolysis. It has been suggested that these pumps play a role in controlling internal pH in coordination with K^+ (a major cellular cation) transport (Nakamura et al., 1984). Another defense against unfavorable pH is internal buffering. The ability of cells to use internal buffering capacity to recover from intracellular pH perturbation depends on the magnitude of the change (Booth, 1999). Cytoplasmic buffering is most likely due to the creation and accumulation of metabolites that have buffering features (Dilworth and Glenn, 1999). For most cells, the buffering capacity is derived through titration of the phosphate groups and carboxylates of physiologically abundant metabolite species (Booth, 1999). This may be a good way to delay adverse pH effects, but can be difficult to maintain as a long-term survival strategy (Dilworth and Glenn, 1999).

Nitrification can be greatly affected by changes in pH. Although all bacteria grow poorly outside the normal physiological pH range of 6.0 to 8.0, nitrifying bacteria are particularly sensitive to pH (Grady *et al.*, 1999). The pH range for growth of pure cultures of ammonia oxidizers is 5.8 to 8.5, and the pH range for growth of nitrite oxidizers is 6.5 to 8.5 (Prinčič et al., 1998). However, a key factor to consider when

defining the preferred pH range for growth of these organisms is the total concentration of ammonia/um and nitrate/nitrous acid. For example, at high but industrially realistic ammonia/um concentrations, sufficient free ammonia will exist in solution at pH 8 to inhibit ammonia oxidizing bacteria (Anthonisen et al., 1976). Therefore, "ideal" pH ranges need to be interpreted in a broader context.

The efficient operation of biological treatment systems depends largely on the interaction between bacteria and the community of various microfauna present. This microfaunal community is dominated by protozoa (ciliates, flagellates, shelled amoeba), fungi, rotifers, and nematodes (Baldwin and Campbell, 2001). Each of these different types of microorganisms exhibits different tolerance of and responses to changes in pH. It has been found that most protozoan species function best at pH levels between 6 and 8, but can tolerate pH levels as acidic as 4.5 (Baldwin and Campbell, 2001). Under acidic conditions, the protozoan cell may incur increased permeability, raising energy costs associated with osmoregulation (Baldwin and Campbell, 2001). As a result, a larger mass of food (bacteria) may be needed to maintain protozoan growth at low pH in wastewater treatment systems.

Higgins and Novak (1997a) established that cation concentrations in the influent of laboratory-scale biological treatment system reactors had a significant effect on the settling and dewatering properties of activated sludge. From this information, it was concluded that there exists a cation balance for a given system, which optimizes settling and dewatering properties. It was suggested that this was especially important for industrial systems, where a cation imbalance is more likely to occur. It was determined that a ratio of monovalent (Na^+) to divalent (Ca^{2+} and Mg^{2+}, in particular) cations greater than about 4, expressed on a milliequivalent basis, resulted in a deterioration in settling and dewatering characteristics (Higgins and Novak, 1997b). It is important to understand and consider this effect when designing source-effect experiments to test the impact of pH on activated sludge system performance, because of the cations introduced with base addition.

The goal of this study was to determine the source-effect relationship associated with a pH upset shock event. For this experiment, pH levels were chosen to illustrate the effect of pH over a wide, but realistic range that could be experienced by wastewater

treatment facilities. Both acidic and alkaline pH levels were studied to provide more complete biological treatment performance results to either type of pH upset.

5.2. RESULTS

5.2.1. pH Concentration Levels

Icxx assays were not determined for the pH source-effect experiment. Instead, the pH maintained in the control reactor for previous experiments averaged between 7 and 8 was used as a basis for selecting shock pHs. The pH levels chosen for this study illustrate the effect of pH over a wide range. An acidic pH of 5 and alkaline pHs of 9 and 11 were chosen, while the control reactor pHs averaged 7.67 ± 15 and 7.58 ± 16 for the 10 and 2 day systems, respectively. A solution containing equal concentrations (on a milliequivalent basis) of NaOH and $Ca(OH)_2$ was used for the base pH adjustment experiments to avoid causing deflocculation through an imbalanced monovalent:divalent ratio. For the acid shocked reactor, 1N sulfuric acid was used instead of HCl to prevent chloride interference during the COD and ion chromatography procedures.

5.2.2. Influent Characteristics

Average influent characteristics over the course of the 10 day and 2 day pH source-effect experiments are given in Table 5.1 and Table 5.2, respectively. The influent quality was relatively constant over the period of the experiments. Influent supplementation was used during this experiment. Consequently, the influent composition reveals that the wastewater used as the feed for the source-effect experiments was medium in strength (Metcalf and Eddy, 2003).

Table 5.1   Characteristics of wastewater used during 10 day pH source-effect
             experiment.

Characteristic	Average ± std dev
Alkalinity (mg/L as CaCO$_3$)	286 ± 21
Total Ammonia (mg/L as N)	23.7 ± 16.1
COD, soluble (mg/L)	537 ± 85
COD, total (mg/L)	698 ± 120
pH	7.5 ± 0.3
TSS (mg/L)	116 ± 37
VSS (mg/L)	91 ± 38

Table 5.2   Characteristics of wastewater used during 2 day pH source-effect
             experiment.

Characteristic	Average ± std dev
Alkalinity (mg/L as CaCO$_3$)	734 ± 367
Total Ammonia (mg/L as N)	14.3 ± 3.4
COD, soluble (mg/L)	639 ± 275
COD, total (mg/L)	889 ± 142
pH	7.6 ± 0.2
TSS (mg/L)	284 ± 176
VSS (mg/L)	99 ± 90

5.2.3. *Effluent Characteristics*

Effluent soluble COD data shows that the most alkaline pH shock severely impacted sCOD removal efficiency for both the 10 and 2 day SRT reactors, as shown in Figures 5.1 and 5.2. There was no significant change in the effluent soluble COD of pH 5 and 9 stressed reactors compared to the control ($\alpha = 0.05$), in either the 2 or 10 day systems. The effluent sCOD remained elevated in the pH 11 stressed reactor relative to the control until cycle 52 (1.3 x SRT) for the 10 day SRT system and throughout the experiment for the 2 day SRT system.
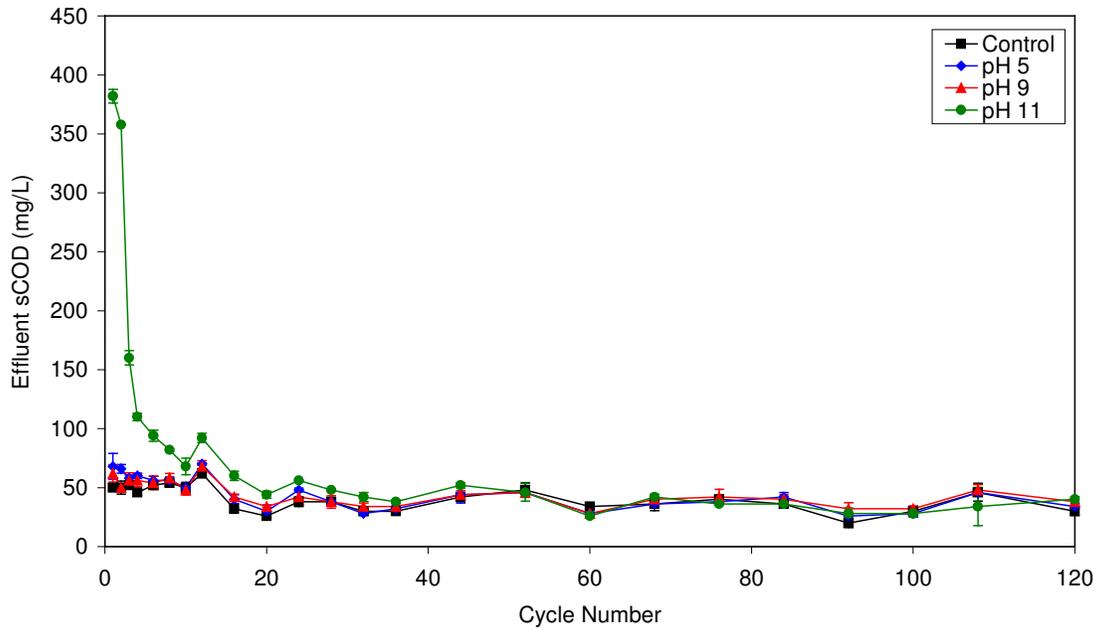
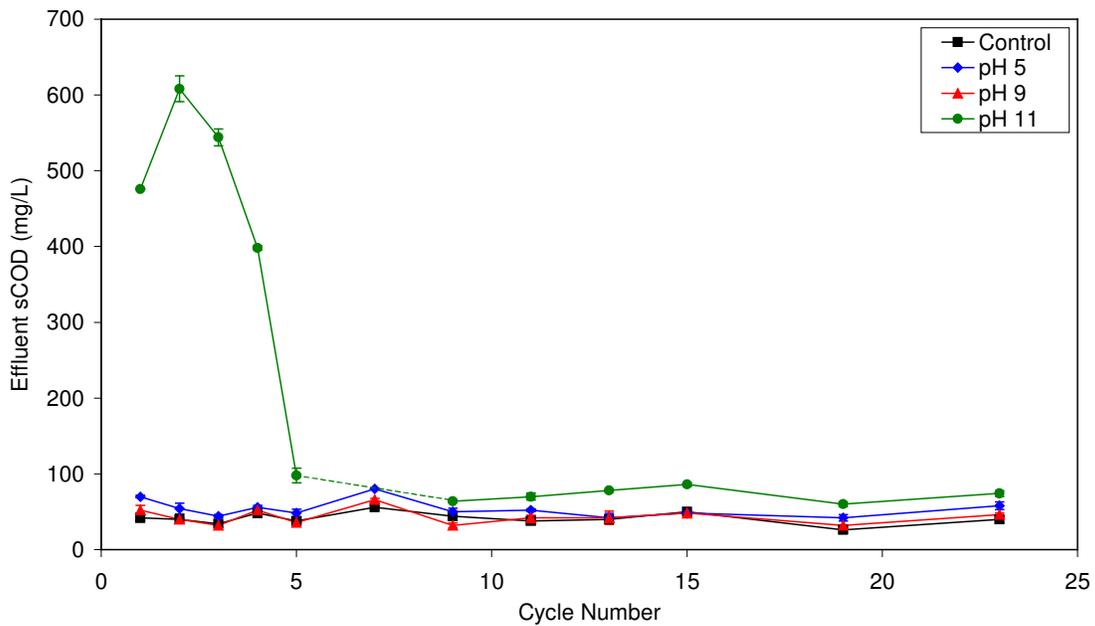Figure 5.1   Soluble effluent COD for 10 day SRT reactors exposed to various pH levels.

Figure 5.2   Soluble effluent COD for 2 day SRT reactors exposed to various pH levels. Dashed line reflects missing data point at cycle 7 for pH 11 stressed reactor.

Effluent TSS was significantly impacted initially by the acidic and alkaline shocks in the 10 day SRT system. In the case of reactor pH 11, the increase in effluent TSS was due to high turbidity caused by deflocculation. Deflocculation was observed beginning with the first cycle after shock in both the 10 and 2 day SRT pH 11 reactor, resulting in effluent TSS concentrations that were both 2.5 times higher than the control. For the 10 day SRT biomass, very high alkaline conditions corresponded with increasing effluent turbidity during the first few cycles (Figure 5.3b); however, by cycle 16 (day 4), there was no statistical distinction between the effluent TSS concentrations for the pH 11 stressed reactor and the control reactor ($\alpha = 0.05$). From that point on, the stressed reactors followed the general trend of the control reactor, but all remained at slighter (but often statistically significant) lower levels. It is unclear why the control system began with a higher than normal effluent TSS level in the 10 day SRT system. The effluent TSS in the stressed 2 day SRT reactors increased immediately after shock (Figure 5.4). The pH 11 reactor in the 2 day SRT system recovered by cycle 5, but effluent TSS levels continued to decrease. It was not until the last cycle that the pH 11 reactor recovered with respect to the control. The acid shock seemed to have the opposite effect of the alkaline pH shock. Effluent TSS for the pH 5 reactor improved for the first nine cycles then recovered to that of the control.

Overall, the pH 11 shocks were found to cause significant deflocculation in both the 2 day and 10 day SRT reactors. In the case of the pH 11 shocked systems, the deflocculation was revealed in the sharp increase in effluent suspended solids and high turbidity of these reactors. Deflocculation was observed beginning with the first cycle after shock in both the 10 and 2 day SRT reactors, resulting in effluent TSS concentrations that were about 2.5 times higher for both the 2 day and 10 day systems. Little deflocculation was seen in either the pH 5 or 9 shocked systems.
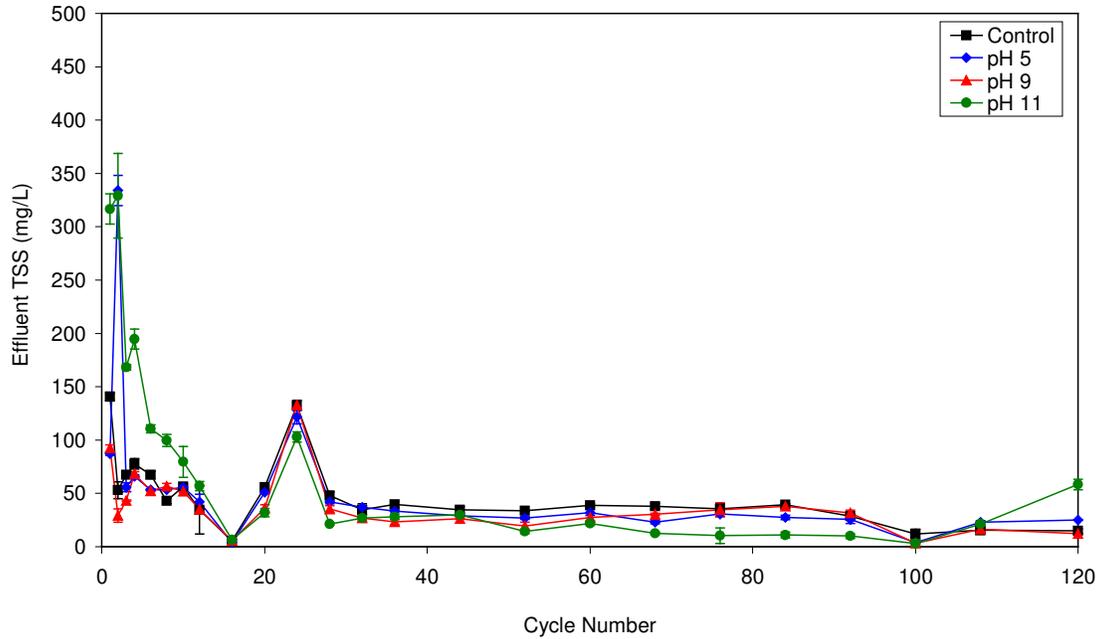
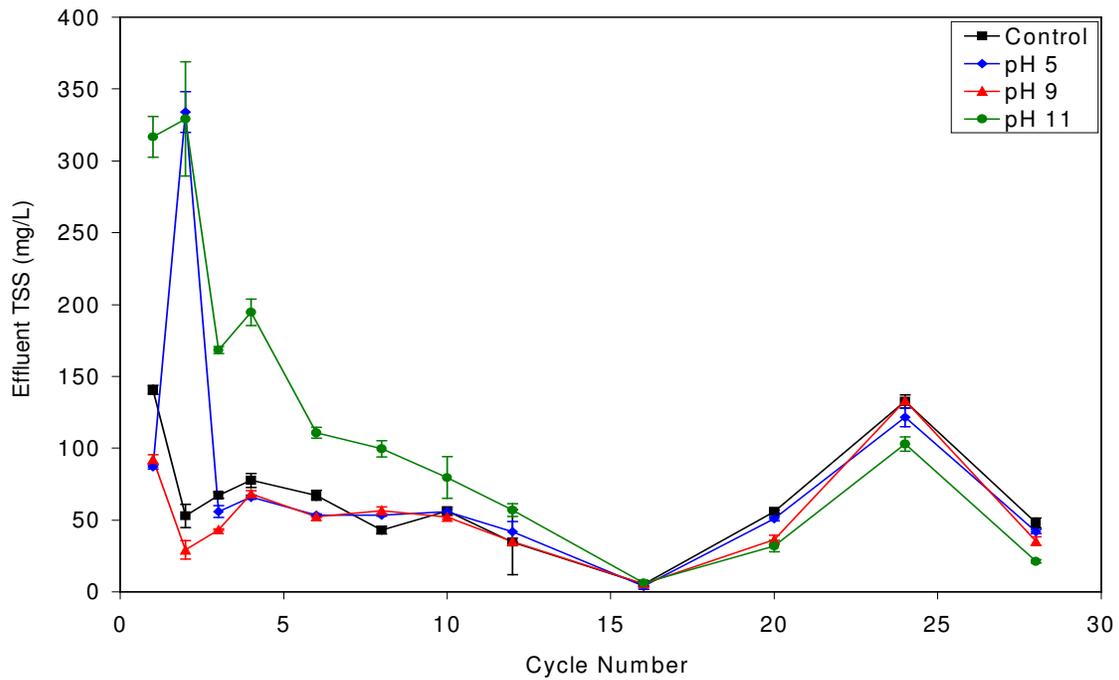Figure 5.3a   Effluent TSS for 10 day SRT reactors exposed to various pH levels.

Figure 5.3b   Effluent TSS for 10 day SRT reactors exposed to various pH levels
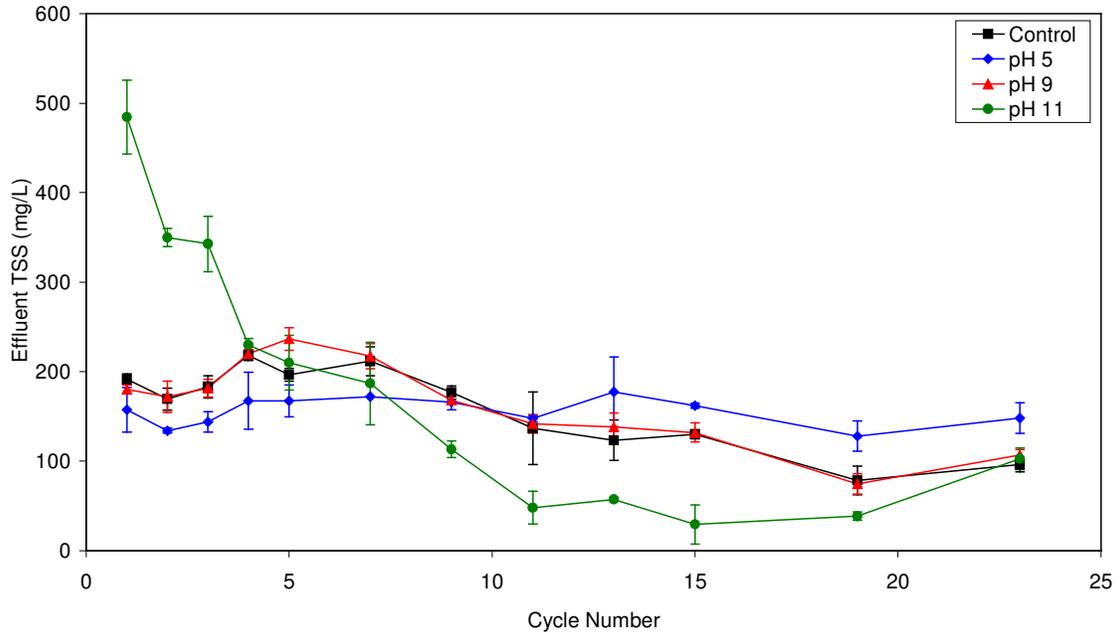(close-up of first 28 cycles).

Figure 5.4   Effluent TSS for 2 day SRT reactors exposed to various pH levels.

Effluent potassium concentration results for the 10 day SRT were inconclusive due to problems with the analytical method. The standard deviation between duplicate injections on the ion chromatograph was so large that the graphs were unreadable during early cycles. Subsequent to this experience, atomic absorption spectrometry was used and yielded more reliable potassium results in samples with high salt concentrations. Effluent soluble potassium levels were measured throughout 2 day SRT experiment using the atomic absorption spectrometry methods (Figure 5.5). There was a large cycle-to-cycle variation that is difficult to explain. It has been proven that dilutions used to analyze potassium samples are not responsible for the variation (Table 5.30 in Appendix D). It is possible that this is a variation due solely to biomass differences from cycle-to-cycle. The pH 11 reactor results show a cycle-to-cycle influx and efflux of potassium at the start of the experiment. At the same time, there was little difference between the pH 5, pH 9 and control reactors. From the first seven cycles results, the pH 11 shock had more of an effect on potassium regulation than the other pH shocks.
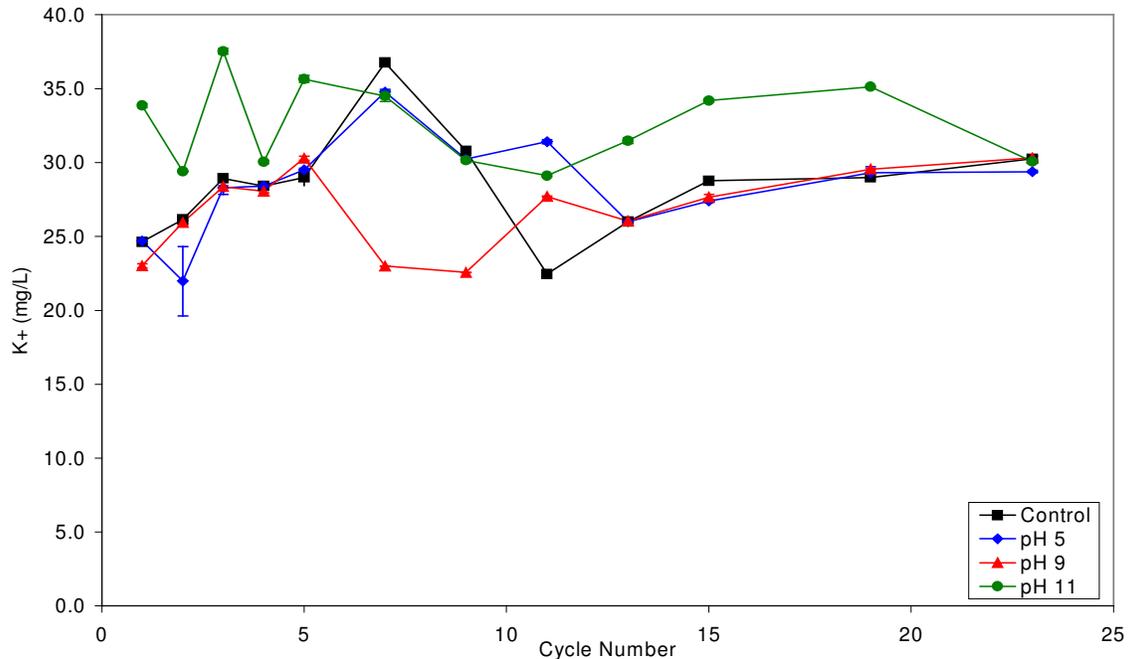
84

Figure 5.5   Effluent soluble potassium levels in the 2 day SRT reactors exposed to various pH levels.

Nitrification was significantly affected by a pH shock of 11. In the 10 day SRT reactors which were previously nitrifying, effluent ammonia concentrations spiked very quickly in the pH 11 reactor (Figure 5.6) and stayed at levels above 20 mg/L for cycles 10 to 44. After cycle 44 (day 11) effluent nitrite levels quickly increased in the pH 11 shocked reactor (Figure 5.7) and continued to stay high until cycle 92 (day 23). The initial interference in nitrification in the pH 11 reactor meant that the ammonia oxidizing bacteria (AOB) were inhibited in the 10-day SRT biomass. From Anthonisen et al. (1976) it is known that AOB and nitrite oxidizing bacteria (NOB) inhibition responses can be consistent with a higher pH corresponding with more free ammonia, which is inhibitory to AOB at free ammonia concentrations from 10 to 150 mg/L as N and inhibitory to (NOB) at free ammonia concentrations from 0.1 to 1.0 mg/L as N. The free ammonia concentrations theoretically present in the mixed liquor were calculated based on measured ammonia and pH values after the method of Anthoniesen et al. (1976) and are provided in Appendix D (Table 5.31). The theorectical free ammonia concentration for

the IC_{50} reactor never rose above 4 mg/L and was quickly diluted to below 0.5 mg/L by cycle 3. Since ammonia oxidation did not recover until cycle 44, it is believed that free ammonia may have had an initial AOB inhibition effect, but was not solely responsible for the extended AOB inhibition. Conversely, since free ammonia inhibits NOB at concentrations substantially lower that those of AOB, the increase in nitrite levels in the IC_{50} reactor after cycle 44 is most likely due to the free ammonia inhibition and not any lags in nitrite dissimilation brought on by a rapid influx of nitrite. It is certainly possible that other effects due to the impact of alkaline pH values on AOB physiology could have complicated this analysis and extended the inhibition effect. Nitrification was interrupted briefly in the pH 5 shocked reactor and the control, but quickly recovered within one or two cycles and again around cycle 20 (day 5) in the control reactor (Figure 5.6) and was not due to any free ammonia inhibition as the levels were practically zero at these times. The reason for the disruption in the control is unknown; however, since it corresponded with what was seen in the pH 5 reactor it is not possible to associate the effluent ammonia spike in the pH 5 reactor with an effect from the acid stress.

Finally, effluent nitrate data (Figure 5.8) were rather confusing prior to cycle 30 for the control, pH 5 and pH 9 reactors, which seemed to be oxidizing effectively all the ammonia and did not have nitrite buildups (Figure 5.7). The low nitrate values reported between cycles 6 and 24 were analyzed on the same day. The inconsistency in this data relative to all other data which indicates nitrification was occurring in the control and other reactors at this time (effluent ammonia, alkalinity) suggests that this data set is unreliable. It is assumed that an analytical problem occurred. From cycle 24 onward, the pH 11 stressed reactor nitrate levels increased slowly concomitant with a decrease in ammonium. The slight nitrite buildup did not prevent nitrite oxidation, as nitrate was formed. Overall, the rate of recovery from deflocculation or effluent COD disruption was more rapid than that from nitrification inhibition in the pH 11 shocked system. Effluent nitrate concentrations in the pH 5 and 9 reactors generally remained below those in the control reactor, suggesting that a modest level of inhibition occurred that was not clearly indicated by the effluent ammonia data.
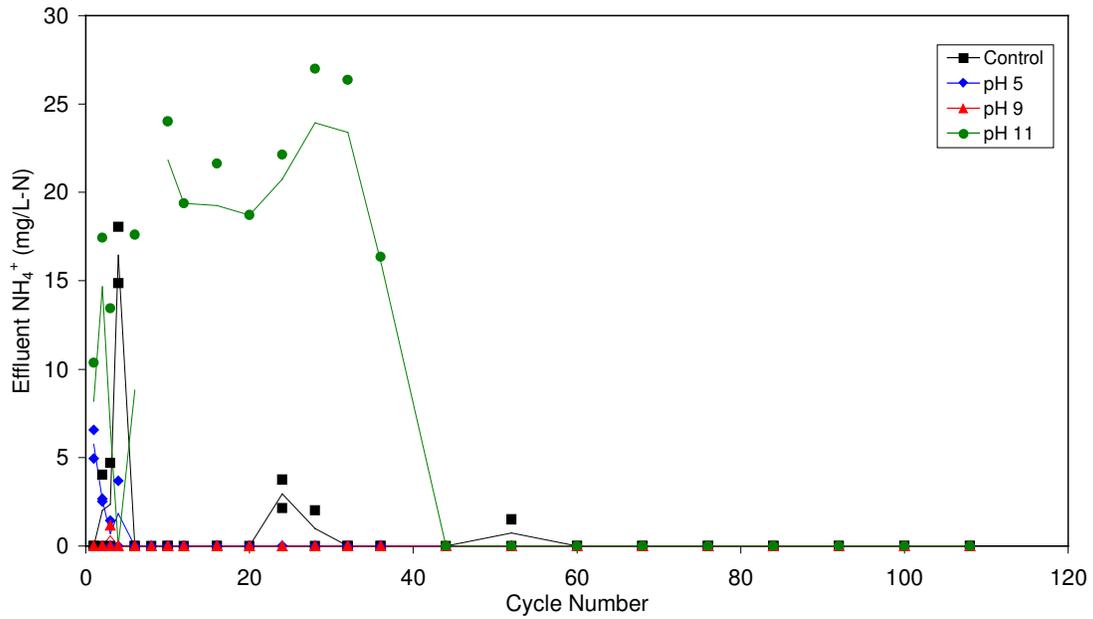
Figure 5.6   Effluent NH_4^+ for 10 day SRT reactors exposed to various pH levels.
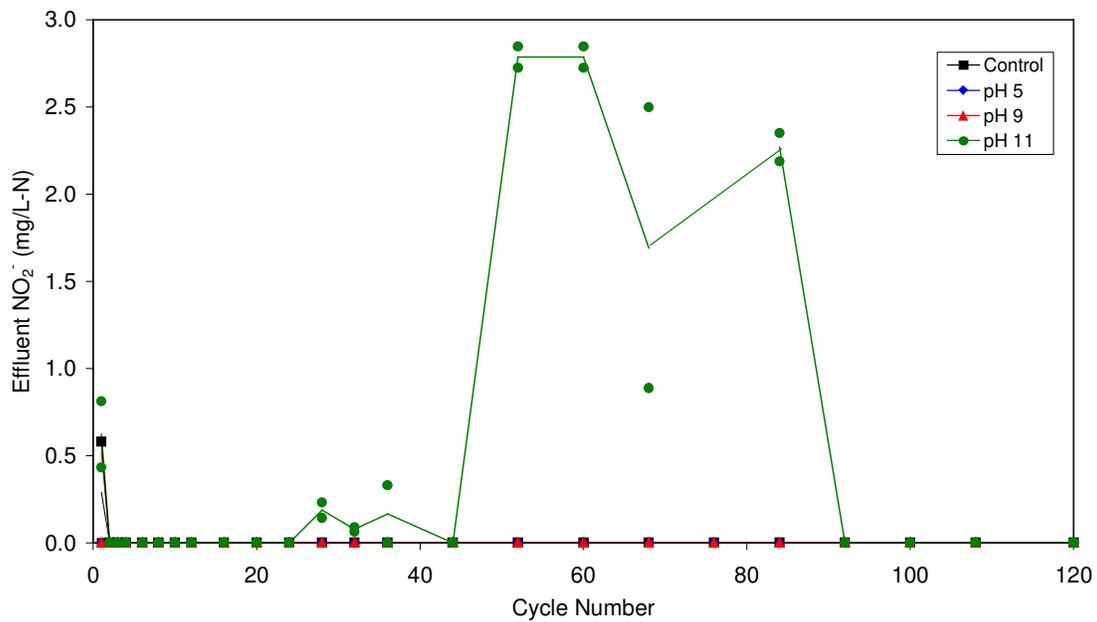

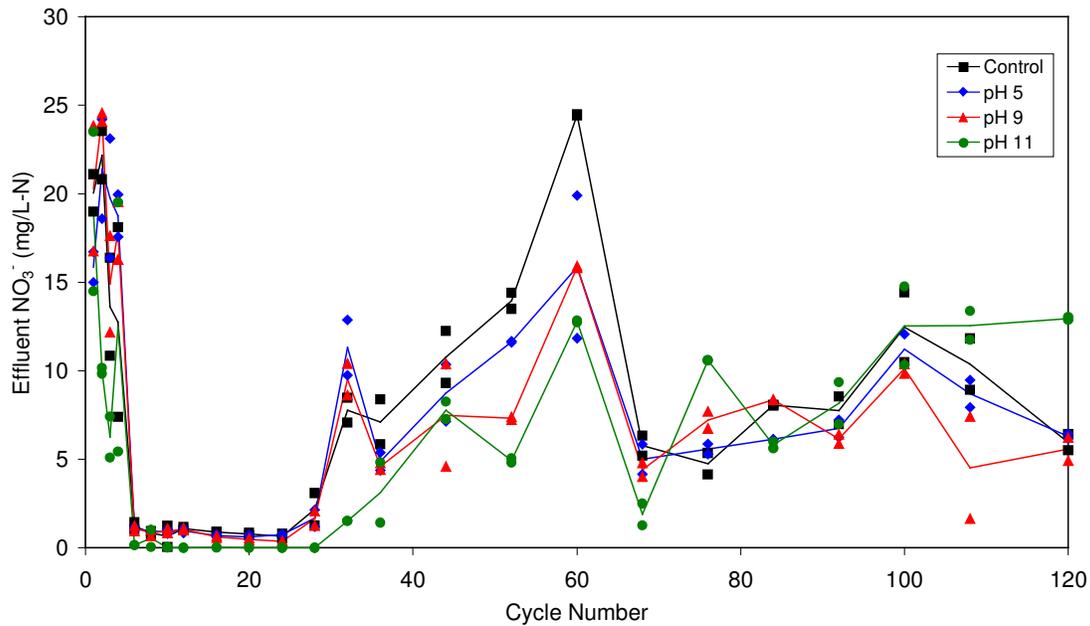Figure 5.7   Effluent NO_2^- for 10 day SRT reactors exposed to various pH levels.

Figure 5.8   Effluent NO$_3^-$ for 10 day SRT reactors exposed to various pH levels.

The 2 day pilot plant reactor was being operated to prevent nitrification and was not nitrifying the day before the experiment began; however, the biomass was partially nitrifying at the beginning of the experiment. Therefore, the 2 day SRT results are interpreted within the context of a nitrifying control. The effects seen for the 10 day SRT reactors were also observed in the 2 day SRT reactors. The nitrification of the 2 day SRT system is surprising, because the reactors were maintained at a low temperature to suppress nitrifiers. The 2 day SRT reactors were also operating at an SRT less than two days (Figure 5.9). Nevertheless, the control reactor was nitrifying enough in that no ammonia levels were measured in the reactor (Figure 5.10). The pH 9 reactor exhibited the least nitrification inhibition effect among the shocked reactors, which was expected due to the similar pHs between the control and pH 9 reactor. The acid shock initially inhibited the AOB, but recovered by cycle 6 where the levels began to follow the recovering control reactor. The extreme alkaline shock greatly inhibited AOB, similar to what was seen in the 10 day SRT system. Recovery, as compared to the control, never occurred in the pH 11 reactor. Nitrification did seem to commence around cycle 6, but

ammonia concentrations increased again around cycle 14. The degree to which ammonia-N accumulation in the pH 11 shocked reactor prior to cycle 6 was much greater than for any other shocked system and probably reflects loss of ammonia assimilation for heterotrophic growth, which was also inhibited as discussed earlier. Nitrite concentrations were consistently detectable in all reactors, including the control, again indicating incomplete nitrification for all systems (Figure 5.11). Nitrate concentrations were consistently below 2.5 mg/L for all the shocked reactors after cycle 3, which reflects the loss of nitrification capacity relative to the control (Figure 5.12). Overall, partial nitrification was occurring throughout the 2 day SRT system and inhibition effects were primarily seen in the acid and pH 11 shocked reactors. This interpretation of inhibition effects is tempered by the fact that other phenomena may have been occurring in the partially nitrifying low SRT system.

The pH range for growth of pure cultures of ammonia oxidizers is 5.8 to 8.5, and the pH range for growth of nitrite oxidizers is 6.5 to 8.5 (Prinčič et al., 1998). This optimal range is determined by three different effects that pH can exercise on bacteria: 1) activation-deactivation of nitrifying bacteria; 2) nutritional effects connected with alkalinity; and 3) inhibition through free ammonia and free nitrous acid (Villaverde et al., 1997). Nutritional effects can occur if carbonate (carbon source) becomes limiting at low pH because alkalinity is converted to CO_2, which can be stripped by aeration. At high pH, alkalinity is primarily in the form of carbonate, and most carbonates are insoluble and can also limit the availability of the carbon source for nitrification. This experiment showed significant nitrification inhibition during extreme alkaline conditions (pH 11) and resulted in very high effluent ammonia levels for the first 40 cycles (10 days).

Prinčič et.al. (1998) found retarded nitrification in low pH environments (pH 6.0), but the low pH nitrification results from this pH shock test showed only minor retardation at the very start of both the 2 day and 10 day SRT acidification experiments, which recovered quickly to control levels. Nitrification in the pH 9 stressed reactor was affected little, because the mixed liquor pH quickly recovered to a pH resembling that of the control.
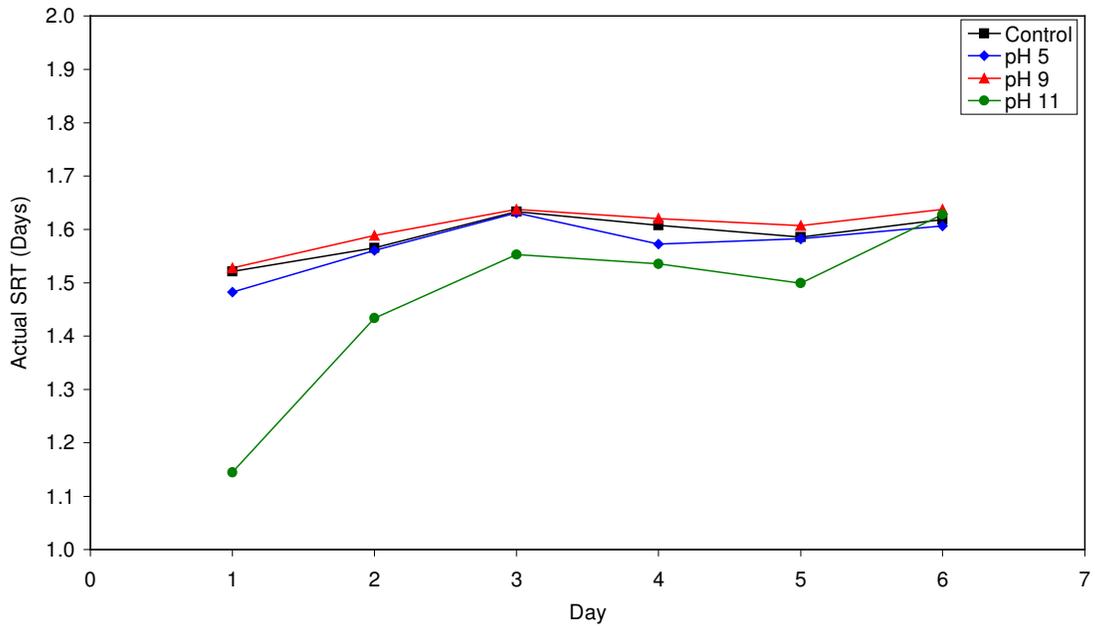
Figure 5.9   Actual SRT of the 2 day SRT reactors exposed various pH levels.
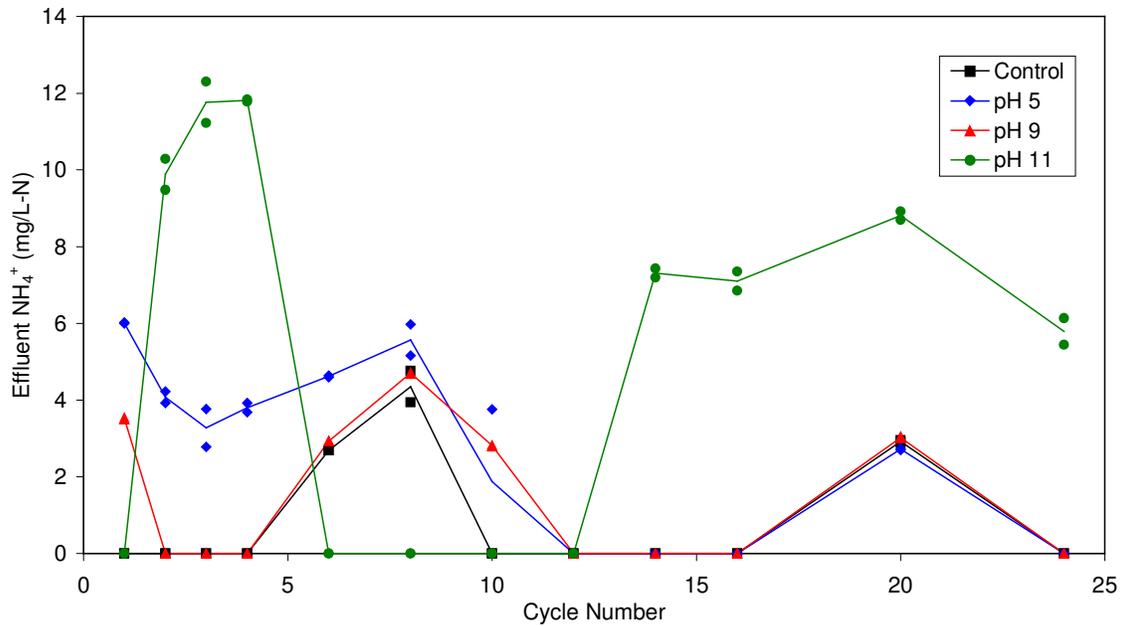

Figure 5.10   Effluent ammonia-N in the 2 day SRT reactors exposed various pH levels.
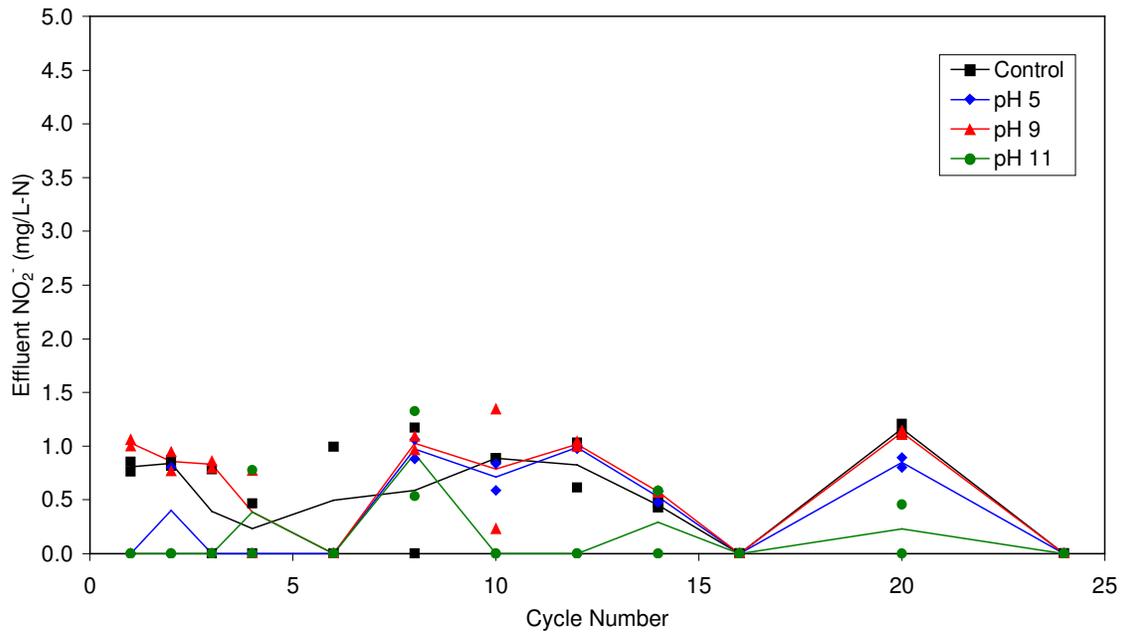
Figure 5.11   Effluent nitrite-N in the 2 day SRT reactors exposed to various pH levels.
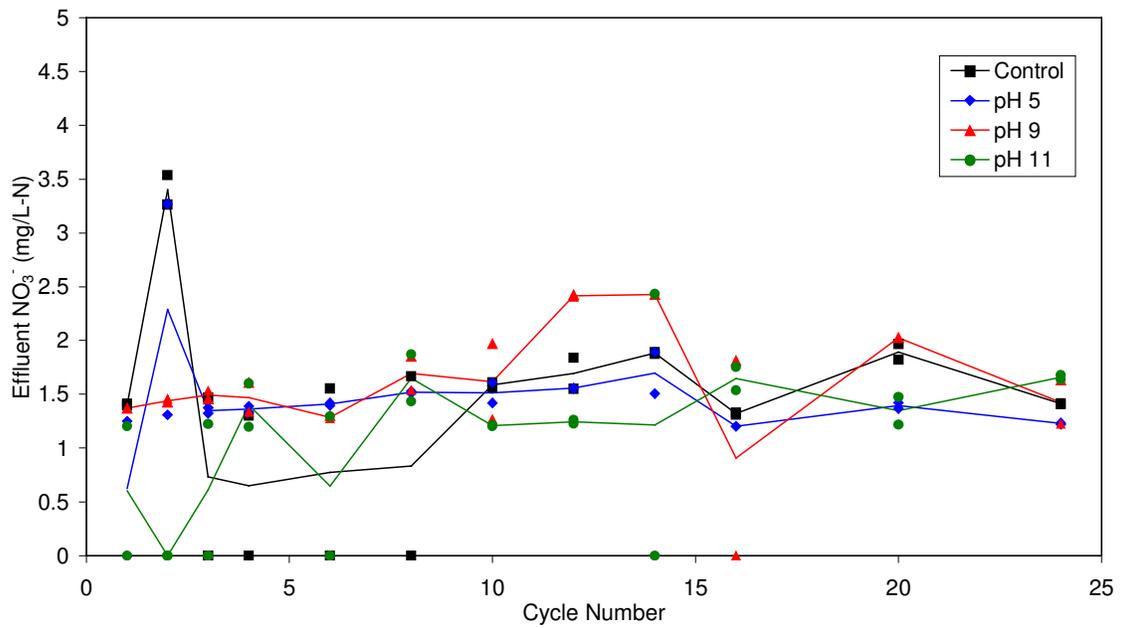

Figure 5.12   Effluent nitrate-N in the 2 day SRT reactors exposed to various pH levels.

Effluent alkalinity concentrations are affected by the degree of nitrification occurring and by the pH of the sample. The pH 11 reactor in the 10 and 2 day SRT systems had high alkalinity concentrations during the early part of the experiments (Figures 5.13 and 5.15), suggesting that ammonia oxidation was inhibited in these systems and/or the high pH of the system caused an increase in the effluent alkalinity. Supposing nitrification was the main cause of the increased alkalinity, the alkalinity in the effluent from the pH 11 reactor in the 10 day SRT system suggests that the ammonia oxidizing bacteria recovered by cycle 42, and this corresponds with the information obtained through effluent ammonia measurements (Figure 5.5). This is explained by the fact that a large amount of alkalinity, approximately 7 mg $CaCO_3$ used per mg NH_4^+-N removed, is used to convert ammonium to nitrite, while only 0.01 mg $CaCO_3$ is used per mg NO_2^--N converted to nitrate. Though correlation between alkalinity and nitrification data is good, it is believed that increased alkalinity levels are a result of the combination of nitrification and high pH effects. In the 10 day SRT system, Figure 5.13 shows that acid shock resulted in decreased effluent alkalinity early on. The slow increase in alkalinity to the control levels in the pH 5 shocked systems correlated with a slow increase in pH from acidic conditions to neutral conditions (Figure 5.14), and suggests that some of this alkalinity loss was due to CO_2 stripping. Slight increases in alkalinity in pH 9 shocked reactor relative to the controls were brief and did not correlate with significant ammonia oxidation inhibition effects.
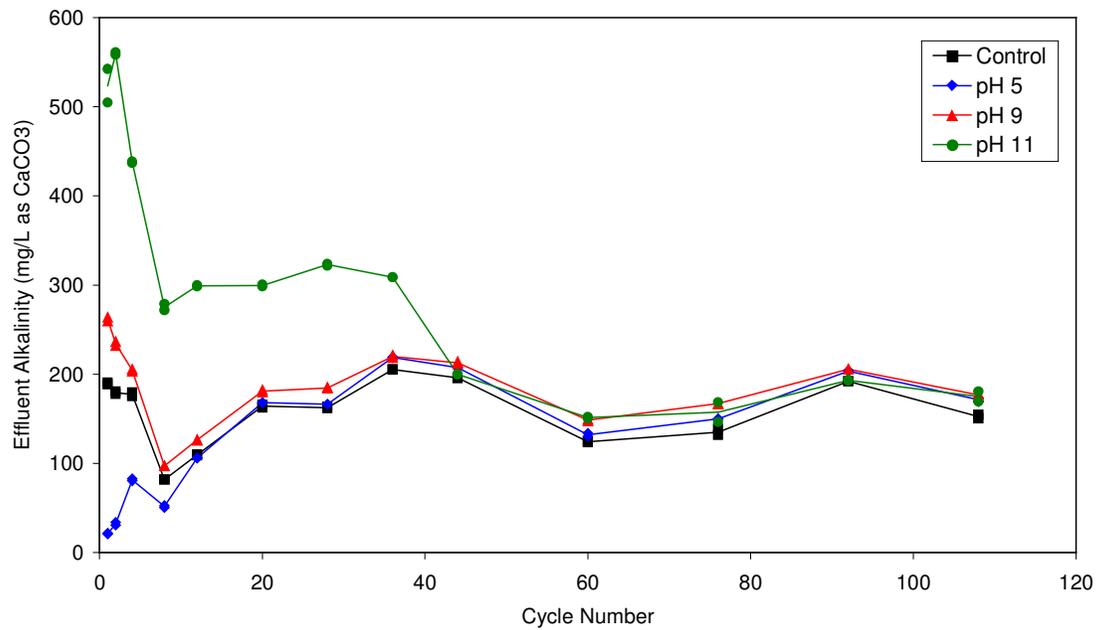
Figure 5.13   Effluent alkalinity for 10 day SRT reactors exposed to various pH levels.
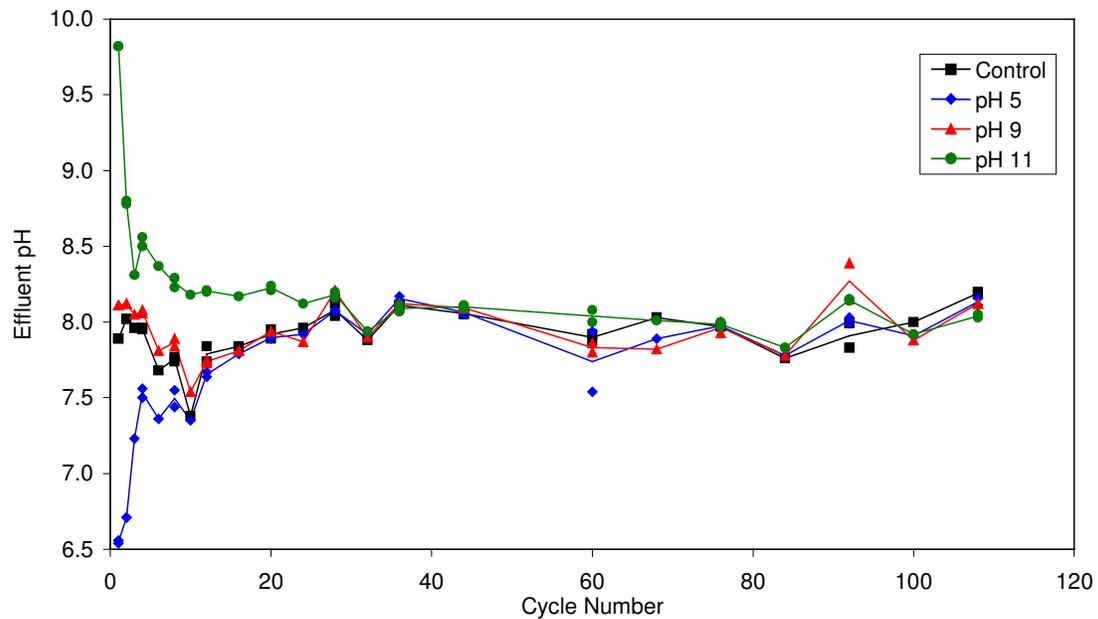

Figure 5.14   Effluent pH for 10 day SRT reactors exposed to various pH levels.

As was stated earlier, alkalinity was monitored because it is consumed during nitrification and should provide an independent measure of correlation with the degree of

nitrification. Alkalinity also corresponds to changes in the pH of a biological system. When looking purely on a nitrification basis, the fact that the 2 day SRT control alkalinity is less that the alkalinities of the pH 9 and 11 reactors means that (the partial) nitrification was affected in these two reactors, because AOB were inhibited (Figure 5.15). The pH 5 reactor had lower levels of alkalinity compared to the control, which indicates that nitrification was actually improved in this reactor. From the previous nitrification data we know that most of these observations are not supported. In this data the effect of alkalinity on pH (Figure 5.16) is believed to outweigh the measured nitrification effects. The slow increase in alkalinity to the control levels in the pH 5 shocked reactor correlates with the slow increase in pH from acidic conditions to neutral conditions, and suggests that most of this alkalinity loss was due to CO_2 stripping. The high levels of alkalinity in the pH 11 shocked reactor correlate to the inhibition of nitrification, but may actually be an additive result of the nitrification effects and the high pH levels. Overall, all 2 day SRT reactors had recovered to the control by cycle 12.
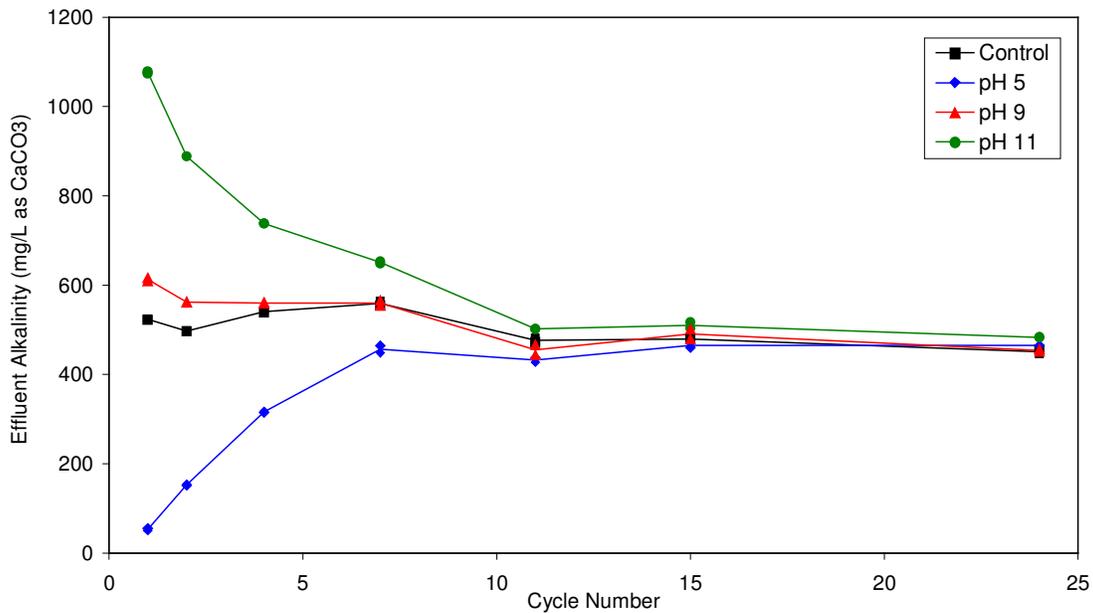

Figure 5.15   Effluent alkalinity in the 2 day SRT reactors exposed to various pH levels.
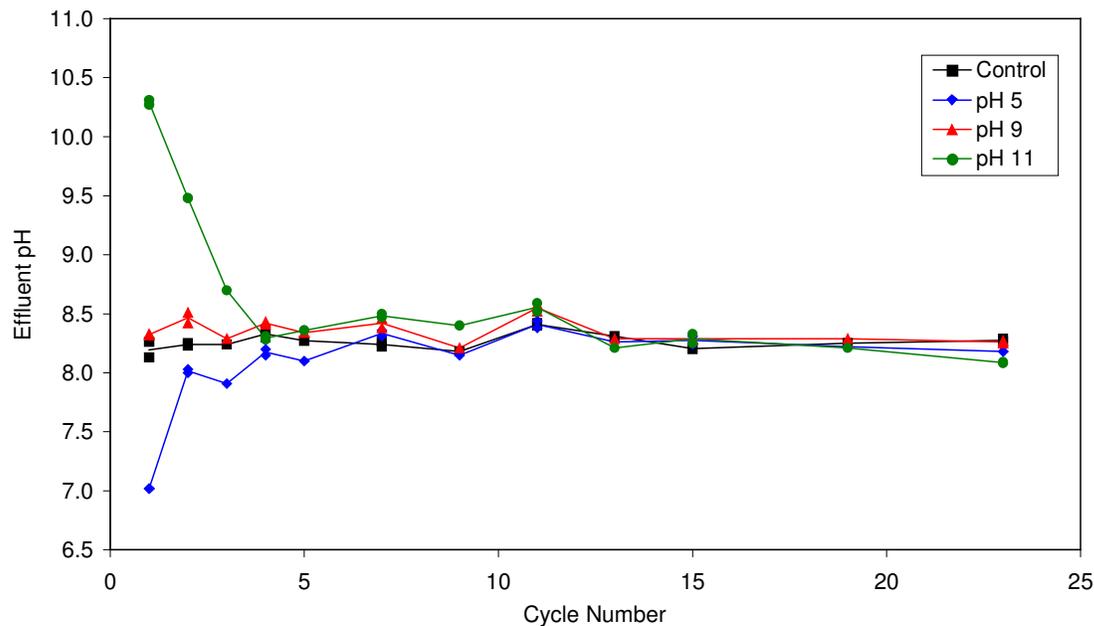
Figure 5.16   Effluent pH in the 2 day SRT reactors exposed to various pH levels.

5.2.4. *Mixed Liquor Characteristics*

The SVI in the 10 day SRT shocked reactors and control were in the excellent range (<125 mL/g) for the first 34 cycles, as shown in Figure 5.17, indicating that acid and base shock did not have an immediate impact on SVI and, therefore, settleability. Despite the excellent SVI values, microscopic evaluation of the pH 11 shocked mixed liquor revealed an increase in the amount of free cells in suspension and an accompanied murkiness in the settled supernatant (consistent with high effluent TSS in Figure 7.3). After cycle 34, there was a large jump in the SVI of the pH 11 shocked reactor, which continued until the end of the experiment. This large SVI increase is most likely not due to the initial pH 11 shock, because the effluent pH had already recovered by that time. Additionally, the SVI increase did not correlate with a dramatic changes in floc size, floc morphology or protozoan abundance (Table E.1 in Appendix E). However, it did correspond with the dynamic increase in nitrite levels in the system (Figure 5.7), when nitrite oxidizing bacteria were apparently inhibited. The reason for this correlation is not clear but may be worth further investigation. Overall, acid and basic shock did not

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appear to have a direct impact on settleability in terms of sludge blanket compaction, but secondary effects related to nitrification inhibition may have impacted settleability.
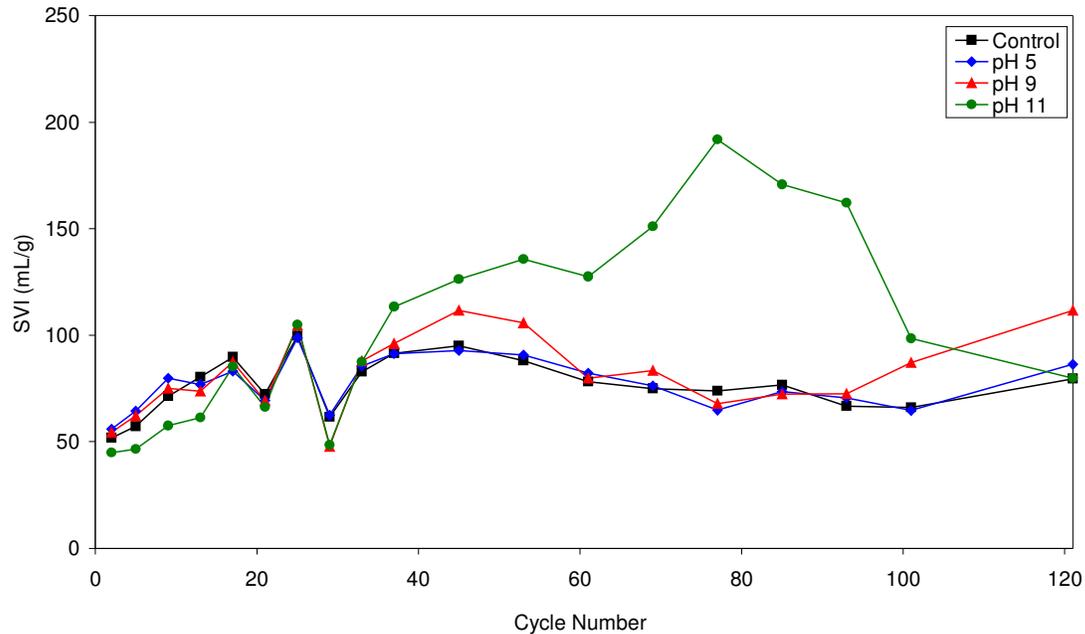
Figure 5.17   SVI values for the 10 day SRT reactors exposed to various pH levels.

In the 2 day SRT reactors, the SVI trends were significantly different than those of the 10 day SRT pH stress experiment (Figures 5.18). The 2 day SRT pH 11 exposed biomass had a significantly lower SVI value, as compared to the control. Improved solids compaction may have occurred due to precipitation of carbonate or phosphate salts, which could have increased the density of the biomass. This hypothesis is supported by the MLVSS/MLSS ratio reported in Table 5.3 (next section). In addition, the supernatant above the settled biomasses in the SVI cylinder was turbid for the pH 11 stressed reactor and consistent with high turbidity observed through effluent TSS measurements. No recovery was seen in the pH 11 reactor. The pH 5 reactor was observed to have a higher SVI measurement, as compared to the control, until recovery at cycle 12. This seems to indicate that an acidic shock has the opposite effect on SVI than an alkaline shock. The SVI increase did not correlate with a dramatic changes in floc size, floc morphology or protozoan abundance (Table E.2 in Appendix E) There was no observed change in the pH 9 reactor.
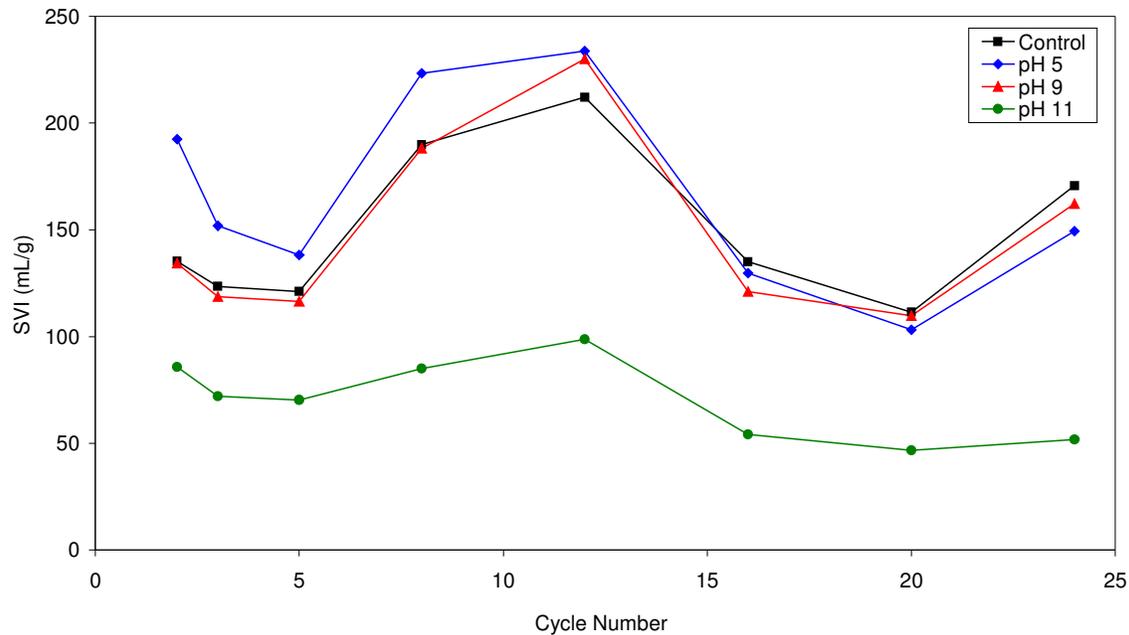
96

Figure 5.18   SVI values for the 2 day SRT reactors exposed to various pH levels.

MLSS and MLVSS data are shown in Table 5.3 and Table 5.4 for the 10 and 2 day SRT reactors, respectively. The concentrations are consistent with the COD load to the systems with nutrient supplementation. Wastage volumes were held constant throughout all experiments and, therefore, the impact of changes in effluent TSS could be directly evaluated. The increase in effluent suspended solids in the 10 day SRT, pH 11 reactor (Figure 5.3) caused a 20 % decrease in biomass (Figure 5.19) for the first portion of the experiment. Accordingly, significantly ($\alpha = 0.05$) lower MLSS and MLVSS concentration were seen for the pH 11 reactor (Figures 5.19 and 5.21). Also, as shown in Tables 5.3 and 5.4, the ratio of MLVSS:MLSS in the pH 11 shocked reactors decreased in both the 10 day and 2 day SRT systems, presumably because of the precipitation of inorganic salts that are less soluble at higher pH levels. Once precipitated, it could take several days to purge the system of these inorganic suspended solids.

In the 2 day SRT system both the pH 5 and pH 11 reactors had a significantly ($\alpha = 0.05$) lower MLSS concentration throughout the experiment, compared to the control and pH 9 reactor (Figure 5.20). The same trend was seen in MLVSS concentrations, though the pH 5 reactor recovered by cycle 8 (Figure 5.22). The pH 11 reactor was affected substantially and recovery was not seen by the end of the experiment. As shown

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in Table 5.4, the ratio of MLVSS:MLSS increased in the pH 5 shocked reactor. This effect cannot be explained, but is interesting in its opposite effect compared to the pH 11 shocked reactor. Again, there was no difference between the pH 9 and control reactors.

Table 5.3.   Mixed liquor concentrations in 10 day SRT reactors exposed to various pH levels.

	MLSS (mg/L)	MLVSS (mg/L)	MLSS:MLVSS Ratio (%)
10 day SRT reactors			
Control	$1,695 \pm 287$	$1,443 \pm 261$	0.85 ± 0.008
pH 5	$1,847 \pm 396$	$1,574 \pm 351$	0.85 ± 0.008
pH 9	$1,879 \pm 392$	$1,580 \pm 353$	0.84 ± 0.009
pH 11	$1,843 \pm 514$	$1,514 \pm 482$	0.81 ± 0.006

Table 5.4.   Mixed liquor concentrations in 2 day SRT reactors exposed to various pH levels.

	MLSS (mg/L)	MLVSS (mg/L)	MLSS:MLVSS Ratio (%)
2 day SRT reactors			
Control	$1,370 \pm 520$	940 ± 334	0.69 ± 0.01
pH 5	$1,118 \pm 334$	877 ± 281	0.78 ± 0.02
pH 9	$1,397 \pm 511$	946 ± 314	0.68 ± 0.01
pH 11	$1,056 \pm 480$	614 ± 202	0.61 ± 0.02

Figure 5.19   MLSS concentration in the 10 day SRT reactors exposed to various pH
levels.

Figure 5.20   MLSS concentration in the 2 day SRT reactors exposed to various pH
levels.
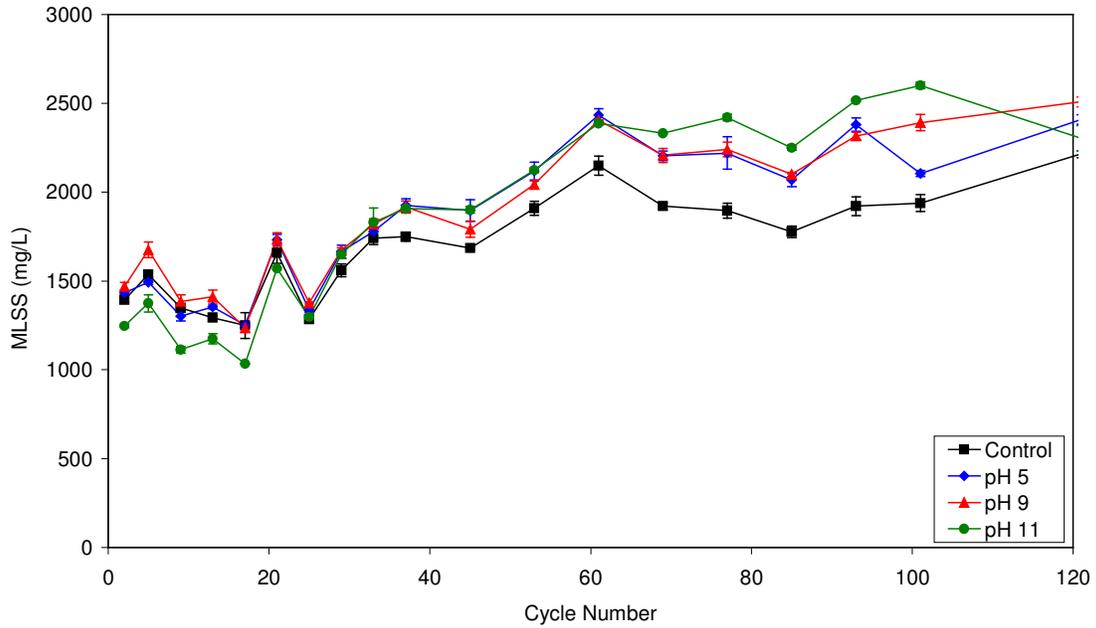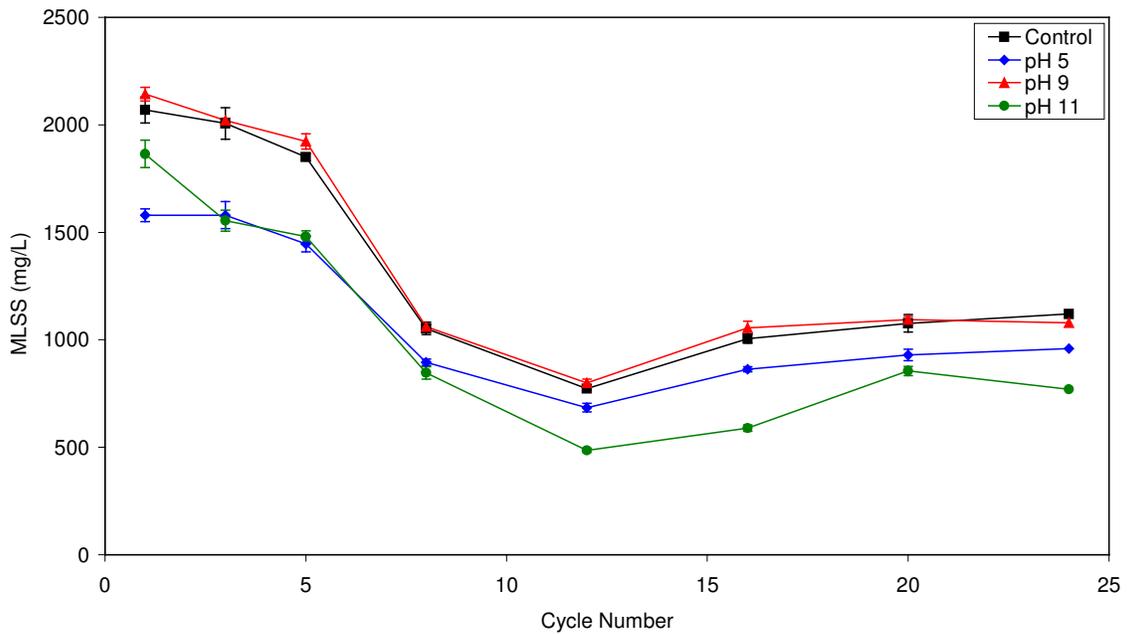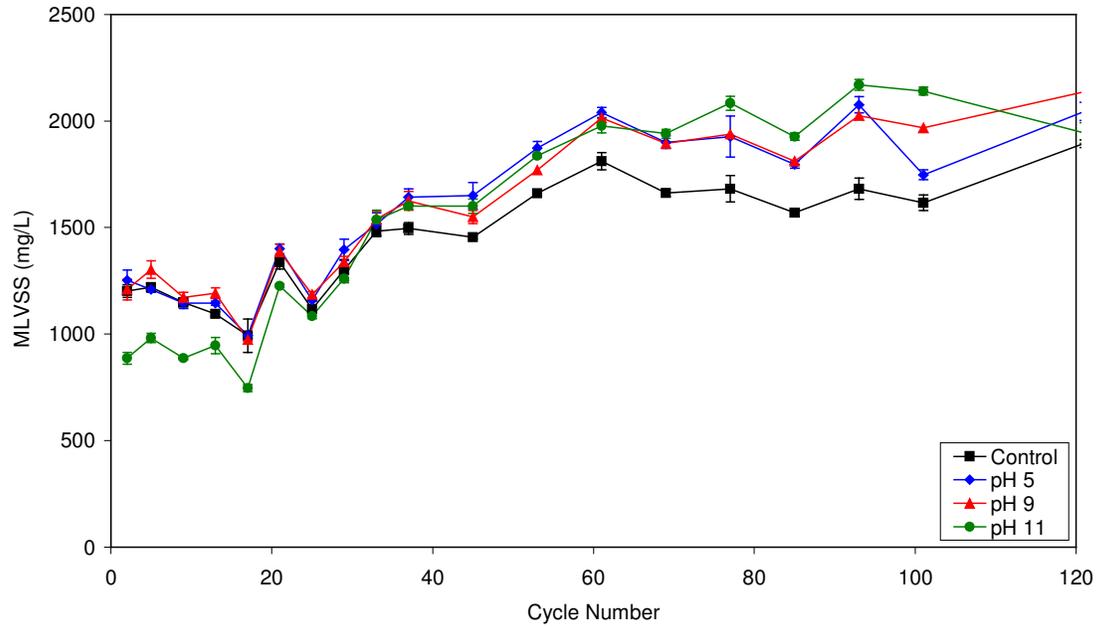
Figure 5.21   MLVSS concentration in the 10 day SRT reactors exposed to various pH levels.
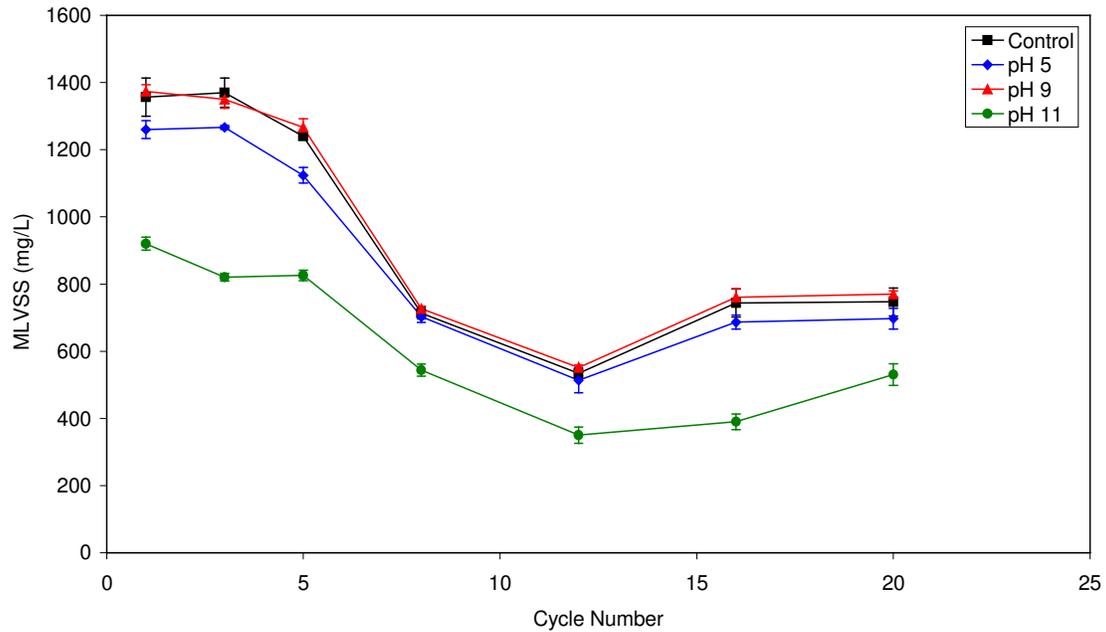

Figure 5.22   MLVSS concentration in the 2 day SRT reactors exposed to various pH levels.

There was a noticeable change in the pH of the 10 day SRT mixed liquor over the first four hours from shock to the end of the first cycle, as shown in Figure 5.23a. The pH 9 shocked reactor recovered first (within approximately 2 hours) to a pH consistent with the control. It wasn't until cycle 60 (day 15) and cycle 17 (day 4.25) that the pH in the mixed liquor of the pH 11 and pH 5 shocked reactors, respectively, recovered to levels consistent with those in the control (Figure 5.23b). For the 2 day SRT experimental system, mixed liquor pH was measured at almost every cycle throughout the experiment (Figure 5.24). The pH 9 reactor was the first to recover to the control, which is expected considering the similarity of pHs between the two reactors. Recovery of the pH 5 and pH 11 reactors occurred at the same time (cycle 8 – day 2). This is interesting to note as the extreme acid and base shocks each occur approximately 3 pH units from the pH of the control. So, it seems that the dilution effects of both shocks are similar. The difference in the 2-day pH recovery profile, where the pH 5 and pH 11 shocked reactors recovered simultaneously, compared to the 10-day pH recovery profile, where there were different recovery times for the pH 5 and pH 11 shocked reactors, may be due to the effects of nitrification on the 10-day SRT system causing a lag in recovery.
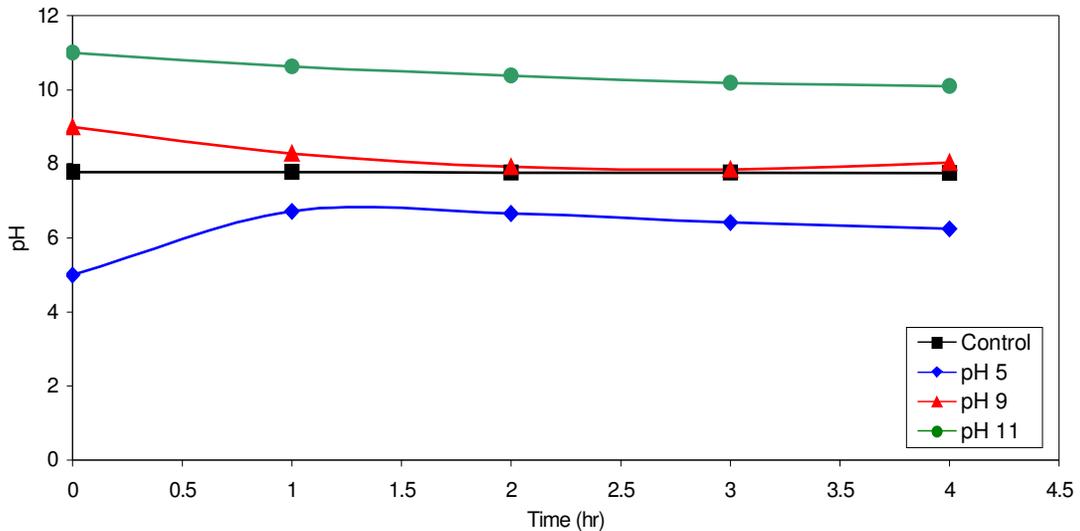

Figure 5.23a.   Mixed liquor pH in the 10 day SRT reactors during first four hours.
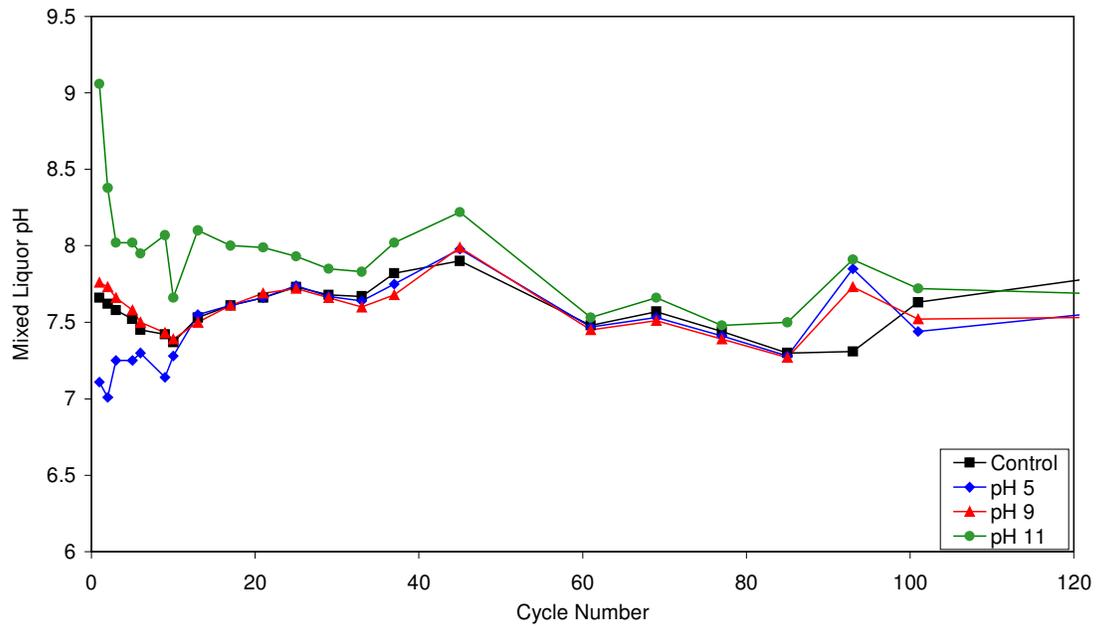
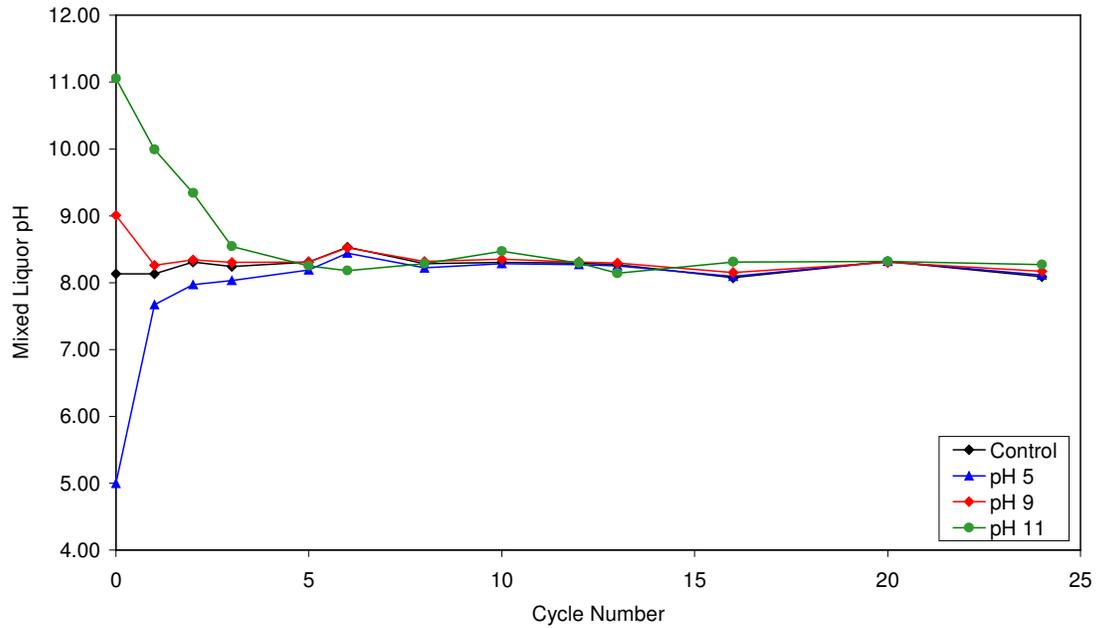Figure 5.23b.   Mixed liquor pH in the 10 day SRT reactors.

Figure 5.24.   Mixed liquor pH in the 2 day SRT reactors.

The specific oxygen uptake rate (SOUR) is a test of biomass viability. The higher the SOUR for a system, the more active the system is. SOUR results

for the first cycle of the 10 day SRT reactors show that both acidic and basic pH shocks decreased respiration, and the degree of respiration decrease correlated with an increase in pH (Figure 5.25). From the 5th cycle to the 25th cycle, the stressed reactors generally have SOURs comparable to those measured in the control reactor. Between the 38th (9.5 days) and 53rd cycle (13.25 days), the SOUR in the control reactor decreased. This coincided with a slight increase in effluent ammonia, which suggests a disturbance in nitrification and may explain the decrease in control SOUR (Figure 5.6). There was a severe inhibition of the pH 11 stressed reactor beginning after cycle 57 and continuing past cycle 80. Although delayed somewhat, this coincides with the point when AOB recovered but nitrite accumulated (Figure 5.7). Complete recovery was never achieved.

SOUR inhibition was profound in the 2 day pH 11 reactor and it generally remained below control SOUR levels, except for cycle 8 (day 2) and cycle 24 (day 6) (Figure 5.26). Though the 2 day SRT biomass was only partially nitrifying during this experiment, this respiration inhibition pattern does correlate with the severe inhibition of AOB seen in Figure 5.9. Additionally, there was no detectable respiration inhibition pattern caused by the pH 9 or pH 5shock. In fact, the pH 5 reactor experienced an increase in biomass respiration initially, though not significantly ($\alpha = 0.05$). The magnitude of the inhibition effect for the pH 11 reactor measured after 6 hours of the initial shock (at cycle 1) was severe and much larger than the effect of any other shock. Though the pH 5 and pH 11 shock were both approximately 3 pH units from the pH of the control reactor, the pH 11 shock was more severe. This is most likely due to the pH 11 shock being much more extreme than the typical growth environment of heterotrophs.
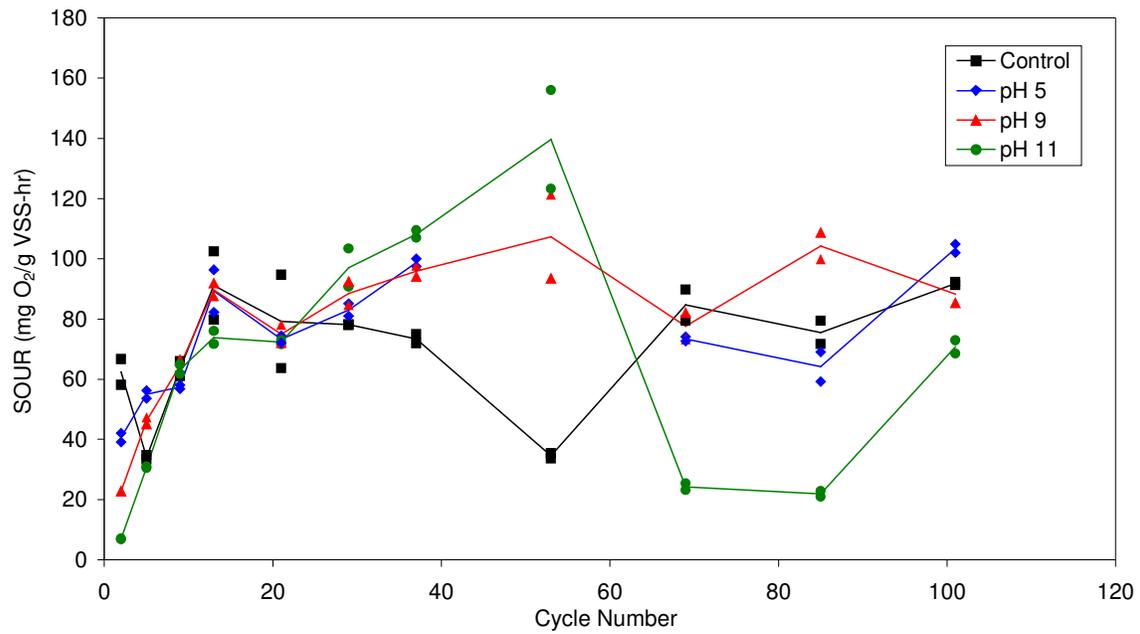
Figure 5.25   SOUR results for 10 day SRT biomass exposed to various pHs.
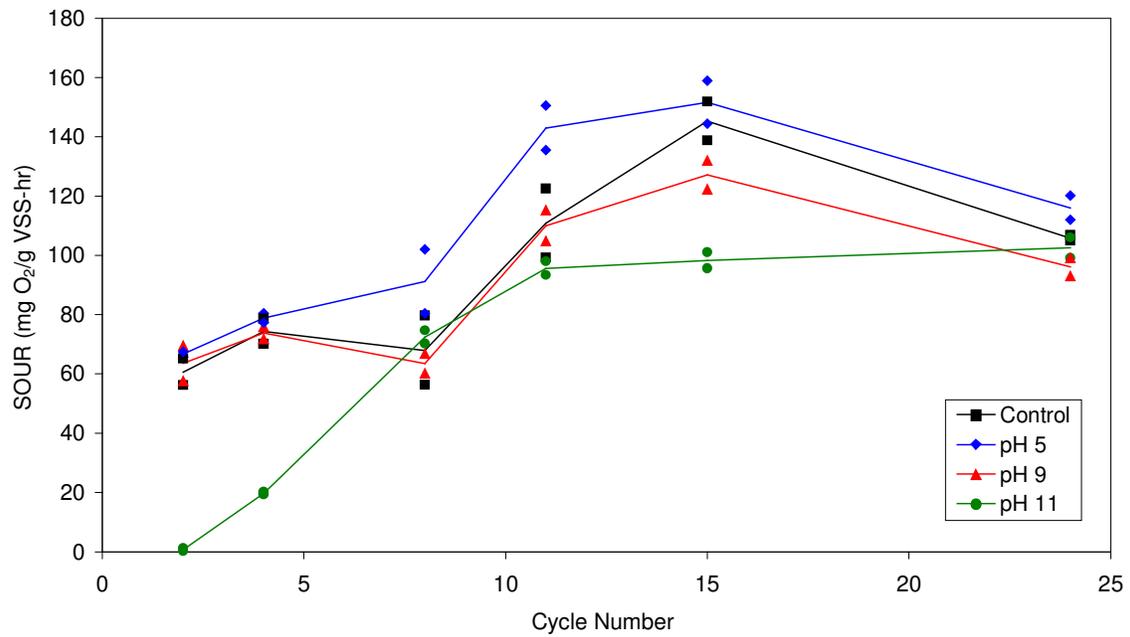
Figure 5.26   SOUR results for 2 day SRT biomass exposed to various pHs.

The nitrate generation rate (NGR) test can be used in conjunction with the effluent data on nitrate, nitrite and ammonia to determine if nitrification is inhibited by the different pH shocks in the 10 day SRT reactors. This test was only run for the 10 day SRT reactors because it was assumed that the 2 day SRT reactors were not nitrifying. Figure 5.27 shows the data from this experiment. The control reactor does not maintain a constant NGR, but the pH 5 and 9 shocked reactors manage to follow the same trend as the control for the first 68 cycles, leading the authors to believe that the fluctuations in these two stressed reactors were not due to pH shock. The pH 11 shocked reactor response seems to suggest that low rates of nitrification were occurring through cycle 85, after which nitrification kinetics improved. This transition point correlates with when nitrite accumulation ceased in the pH 11 shocked reactor.
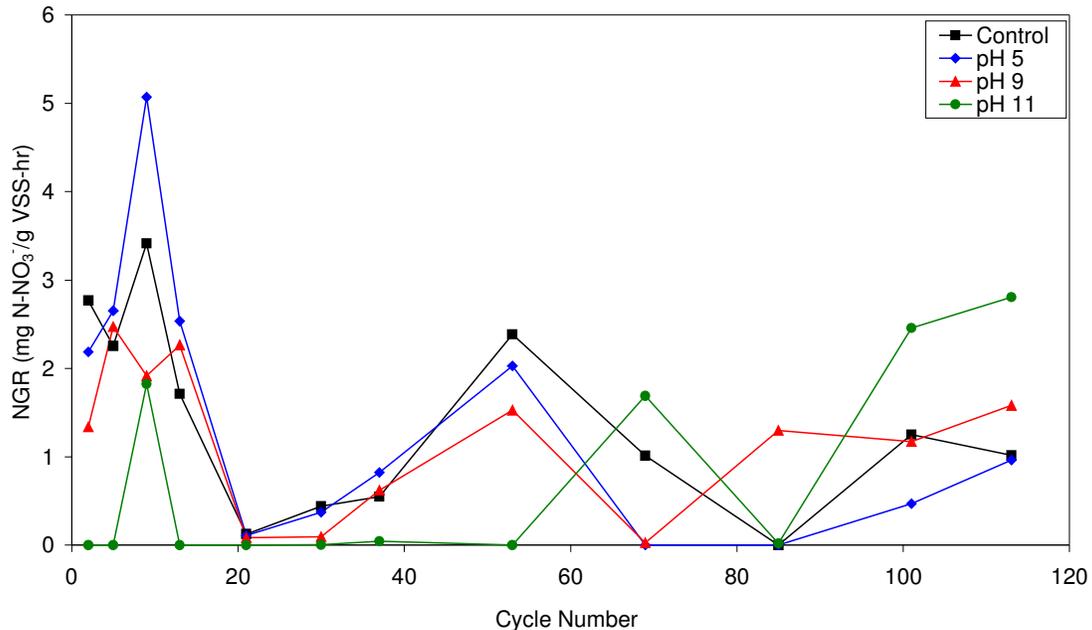
Figure 5.27   NGR results for 10 day SRT biomass exposed to various pHs.

5.3.   SUMMARY OF RESULTS/CONCLUSIONS

1) pH shock only caused significant negative effects on biosolids settleability in the pH 11 stressed reactors, as seen in the SVI data. Small effects were seen in the pH 5 stressed reactors

2) Ammonia oxidation was significantly affected by pH 11 shock, even in a partially nitrifying system. Unfortunately, few concrete conclusions can be drawn regarding the impact of pH on nitrogen metabolism in the 2 day SRT system, because the biomass was partially nitrifying.

3) Soluble COD removal efficiency decreased when shocked at pH 11. It is unknown if the mechanism that caused this effect is the same as that responsible for deflocculation.

4) Significant deflocculation initially occurred in response to the pH 11 shock with effluent TSS increasing significantly after the shock. This response may explain, in part, the elevated effluent sCOD performance during this same time.

6. ENGINEERING SIGNIFICANCE

Through the design of a modified respirometric assay, it was possible to better monitor for inhibitory industrial wastewaters discharging to HRWTF. It was found that all the contributing industries tested had inhibitory properties and substantial day-to-day variation. It was also discovered that industries with very small flow contributions can have significant inhibition effects. The modified respirometric assay enables HRWTF to identify possible industrial sources of toxicity/inhibition and to possibly defer permit violation fines to those industries.

The study into the effects of cyanide (zinc-cyanide complex) on activated sludge revealed a significant initial inhibition of respiration and nitrification, while sCOD removal, effluent TSS and dewaterability were unaffected. Recovery was observed in less than 2 X SRT for the nitrifying biomass and in 3 X SRT for the non-nitrifying biomass.

The study of the effects of acid and basic pH shock on activated sludge revealed that the extreme basic shock (pH 11) affected the settleability, nitrification, sCOD removal, and effluent TSS levels. The pH 5 shock effected biomass respiration, nitrification, effluent TSS levels, and mixed liquor total and volatile suspended solids, but recovered quickly. pH 9 shock had no observed treatment effects.

In conclusion, the evaluation of the source-effect relationship in relation to chemical toxicity on activated sludge is an important step in the understanding of upset events. Effluent violations and extended plant recovery are major consequences of upset events. With treatment plant deterioration, effluent discharged into the receiving natural water body may be toxic to aquatic and human life. Operators are often faced with a reactive, rather than proactive response to upset events when these toxic or inhibitory substances reach the bioreactor. With knowledge of the source, and information on source-effect relationships from this study and others like it, operators have the potential to limit treatment deterioration by applying the necessary control measures.

A few control strategies can be suggested for at-risk wastewater treatment plants. For treatment plants, like HRWTF, following the management strategy (outlined in the literature review in section 3.1) of collecting daily influent samples from each industry and storing them for possible testing if loss of treatment occurs is an excellent way to

identify problematic discharges. Also, continuous testing of samples over time to create a large database of results can help in determining any patterns to upset events. It is also suggested that wastewater treatment plants look into the use of biosensors submerged in incoming waste streams for warning of approaching inhibitory/toxic chemicals. The WERF source-effect studies completed herein will eventually go to the engineering of a biosensor similar to this. It is also suggested that information on common effects from certain chemical sources be documented by operators. These effects should be correlated with appropriate corrective measures to be implemented if these effects were to develop. So, if it were known that a certain chemical would be entering the treatment plant, operators would know what effects to anticipate and what corrective measures should be applied.

Lastly, there are more experiments that can be conducted to further understand the findings of this investigation. A study to determine the difference between the short-term respirometric assay results presented in Section 3 and longer-term tests that may take into account any dampening effects that might be seen. It is also suggested that a similar pH source-effect experiment be completed using lower acidic shocks that would be more applicable to industrial wastewater treatment plants, instead of the domestic wastewater treatment plants that were targeted in this pH source-effect experiment. It would also be interesting to repeat the pH source-effect experiment where shocks would occur as previously completed. Immediately after initial effects were observed, the addition of acid or base to each reactor to return to control pH levels would be applied. The study would investigate if returning the shocked reactor pHs to that of the control can suppress any treatment effects.

7. ACKNOWLEDGEMENTS

8. REFERENCES

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