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BIODEGRADATION OF AN ORGANOPHOSPHATE CHEMICAL WARFARE AGENT SIMULANT BY ACTIVATED SLUDGE WITH VARYING SOLID RETENTION TIMES

THESIS

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DEPARTMENT OF THE AIR FORCE
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AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

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BIODEGRADATION OF AN ORGANOPHOSPHATE CHEMICAL WARFARE AGENT SIMULANT BY ACTIVATED SLUDGE WITH VARYING SOLID RETENTION TIMES

THESIS

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In Partial Fulfillment of the Requirements for the

Degree of Master of Combating Weapons of Mass Destruction (Chemical Option)

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March 2013

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Abstract

Since World War II, organophosphates (OPs) have been used as chemical warfare agents for their lethal capacity to irreversibly inhibit acetyl cholinesterase (AChE) enzymes. Although many efforts have been taken to prevent their misuse against military and civilian targets, a determined adversary could still obtain and deploy these weapons and injure, kill, or instill terror against others. Should such a chemical attack take place, standard operating procedure dictates decontamination efforts be swiftly employed for the safety of contaminated victims. This decontamination is often accomplished with copious amounts of water, bleach and /or soap. Some communities plan for decontamination water to be sent to a wastewater treatment facility. However, few studies have been done to determine the fate of these OP chemical weapons in the activated sludge or under what conditions this removal is optimal.

This study examined the fate of malathion, a surrogate compound for studies on the organophosphate VX, in bench-scale sequencing batch bioreactors being operated under varied solid retention times (SRTs) to simulate the activated sludge process in wastewater treatment facilities. Results show that a constant influent of malathion will be removed from the effluent. However, the heterotrophic bacteria undergo a stress response which temporarily inhibits malathion removal, the extent of which increases with increasing SRTs. After this stress response takes place, the activated sludge is able to resume a high degree of malathion removal, even with increased influent concentration; however, this is done so at the expense of acceptable chemical oxygen demand (COD) removal in the effluent, suggesting malathion may be toxic to the heterotrophic bacteria in activated sludge.

Acknowledgments

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Allen K. Janeczko

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BIODEGRADATION OF AN ORGANOPHOSPHATE CHEMICAL WARFARE AGENT SIMULANT BY ACTIVATED SLUDGE WITH VARYING SOLID RETENTION TIMES

I. Introduction

Nerve Agent Chemistry and Toxicology

Nerve agents fall under a class of chemical compounds known as organophosphates (OPs). In industry, this class of compounds is often used for insecticides, fungicides, herbicides and plant growth regulators such as dichlorvos, iprobenfos, glyphosate, and chlorphonium (Salem, 2008). However, for military applications, OPs have been used for their neurotoxic capacity to inhibit the acetyl cholinesterase (AChE) function in humans (Robert, 1992). For vertebrates, an excitation of nerve cells influences the release of neurotransmitters, such as acetylcholine (ACh), at the nerve terminus. The Ach then crosses the synaptic cleft to interact with a receptor on the postsynaptic membrane of a neuron or muscle to cause a response in the membrane (Robert, 1992). The transmitter must then be quickly removed to prevent abnormal excitation of the membranes (Robert, 1992). In the case of acetylcholine, it is hydrolyzed by acetyl cholinesterase into choline and acetate as graphically displayed in Figure 1.

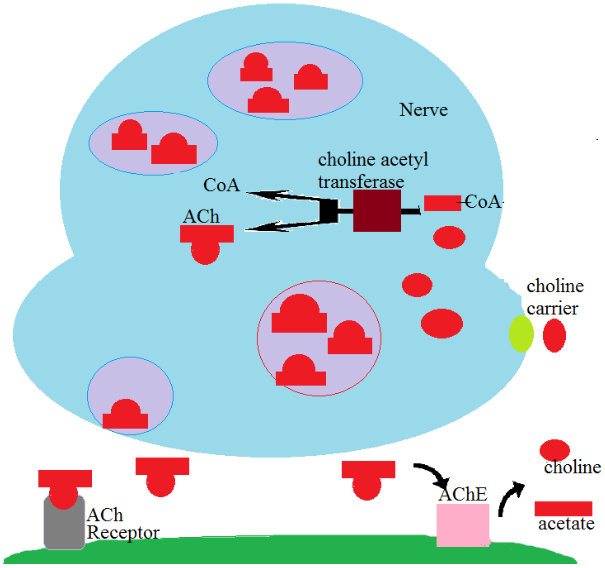


Figure 1: Cholinergic Enzymes and Transporters (modeled after Rand, 2007)

Nerve agents induce their toxic effects by forming a strong bond with a serine hydroxyl group in the active site of AChE which deactivates the enzyme. This process can be reversed if proper treatment is given in a timely manner; however, failure to do so results in a nerve agent being irreversibly bound, or "aged", to the AChE in which the enzyme cannot be reactivated. Should enough AChE be inhibited, ACh will not be hydrolyzed fast enough and excessive postsynaptic action potentials are produced to cause overstimulation in an effected organ (Nerve Agents, 2008). This cholinergic crisis occurs in organs with muscarinic or nicotinic receptors. Muscarinic sites of action occur in the glands, pulmonary and gastrointestinal system muscles, and efferent organs of cranial nerves whereas nicotinic sites primarily affect the skeletal muscles and autonomic ganglia (Nerve Agents, 2008). Throughout the body, overstimulation in these receptors produces a variety of symptoms. These include miosis, rhinorrhea, excessive sweating and secretions in lungs, nasal and oral glands, bronchoconstrictions, chest tightness, increased fasciculation, flaccid paralysis and twitching of the muscles. In the central nervous system (CNS), acute severe intoxications lead to loss of consciousness, convulsions, and depression of respiratory function which can lead to death. Symptoms from lesser doses include forgetfulness, irritability, nightmares, insomnia, and decreased comprehension (Nerve Agents, 2008).

Treatment for nerve agent exposure consists of properly administering three drugs: atropine, pralidoxime chloride (2-PAM-Cl), and diazepam. Two mg of atropine acts to bind to muscarinic receptors and "block" them from further ACh stimulation (Lillie, 2005). Six hundred mg of 2-PAM Cl is used to reactivate AChE which has been inhibited by nerve agents. However, 2-PAM Cl is only effective on AChE which has not been irreversibly bound to an organophosphate. Together, these make the MARK I kit which is the current field use treatment for nerve agent exposures (Lillie, 2005). Diazepam is an anticonvulsant which is used in cases of moderate to severe exposures to prevent any brain damage. As a pre-treatment for military that may be exposed to nerve agent, such as soman, pyridostigmine bromide (PB) can be used (Lillie, 2005). Pyridostigmine bromide is in a class of compounds called carbamates which, like organophosphates, binds to AChE. However, PB differs in that it does not bind to AChE irreversibly and aging will not occur (Lillie, 2005). As a result, the temporarily blocked AChE is not susceptible to nerve agent attack. Atropine and 2-PAM Cl are still necessary to reactivate the AChE sites that were protected by PB (Lillie, 2005).

History of Nerve Agents

Organophosphates first came to rise in the mid-nineteenth century with the synthesis of tetraethyl pyrophosphate (TEPP) in the De Clermont laboratory in France (Chauhan, 2008). However, their highly toxic capabilities had not been recognized until the 1930s when they became used as a chemical warfare agent (CWA) known as nerve agents. Nerve agents are a highly lethal neurological group of chemical weapons first synthesized in 1936 by Dr. Gerhard Schrader of I.G. Farben in Germany through his accidental success in synthesizing tabun while attempting to develop new insecticides (Salem, 2008). Sarin was synthesized two years later by Dr. Schrader and designated as T-144 or Trilon-46 in Germany and later as GB by the United States (Salem, 2008). During WWII, Germany stockpiled sarin in bombs, shells and rockets, but the weapons were never employed during the war for reasons that are unknown. In 1945, the allied forces became aware of Germany's nerve agent program and both the United States and the Soviet Union took this technology and made nerve agent development a primary focus (Salem, 2008; Hoening 2002). Later, in 1952, Dr. Ranajit Ghosh discovered what is now known in the military as VX while conducting research into pesticides (Hoening, 2002). The older "G" agents are more volatile and evaporate quickly whereas the newer VX has the viscosity of motor oil and is far more persistent.

Since WWII, there have been several incidents of CWA applications around the world. To name a few: the Yemen Civil War (1963-67), Soviet's invasion of Afghanistan (1979-89), Iran-Iraq War (1980-88), Libya against Chad (1987), and Iraq against the Iraqi Kurds (1988) (Salem, 2008). In 1975, the United States ratified the 1925 Geneva Protocol and ceased development of chemical weapons for offensive applications

(Hoening, 2002) and later the Chemical Warfare Convention which called for the destruction of all chemical stockpiles (Hoening, 2002). Unfortunately, CWAs still remain a threat and show no sign of disappearing.

Nearly two decades ago, U.S. soldiers were unknowingly exposed to low levels of sarin and cyclosarin after the destruction of two Khamisiyah munitions sites in Iraq during the Persian Gulf War on March 4th and 10th 1991 (Bullman, 2005). This resulted in almost 100,000 soldiers being exposed to asymptomatic doses of sarin gas (Bullman, 2005). Months after returning from Operation Desert Storm, there was an unusually high number of Gulf War veterans reporting symptoms such as fatigue, musculoskeletal discomfort, skin rashes and cognitive dysfunction (Broderick, 2011). The exposure to low doses of sarin and cyclosarin as well as other possible etiological agents is the suspected cause of those suffering from an illness known today as Gulf War Illness.

Two highly publicized examples of terroristic employment were conducted in 1994 and again in 1995 by a Japanese religious doomsday sect known as the Aum Shinrikyo. According to the "Chronology of Aum Shinrikyo's CBW Activities" (2001), on June 27, 1994, the Aum Shinrikyo conducted the first abuse of nerve agent gas against an innocent civilian public in Matsumoto, Japan. The targets were three judges hearing a case of fraud against the cult with the primary goal being to delay the ruling (Tu, 1995). The cult conducted its attack using sarin dispersed with a heating pot to vaporize and a fan equipped to a van to disperse the gas. The attack resulted in seven fatalities, 144 injured and 126 complaining of various symptoms.

The Japanese police raided the Aum Shinrikyo's compound facilities in Kamikuishiki and several other locations on March 22, 1995 in an attempt to disrupt a second attack (Tu, 1995). The raids resulted in discovering a variety of precursors which could have led to the production of sarin, VX, mustard gas and several biological weapons. However, no intact sarin was found (Tu, 1995). A second attack took place at the Kasumigaseki district subway system where many government buildings, such as the Tokyo Metropolitan Police Headquarters, were located (Tu, 1995). The attack applied simple dispersion of approximately 30% pure sarin in eleven plastic bags (eight of which released agent) pierced by umbrellas in five subway cars stopped in the district at approximately 8 a.m. (Salem, 2008; Tu, 1995; Chronology of Aum Shinrikyo's CBW Activities, 2001). As a result of the attack, there were twelve fatalities, 1039 injuries and approximately 4460 reported to the hospital. Out of the approximately 5,500 victims, only about 70 were found to be at least moderately ill where the rest showed no physical symptoms of intoxication (Chronology of Aum Shinrikyo's CBW Activities, 2001).

The most recent exposure to nerve agents documented occurred in May 2004 when a U.S convoy came across an old Iraqi artillery shell which was a binary weapon rigged to be used as an improvised explosive device (IED) and detonated before the ordnance team could disarm it. The exposure was mild and additional decontamination of the area was deemed unnecessary (Arraf, 2004).

With the developments of the growing conflict in the Arab state of Syria, the large and well developed chemical weapon program in the country has become a growing focal point of chemical weapon counter-proliferation. Should the dictator of Syria, Bashar Assad, and his regime fall, the possibilities of Syria's chemical arsenal being shipped to a violent rogue nation or stolen by terrorist groups such as al-Qaeda would become a cause for concern. As a result, it becomes increasingly important to better understand and improve upon the current methods for which nerve agents are decontaminated and processed should an attack take place.

Nerve Agent Decontamination

Table 1 outlines the characteristics of each of the trademark nerve agents: tabun (GA), sarin (GB), soman (GD) and VX. Each is highly toxic for its capability to inhibit ACh. However, VX is the most dangerous. First, VX has an LD_{50} (dosage causing death in 50% of an exposed population) of only 5mg/70-kg man which is two orders of magnitude lower than its closest G-agent (soman with LD_{50} of 350mg/70-kg man) (Lillie, 2005). Secondly, its low volatility of only $10.5 mg/m^3$ makes this agent the least likely of the four to enter into the gaseous form and hence, be more persistent in the environment. Finally, VX has a hydrolysis half-life of 1,000 hours which far exceeds any of the G-agents. These factors are important in considering the degradation of nerve agents that may be processed in a municipal wastewater treatment plants. It is for these reasons VX was of primary interest in this research.

Table 1: Chemical and Physical Properties of Nerve Agents (Munro, 1999).

	Agent			
Property/Parameter	**GA (Tabun)**	**GB (Sarin)**	**GD (Soman)**	**VX**
Chemical Formula	$C_5H_{11}N_2O_2P$	$C_4H_{10}FO_2P$	$C_7H_{10}FO_2P$	$C_{11}H_{26}NO_2PS$
CAS #	77-81-6	107-44-8	96-64-0	50782-69-9
Molecular Weight	163.1	140.1	182.2	267.4
Physical State	Oily liquid	Liquid	Liquid	Oily liquid
Color	Colorless to brown	Colorless	Colorless	Light amber/amber
Melting point (^{o}C)	-50	-56	-42	-39
Boiling point (^{o}C)	220-246	158	198	298
Density liquid (g/ml)	1.0733 25 ^{o}C	1.102 20 ^{o}C	1.022 25 ^{o}C	1.008 20 ^{o}C
Vapor pressure (mmHg 20 or 25 ^{o}C)	0.037 @ 20 ^{o}C 0.07 @ 25 ^{o}C	2.10 @20 ^{o}C	0.40 @ 25 ^{o}C	0.0007
Volatility (mg/m3)	610	22,000	3,900	10.5
Vapor density (air =1)	5.6	4.9	6.3	9.2
Water solubility (g/L)	98 @ 25 ^{o}C	Miscible	21 @ 20 ^{o}C	30
Hydrolysis rate (half-life)	8.5 hr (pH 7)	39 hr (pH 7)	45 hr (pH 6.6)	1,000 hr (pH 7)
Henry's Law constant (H, atm X m3/mol)	1.52 X 10^{-7}	5.4 X 10^{-7}	4.6 X 10^{-6}	3.5 X 10^{-9}
Log Kow	0.384	0.299	1.824	2.09
Log Koc	2.02	1.77	1.17	2.5

Should a chemical incident occur, standard procedure dictates all contaminated personnel are to be decontaminated (Medical Management of Chemical Casualties Handbook, 2007). This is accomplished through applying soap and water, or a 0.5% hypochlorite bleach solution along with large quantities of water (Medical Management of Chemical Casualties Handbook, 2007). Furthermore, a significant amount of this wastewater used in decontamination may be lost and enter the streamline of the millions of gallons flowing toward a wastewater facility on a daily basis. Should intact or degradation products of nerve agents enter the wastewater treatment process, it remains unclear if these compounds will be completely degraded, partially degraded, or flow through the plant unaffected.

Wastewater Treatment Plant Operations

Most communities have municipal wastewater treatment systems to which decontamination wastewater may be sent. Although information on the fate of VX in a wastewater treatment plant is scarce, a recent study by Schuldt (2012) found that ethyl methylphosphonic acid (EMPA), a hydrolysis product of VX, can pass through a treatment plant largely unchanged. Many municipal wastewater treatment facilities start with a preliminary treatment where wastewater passes through bar screens to physically remove large debris (Figure 2). The wastewater flows to a settling tank where it is held to allow solid particles to settle to the bottom (water.me.vccs.edu). Next the settled wastewater flows to an aeration tank where micro-organisms, collectively known as activated sludge, consume dissolved compounds and nutrients in the water. Over time, the sludge will remove the available food sources and reproduce (Droste, 1997). After the microbial activity, the activated sludge is allowed to settle to the bottom where some is recycled to the aeration basin to treat incoming water and the rest is sent to a solids handling area where they may be removed to landfills or have their high nutrient content utilized in land applications such as farming. The amount of solids that gets wasted is dependent on each facility's solid retention time (SRT) which determines the solids removal rate from the system as per Equation 1 (Droste, 1997).

$$\theta_x = \frac{VX_v}{solids\ removal\ rate\ from\ the\ system(\frac{mg}{day})} \tag{1}$$

where:
θ_x= solid retention time (days)
V=Volume of aeration basin (L)
X_v= Total suspended solids (mg/L)

The SRT is the average amount of time the sludge spends in the aeration basin before being wasted (Droste, 1997). It is also an important design parameter in the activated sludge process since it controls other process parameters such as effluent water quality and oxygen demand (Wu, 2011). The micro-organisms in this process require a certain amount of time in order to consume incoming substrate and reproduce. If the SRT is set low, the sludge will be less starved and undergo less endogenous decay (Droste, 1997). Additionally, if set too low, the sludge will not be able to sustain itself and be wasted faster than it reproduces, consequently lowering the quality of the effluent unless the bacteria is resupplied from outside the aeration basin (Droste, 1997). At the other extreme, higher SRTs create a more starved environment for the activated sludge, impacting the settling ability of the sludge as well as the amount of sludge that can be produced in the system (Droste, 1997). Some municipal wastewater treatment facilities may favor a higher SRT to allow more bacteria to be available to degrade excess dissolved substrate or an increase in the daily processing volume. Furthermore, having an excess of bacteria present gives an added benefit to the facility by sustaining reasonable water treatment if a toxicant enters the plant and deactivates a portion of the biomass (Droste, 1997). The remaining wastewater then goes through a disinfecting step with chlorine or ultraviolet light before being released into a local water reservoir (Droste, 1997).

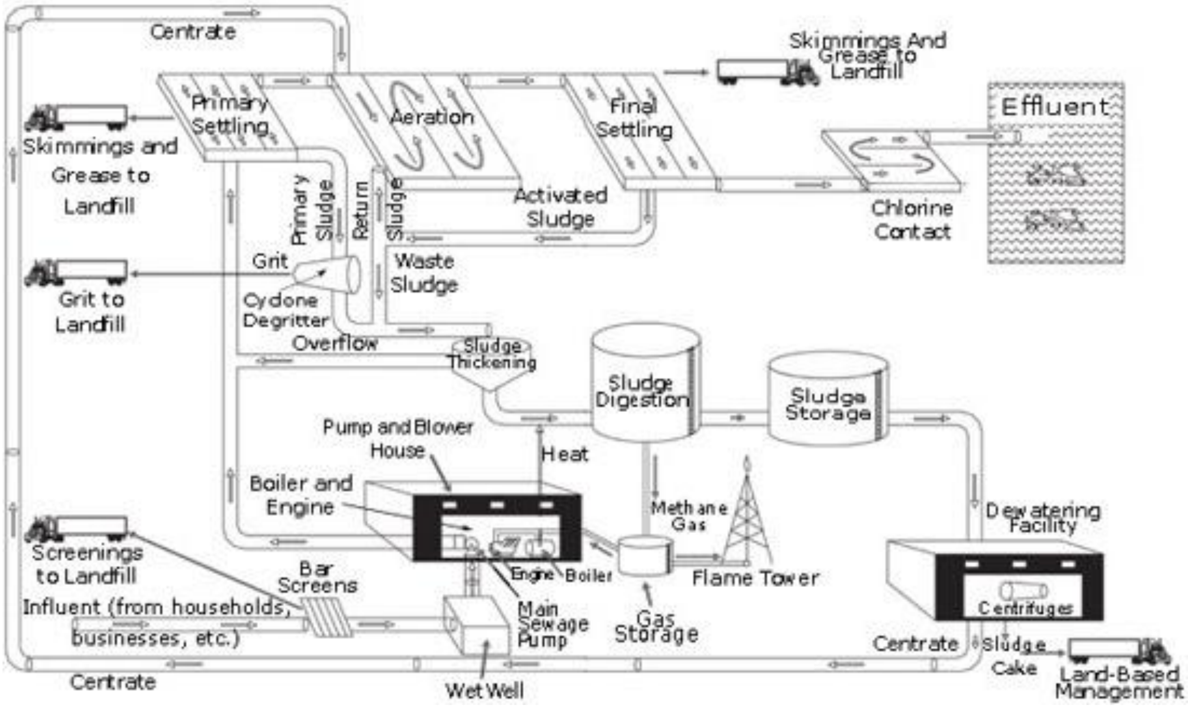


Figure 2: Wastewater Treatment Plant Process (www.nyc.gov)

The activated sludge process is the primary means of degrading dissolved compounds in the wastewater which have passed through the initial bar screens and primary settling tanks. The heterotrophic bacteria present in the sludge consume organics as both a carbon and energy source while the nitrifying (autotrophic) bacteria consume inorganic carbon sources and utilize reduced nitrogen sources, such as ammonia or nitrite, for energy (Vaccari, 2006). Furthermore, ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) convert ammonia to nitrite and nitrate through reactions shown in equations 2-4.

$$NH_3 + O_2 + 2e^- + 2H^+ \text{ - -> } NH_2OH + H_2O \qquad (2)$$

$$NH_2OH + H_2O + 1/2\ O_2 \text{-->} NO^{2-} + 2H_2O + H^+ \qquad (3)$$

$$NO^{2-} + 1/2O_2 \text{-->} NO^{3-} \qquad (4)$$

AOB are also known to co-metabolize organic compounds as well. Ammonium monooxygenase (AMO) in nitrifying bacteria such as *Nitrosomonas,* and *Nitrosospira* is responsible for the production of not only hydroxylamine, but it can also insert oxygen into the C-H bonds to catalyze hydrolysis (Hyman, et al., 1988). The AMO can catalyze oxidation of alkanes up to C_8 to alcohols and also to epoxides for alkenes up to C_5 (Hyman et al., 1988). This enzyme has also been found to degrade several organic compounds (Vader et al., 2000; Shi et al., 2004; Ren et al., 2007). It is conceivable that AMO co-metabolism, alongside with heterotrophic metabolism, may be involved in the degradation of VX as well as any of the other OP nerve agents.

Problem Statement

Numerous studies have already been conducted for determining the toxicity and characteristics of nerve agents. However, research falls short in analyzing the fate of these compounds in municipal wastewater treatment plants. They may breakdown into less harmful products, sorbs to the activated sludge, remain unaffected, or a combination thereof. In the event of a chemical weapon attack, there is the possibility that the water being used in the decontamination may be sent or lost to a wastewater treatment plant where the risk of the CWA or its degradation products, some of which also exhibit toxicity, may not be limited to the initially exposed individuals. If wastewater treatment operations do not sufficiently remove these compounds, the surrounding environment and ecosystem could be affected. If sorption were to occur, the toxic OPs may follow disposed waste sludge where it may be applied as fertilizer in agricultural areas. Furthermore, should degradation not occur by the heterotrophic or nitrifying bacteria, the toxic OPs may leave the facility in the effluent and enter a receiving body of water. Both routes of escape could pose dangers to the local population. It is therefore important to understand and maximize the efficiency of wastewater treatment plants in handling OPs to prevent their accidental spread to an unsuspecting population.

Research Objectives/Hypothesis

The purpose of this study is to examine the effectiveness of the degradation of malathion, an OP insecticide and surrogate for VX, in bench scale activated sludge sequencing batch reactors by varying the solid retention time (SRT) of the activated sludge. Theoretically, a longer SRT should result in a higher substrate removal (Racz et al., 2012). This study also aims to identify the presence of any metabolites or degradation products which may persist within an activated sludge system after being exposed to the VX surrogate. My hypothesis is a SRT extension will enhance malathion degradation.

Scope and Approach

This research used multiple 2.0 L activated sludge sequencing batch reactors to replicate the microbial activity that takes place within the activated sludge process under different SRTs. These batch reactors are seeded with activated sludge provided by the Fairborn Water Reclamation Facility (FWRF) in Fairborn, Ohio and fed simulated wastewater, containing chemicals commonly found in municipal wastewater facilities. Sequencing batch reactors are a type of aerobic biological treatment which focuses primarily on the activated sludge process displayed in Figure 3. The process starts with a fill stage of the influent followed by aeration and metabolic reactions exercised by the microbial community. The reactor then ceases aeration to allow the biomass to settle and remove the treated effluent as well as waste sludge before the process repeats itself (Droste, 1997).

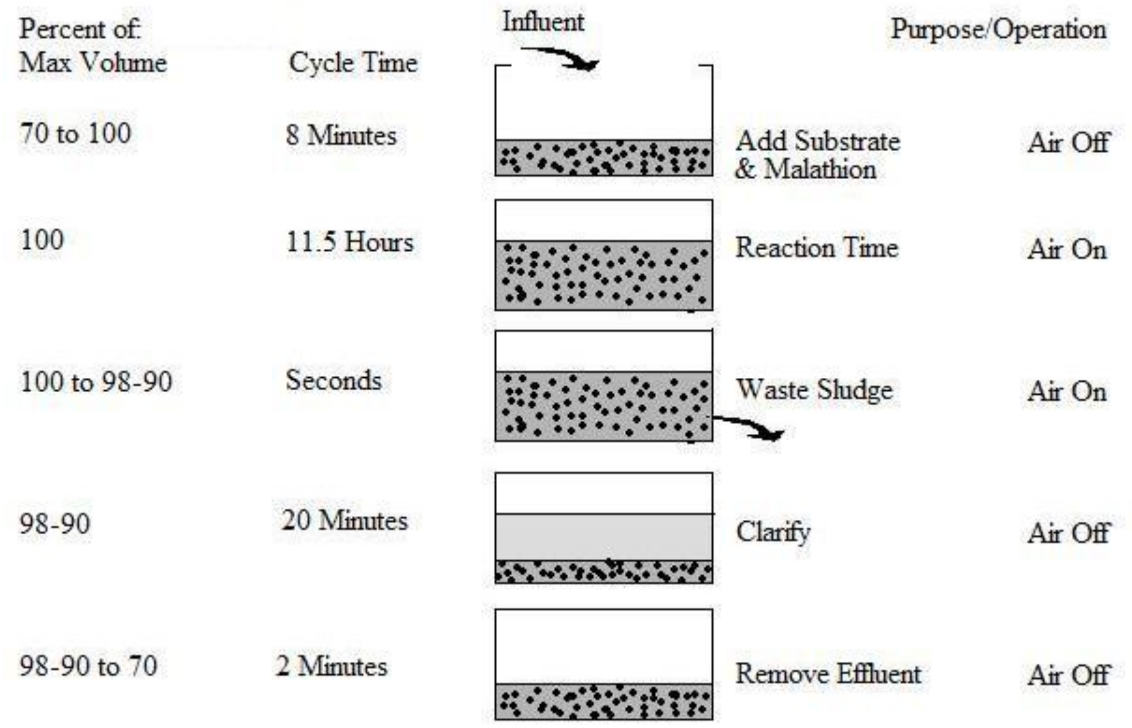


Figure 3: Sequencing Batch Reactor Operating Cycle

This work will build upon batch test experiments determining the ability of activated sludge to sorbs and biodegrade malathion (Walters, 2013). Malathion was chosen as a surrogate for VX due to several characteristics they both share (Appendix A). One such characteristic is their similarities in both physical appearance and chemical structure, and consequently mode of toxicity, although malathion is much safer to handle than VX. Secondly, VX and malathion have octanol-water partition coefficients around 2.0, suggesting they are similar in polarity and possibly their tendency to adsorb to the activated sludge. The later condition was examined in a study by Walters (2013), which found that malathion sorption to activated sludge was negligible with respect to both contact time as well as concentration of sludge in the system. Lastly, both compounds have low vapor pressures and hydrolysis rates thus lowering the differences in their removal by volatilization and hydrolysis and allowing the removal in an activated sludge system by biodegradation to better reflect similar conditions with VX. The results of this study will provide further insight of the fate of OPs which enter the wastewater treatment facility over a prolonged period of time as well as whether changing the SRT will influence the degradation of OPs.

Significance

Should a CWA incident occur, decontamination wastewater may flow into a wastewater treatment plant. It is important for an operator of a wastewater treatment facility to know what changes can be made in the standard operating procedure to safely treat CWA contaminants without doing so at the expense of effluent water quality. If the activated sludge process cannot completely biodegrade the CWA, regardless of changes in SRT or any other parameters under operator control, it may escape the wastewater treatment plant in the wasted sludge, aqueous effluent, or both. Consequently, significant health concerns may arise in areas where streams containing discharged wastewater are downstream potable water sources. If there is a time dependence of effluent quality as a result of the constant exposure to a CWA in a municipal wastewater treatment plant, warning and relief aid may be given to those possibly at risk of toxicity from the discharged effluent in a timely manner.

II. Scholarly Article

Abstract

This study investigated the fate of malathion, a surrogate compound for VX studies, in multiple activated sludge sequencing batch reactors (SBRs), each being operated under a different solid retention time (SRT) for 30 days. Each reactor was provided with synthetic feed to simulate wastewater and malathion at a concentration of 5 mg/L once every 12 hr. Removal of malathion from the effluent only varied with SRT during a short lived stress response within the first week. The stress response temporarily inhibited malathion removal in the activated sludge, the extent of which increased with increasing SRTs. This behavior may have resulted from a toxic chemical such as malathion or a metabolite of it, being present in higher quantities in the system longer with increasing SRTs. Each SBR was capable of resuming high malathion removal by day 10 and continued to remove malathion as the influent malathion concentration had increased from 5 mg/L to 20.8 mg/L. During the last 7 days of the experiment, effluent malathion concentrations had reached a possible threshold limit of 266 ± 62 µg/L. Malathion appears to have a toxic effect on the heterotrophic bacteria in the activated sludge.

Key words: Organophosphate, malathion, activated sludge, solid retention time

Introduction

Nerve agents, arguably the most toxic class of chemical warfare agents (CWAs), are all a part of a class of compounds known as organophosphates. Organophosphates have consistently been used in agriculture for their insecticide application; however, their military appreciation was not recognized until the 1930s (Salem, 2008). The toxicity of this class of CWAs is attributed to binding of the compound to the active site of AChE and thus deactivation of the enzyme (Nerve Agents, 2008). Several forms of nerve agents have been weaponized, but the most toxic include tabun (GA), sarin (GB), soman (GD), and VX. Although efforts have been made to prevent their misuse, such as the 1925 Geneva Protocol and the Chemical Warfare Convention, a determined organization may yet succeed in obtaining and weaponizing these compounds in order to kill or terrorize innocent lives.

Should any nerve agent be employed against civilian or military personnel or infrastructure, standard operating procedures dictate decontamination efforts be executed. This is often accomplished with copious amounts of water, coupled with soap or a hypochlorite bleach solution (Medical Management of Chemical Casualties Handbook, 2007). Some of this water/CWA solution is then removed and may potentially be sent to a local municipal wastewater treatment plant. However, few studies have been conducted to determine the effectiveness of wastewater facilities to degrade these compounds to non-toxic compounds. One such study found that EMPA, a hydrolysis product of VX, degraded 28% possibly via cometabolism during batch tests in the presence of nitrifying activity (Schuldt, 2012).

Many wastewater treatment plants utilize biological degradation characteristics of several bacteria (collectively known as activated sludge) to degrade organics that cannot be simply filtered out of the influent. Also, due to their hydrophobic tendencies, they may sorb onto the activated sludge (AS) biomass (Bondarenko and Gan, 2004). If nerve agents cannot be completely degraded through the biological activity in wastewater treatment facilities, they can both escape with the plant's effluent or the wasted sludge and possibly induce toxic effects on the receiving ecosystem.

VX was the nerve agent of interest for this study. It has physical characteristics that make it more dangerous than the G-agent counterparts and thus worst case scenario of a CWA attack. First, VX has a LD_{50} of a 5 mg/70 kg person making it the most lethal of the nerve agents (Lillie, 2005). Second, VX is the most persistent with a hydrolysis rate of 1,000 hours and a low volatility of 10.5 mg/m^3, making it the least likely to enter the vapor form (Munroe, 1999). Since testing of VX is outside the capacity of our laboratory, malathion, a commonly used organophosphate insecticide, was used as a surrogate for VX in this experiment. Malathion was chosen because it has several characteristics similar to VX (Appendix A). The first being a chemical structure similar to that of VX, but is far less toxic and thus safer to handle. It also has an octanol-water partition coefficient close to that of VX, which may yield similar sorption kinetics between the two. Walters (2013) found that sorption of malathion to the activated sludge is not a significant method of removal, with respect to both contact time and sludge concentration, and thus may not be a significant factor in VX removal either. Like VX, malathion has a low vapor pressure and a slow hydrolysis rate consequently leading to less degradation to occur via volatilization or hydrolysis, making biodegradation the primary means of removal. Walters (2013) concluded malathion was able to degrade ~87% in a 12 hr batch test with aerated activated sludge. However, that efficacy may not hold constant over multiple cycles receiving a constant amount of malathion. Furthermore, the consistent presence and/or accumulation of malathion and any non-degraded byproducts could impact the bacterial community by causing a shift in the microbial population, bacterial death, or both. Consequently, effluent quality may lessen and more malathion may escape the wastewater treatment facility.

This study determined the capability of activated sludge to degrade malathion in sequencing batch reactors (SBRs). The solids retention time (SRT) was the parameter of interest for this study. It is an important design parameter in the activated sludge process since it controls other process parameters such as effluent water quality and oxygen demand (Wu, 2011). A low SRT causes the sludge to be less starved and undergo less endogenous decay while still sustaining acceptable nutrient removal (Droste, 1997). On the other hand, higher SRTs create a more starved environment for the activated sludge, impacting the settleability of the sludge as well as the amount of sludge that can be produced in the system (Droste, 1997). Some municipal wastewater treatment facilities may favor a higher SRT to allow more bacteria to be available to degrade excess substrate or an increase in the daily processing volume (Droste, 1997). Furthermore, having an excess of bacteria present gives an added benefit to the facility by sustaining reasonable water treatment if a toxicant enters the plant and deactivates a portion of the biomass (Droste, 1997).

Materials and Methods

Sequencing Batch Reactor Operation

Three reactors were operated under conditions adopted from Racz et al. (2010a). Specifically, 2.0 L SBRs were constructed and seeded with an activated sludge sample extracted from the Fairborn Water Reclamation Facility (FWRF), Fairborn, Ohio. In order to sustain simultaneous chemical oxygen demand (COD) removal and nitrification activity, two feed sources were used, feeds A and B respectively. Feed A consisted of (per liter deionized water) 71.4 g $NaHCO_3$ while feed B consisted of the following: 3.0 g peptone, 1.25 g $NaCH_3COO$, 2.26 g NH_4Cl, 6.86 g $MgCl_2 * 6H_2O$, 1.72 g $CaCl_2 * 2H_2O$, 0.6675 g KH_2PO_4 and 20 mL of a trace element solution adapted from Hesselmann et al. (1999). This trace element solution consisted of the following (per liter of deionized water): 5.46 g citric acid, 4.0 g hippuric acid, 0.72 g $Na_3NTA * 2H_2O$, 0.3 g $Na_3EDTA * 4H_2O$, 3.0 g $FeCl_3 * 6H_2O$, 0.5 g H_3BO_3, 0.3 g $ZnSO_4 * 7H_2O$, 0.24 g $MnCl_2 * 4H_2O$, 0.14 g $CuSO_4 * 5H_2O$, 0.06 g KI, 0.06 g $Na_2MoO_4 * 2H_2O$, 0.6 g $CoCl_2 * 6H_2O$, 0.6 g $NiCl_2 * 6H_2O$, and 0.06 g $Na_2WO_4 * 2H_2O$. Feed A acted both as an inorganic carbon source for the nitrifying bacteria as well as to adjust the pH of the mixed liquor to a pH of 6-7.5 to minimize hydrolysis of malathion. Feed B contained the organics, volatile fatty acids, and micro and macronutrients found in wastewater. Sodium acetate was added to feed B to represent the presence of volatile fatty acids (Kindaichi et al., 2004) which is readily metabolized by heterotrophs (Tam et al., 1992) and peptone to act as a complex carbon, nitrogen, and energy source for heterotrophic bacteria in aquatic environments (Racz et al., 2010a; Goel and Noguera, 2006). For the first fifteen days each reactor had an operation procedure consisting of two, 12 hour cycles per day with each cycle consisting of three stages. The first stage consisted of an aeration period of 11.5 hr with compressed air to ensure sufficient oxygen contact with the sludge to maintain a proper dissolved concentration of approximately 7 mg/L. During this phase, each SBR wasted enough mixed liquor to operate with SRTs of 9.5, 25 , and 50 days respectively with the sludge wasting taking place at hour eleven. The second stage ceased aeration for 20 minutes to allow for the suspended biomass to settle to the bottom of the reactor. The third and final stage decanted 670 mL from the top of the settled reactor mixture and resupplied, via peristaltic pump, with a fresh source of simulated wastewater consisting of 524 mL deionized water, 8mL of feed A, 38 mL of feed B, and 100 mL aqueous malathion solution at 100 mg/L restoring the final volume of the reactor to 2.0 L before restarting the cycle. This brought the final malathion concentration to 5 mg/L plus any malathion that may not have been degraded or removed through the effluent or mixed liquor discharges.

From day 16 to day 30, each reactor was fed a 500 mL aqueous solution of malathion at 100 mg/L, along with the feed A and B volumes mentioned earlier, to a final malathion concentration of 20.8 mg/L plus any malathion that may not have been degraded or removed through effluent or mixed liquor discharges, bringing the final volume of each reactor to 2.4 L. The third stage of each cycle had wasted 1170 mL, resulting in the same initial volume that had been used in the first half of the experiment prior to feeding. Mixed liquor wasting was adjusted for this increase in volume to retain the original SRTs each SBR had been operating under.

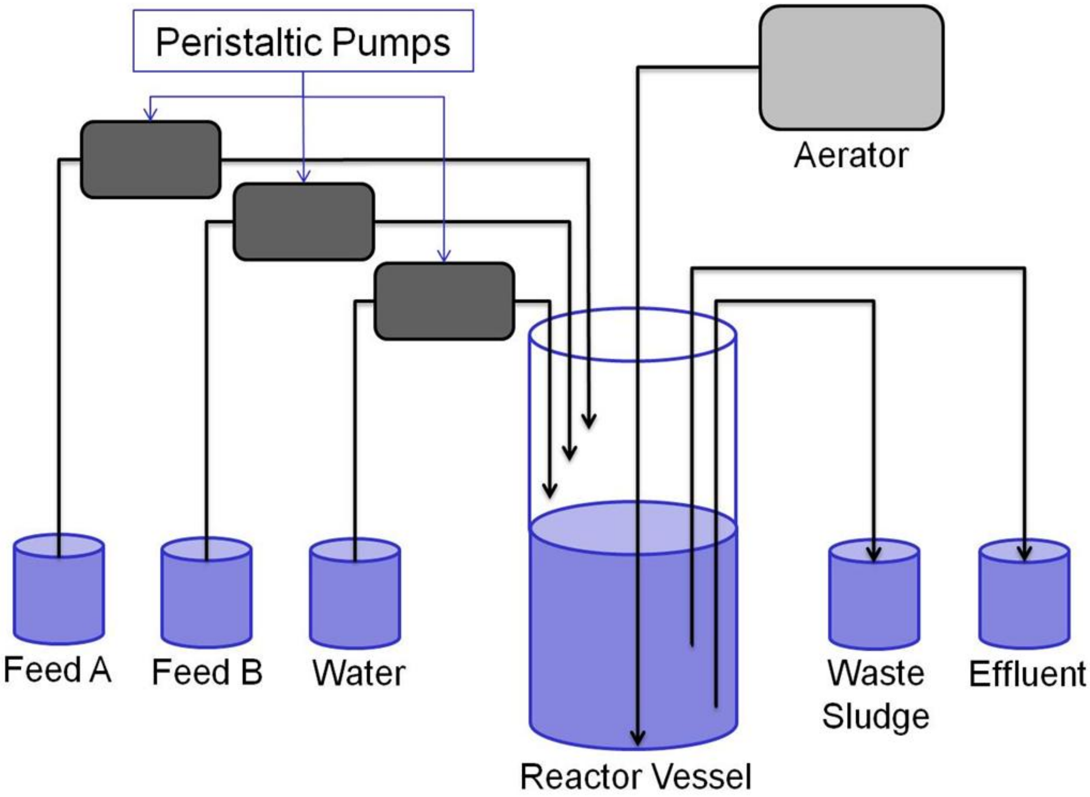


Figure 4: Diagram of Sequence Batch Reactors

Extraction and Measurement of Malathion

Malathion was measured in only the effluent since sorption to the biomass was not a significant factor in malathion removal (Walters, 2013). Malathion in the effluent was determined by first passing a sample of the effluent through a Whatmann filter paper, then adding 2 mL of methylene chloride to 10 mL of the filtrate, vortexing, and allowed to settle for one hour. After such time, 1 mL of the methylene chloride was collected and spiked with an internal standard of 30 µL from a 1000 mg/L dimethyl methylphosphonate (DMMP) stock solution prepared in methylene chloride. These samples were vortexed and analyzed by a gas chromatography/ mass spectrometer (GC/MS) and quantified against calibration curves of known standards prepared in the same manner. All measurements were conducted in duplicate.

GC/MS

An Agilent GCMS-7890A/5975C inert with an Agilent 19091S-433 column (0.15µm film thickness, 0.25mm id. X 30m length) attached was used for detecting malathion and dimethyl methyl phosphonate, and any metabolites present in the effluent. The mass-spectrometry was performed using the selected ion monitoring (SIM) mode with a *m/z* = 125 and 124 for malathion and the two peaks from DMMP respectively to detect and quantify malathion in each sample while the total ion chromatography (TIC) mode was used to determine the presence of any malathion metabolites in the effluent. The column was held isothermally at an initial temperature of 50 ^{o}C for three minutes and temperature-programmed at 20 ^{o}C/min to 260 ^{o}C during the SIM mode. In the TIC mode, the column was held isothermally at an initial temperature of 35 ^{o}C for three minutes and temperature-programmed at 10 ^{o}C/min to 260 ^{o}C. Calibration curves were made using the ratio of malathion peak areas to the sum of the two peaks for DMMP which occur due to dimerization. Injection volume was 4µL.

Non-Malathion Analytical Methods

Concentrations of chemical oxygen demand (COD), NH_3-N, NO_3-N, and NO_2-N were measured using Hach methods 8000, 10031, 10020, and 8153, respectively. Total suspended solids (TSS) and volatile suspended solids (VSS) were measured using standard methods (APHA, AWWA, WEF, 1998). All measurements were conducted in duplicate.

Results and Discussion

Long-term Malathion Removal Monitoring

Figure 5 gives the malathion concentration in the effluent throughout the experiment for each SBR. Initially, lower SRTs were more favorable for degrading malathion. By two and a half days of supplying each reactor 100 mL of a 100 mg/L aqueous malathion solution each, the SBR with an SRT of 9.5 days had degraded malathion to a concentration of 1805 ± 174 µg/L in the effluent. The SBR with an SRT of 25 days had an effluent malathion concentration of 1853 ± 182µg/L, and the malathion concentration in the effluent from the 50 day SRT SBR was 2318 ± 65 µg/L. However, from the third to eighth days of feeding each reactor malathion in this fashion, the activated sludge underwent a notable decrease in its capability to degrade malathion.

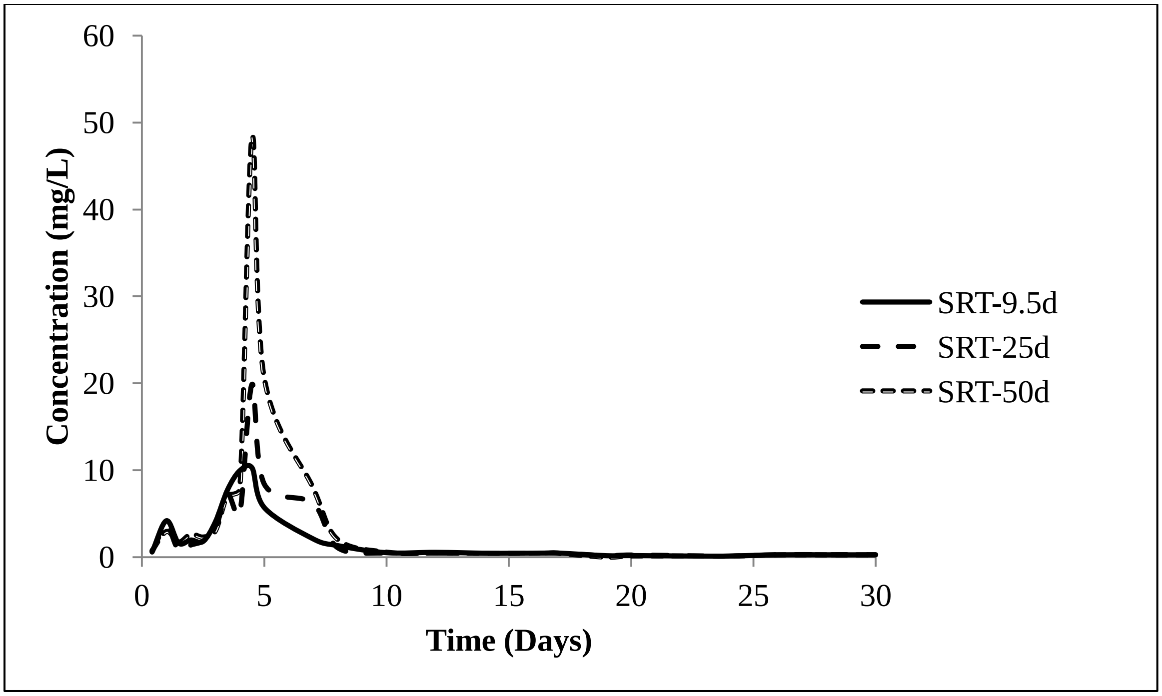


Figure 5: Concentration of malathion (mg/L) over time (days) in each SBR being fed malathion to a final concentration of 5mg/L (days 0-15) and 20.8 mg/L (days 16-30) plus any residing malathion from previous cycles.

Qualitatively, it is apparent that activated sludge has undergone some form of stress response. The spike in malathion concentration at 4.5 days increased with increasing SRT, although we expected it to be lower with increased SRT due to the presence of a more diverse community of bacteria. Since more mixed liquor is wasted in the lower SRTs, more of the chemical, either malathion or a metabolite of it, causing this apparent stress response is removed from the system during sludge wasting and therefore is not available to inhibit the activated sludge from degrading malathion. From the spike in malathion concentration until day 10, each SBR was gradually able to resume removing malathion, though concentrations in the effluent remained higher with longer SRTs.

From days 10 to 15, the malathion concentration in the effluent had reached a plateau of approximately 474 ± 47 μg/L in each SBR. There may have been a short lived impact on some of the AOB in the SBRs operated at an SRT of 25 days at day 5, closely coinciding with the malathion spikes; however, the average range of NH_3-N and NO_3-N concentrations from measurements taken prior to introducing malathion suggests the nitrifying bacteria were largely unaffected by the presence of malathion (Appendix B).

The heterotrophic bacteria were the most influenced by the presence of malathion in the activated sludge. COD concentrations in the effluent had immediately increased upon addition of malathion; however, like malathion, the COD present in the system reached peak concentrations in days 3 to 8, the highest being at day 3 where each SBR had a COD of 99.1 ± 4.6 mg/L in the effluent (Figure 6). By the end of day fifteen, COD removal had declined from nearly complete removal from an initial COD of 114 mg /L after first being fed malathion to effluent concentrations of 65 mg/L, 48 mg/L, and 52 mg/L in SRT-9.5, SRT-25, and SRT-50 respectively. This, along with the impact on malathion concentration, may suggest some of the heterotrophic species in the activated sludge are dying off, leading to an increase in population density for the bacteria capable of metabolizing the substrate feeds and malathion or those not impacted by malathion. Alternatively, rather than certain species being affected while others are not, the malathion may have also acted to temporarily inhibit catabolic functions in the activated sludge, resulting in an increase in COD concentration. The spikes in malathion and COD concentrations may also be attributed in part to a stress response the bacteria are exercising by releasing soluble microbial products (SMP), due to cell lysis, or releasing extracellular polymeric substances (EPS). Both SMPs and EPSs accumulate and are typically utilized in a SBR later on (Ni, 2010). They also have been found to contribute to biofouling in membrane bioreactors (Ni, 2010). The EPS however, has been observed to serve as a protective barrier against some charged or hydrophobic compounds by sorption and/or reactions with the toxic compound to prevent penetration by the activated sludge (Henriques, 2007). Furthermore, EPS concentrations in activated sludge have been found in several studies to be lower in facilities operating at longer SRTs (Ahmed et al., 2007; Masse et al., 2006). As a result, the higher malathion concentration present with increasing SRTs may be attributed to a decrease in EPS present with increased SRTs. Should this be the case, it may be possible the malathion in the system is not only being biodegraded or abiotically degraded, but trapped within an EPS matrix and physically removed during the daily wasting of the sludge. According to Racz (2012), sorption characteristics may change among biomasses since EPS characteristics, which can be important to sorption and can vary between different biomasses. If EPS sorption is a significant factor in malathion removal, the characteristics of the EPS with the bacteria not subject to heat inactivation may have a higher potential for sorption than when heat is applied, as was the case in Walters (2013) which found sorption not to be a significant factor in malathion removal. A stress response resulting in the production of EPS for malathion removal would require energy and may have been a contributing factor in the reduction of COD removal due to a hypothesis proposed by Love (2002) where energy is redirected from growth to biosynthesis. Deflocculation also occured in each reactor over time, a result which is known to occur under some stressed conditions (Villain and Marrot, 2013). As a result, the malathion stressed activated sludge along with the varied floc characteristics among the varying SRTs appear to have affected each reactors ability to respond to the stress and degrade malathion. If there is an EPS matrix present as a result of exposure to malathion, methylene chloride was not capable of extracting it from the EPS when applied to activated sludge filtered from the mixed liquor.

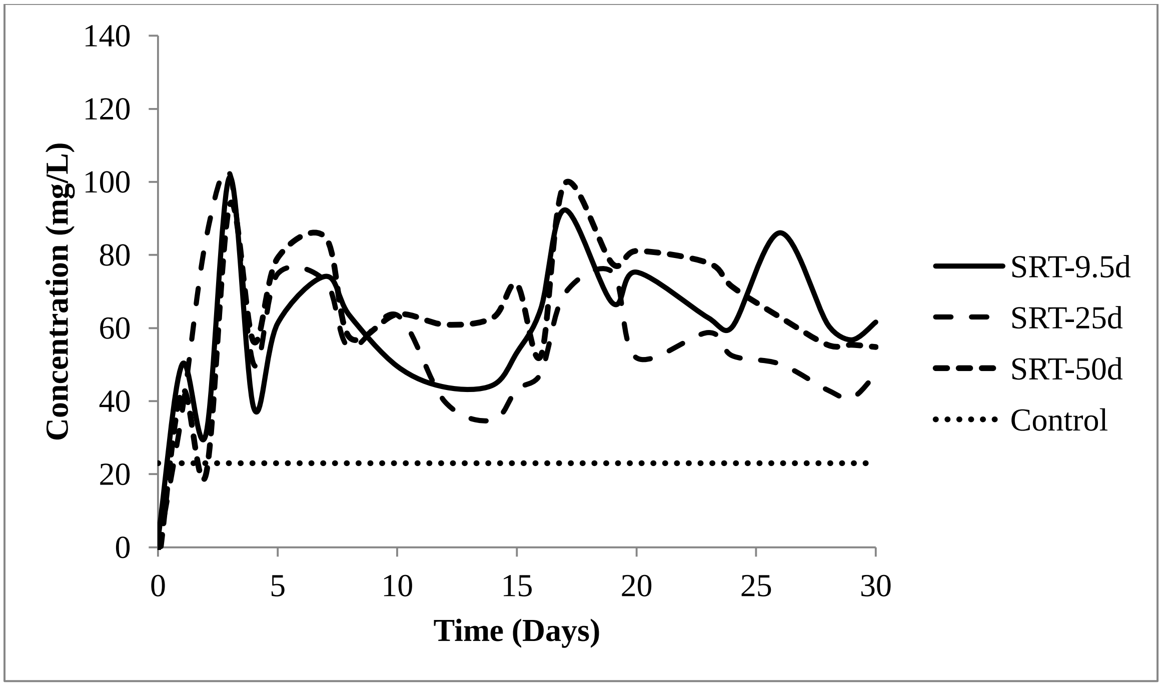


Figure 6: Concentration of COD (mg/L) over time (days) in each SBR being fed malathion to a final concentration of 5mg/L (days 0-15) and 20.8mg/L (days 16-30) plus any residing malathion from previous cycles.

When the influent malathion concentration was increased from 5 mg/L to 20.8mg/L each cycle, the activated sludge maintained its ability to remove malathion from the effluent. From day 24 to 30, malathion concentrations in the effluents were 266 ± 62 µg/L for each SBR operated under different SRTs. With the increase in malathion influent feed, COD effluent concentrations in each reactor remained high, with each reactor having a concentration of 61 mg/L, 47 mg/L and 54 mg/L in SRT-9.5, SRT-25, and SRT-50 respectively at the end of the experiment, further suggesting malathion has a negative impact on the heterotrophic bacteria present in the activated sludge . As in the 5 mg/L influent malathion condition, NH_3-N and NO_3-N concentrations did not vary from measurements taken prior to introducing malathion suggests the nitrifying bacteria were largely unaffected by the presence of malathion (Appendix B). The decrease in NO_3-N concentrations during the second phase of the experiment is primarily due to the dilution of NO_3-N in going from 2.0 L to 2.4 L in each reactor with the increase in malathion influent.

Throughout both phases of malathion degradation, four metabolites were regularly observed in all three reactors (Figure 7) . The first of which was thymine, which may have come from the degradation of peptone. The other three were phosphorodithioic acid O,O,S-trimethyl ester, O,S,S-trimethyl ester, and phosphorothioic acid O,O,S-trimethyl ester, the first two coming from malathion and isomalathion, an impurity found in malathion batches. The presence of the latter two throughout the experiment suggests the bond between the first and second carbons after the P-S bond could be susceptible to the AMO from the nitrifying bacteria or any of the oxygenase enzymes from the heterotrophs (Figure 8). This particular site may have also been a target in malathion degradation due to the electron withdrawing phosphorous section pulling electron density away from the first carbon while the esters present on the two tails of malathion pull electron density away from the second, making this bond an appealing site for enzymatic activity. Neither of the phosphorodithioic acids were quantified at any point in this study.

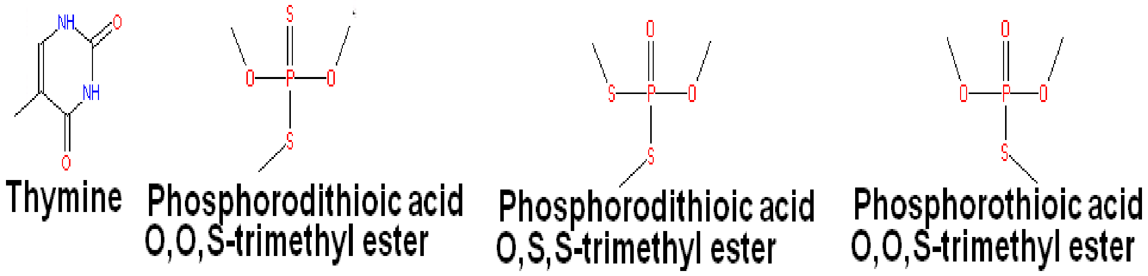


Figure 7: Malathion Metabolites in SBRs

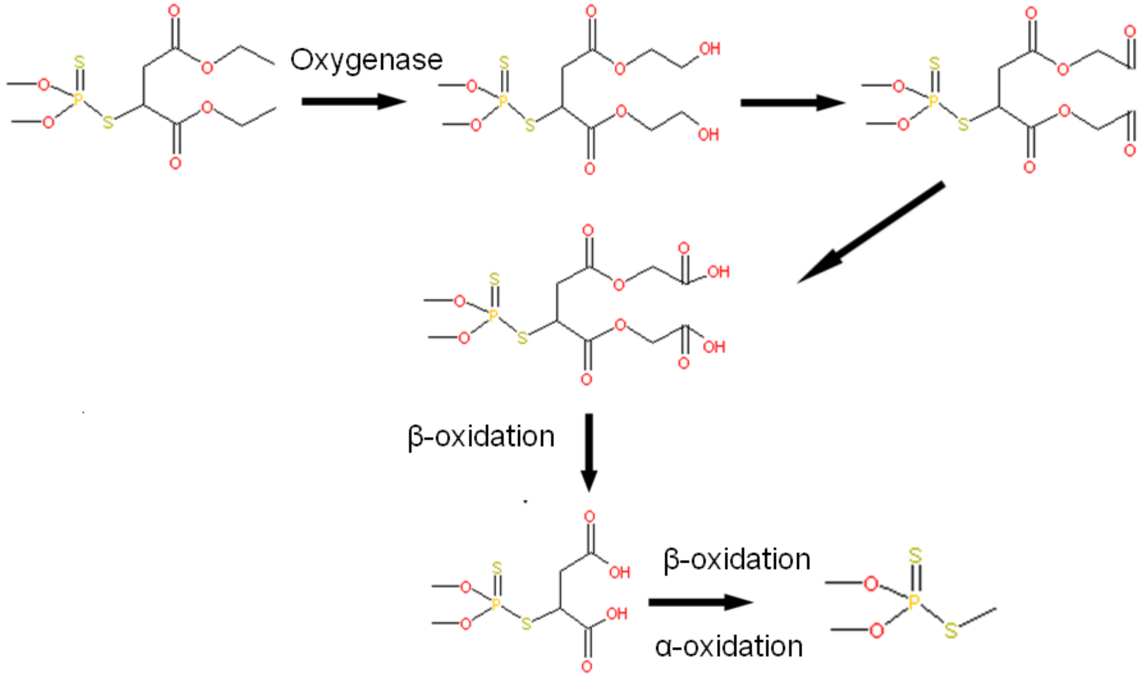


Figure 8: Proposed Degradation Pathway of Malathion.

Conclusions

This study has provided insight to the fate of malathion, a surrogate compound for VX studies, in a municipal wastewater treatment facility and demonstrates malathion may be largely removed eventually from the plant effluent, regardless of which SRT the plant is being operated under. However, constant exposure to VX may trigger a stress causing a temporary inhibition in OP removal, the extent of which increased with increasing SRTs, before the activated sludge had restored its capacity to remove the OP. As a result, this study concludes wastewater treatment facilities operated under lower SRTs may best favor VX removal since it is only during the temporary stress response where varying SRTs impacts OP removal. Consequently, the presence of this VX surrogate resulted in a decrease in COD removal under each SRT while having no impact on NH_3-N oxidation, suggesting VX is toxic to some of the heterotrophic species in the activated sludge. As a result, organic carbon sources in the effluent may pass through the plant unchanged, along with some of the phosphorodithioic acid metabolites, resulting in poor effluent quality and potential downstream toxicity.

III. Conclusions

Chapter Overview

This chapter discusses the findings in this study to answer the research questions found in Chapter I. The results section serves to summarize and compliment the discussion included in the scholarly article. A brief discussion pertaining to the significance of this study follows a review of the findings. Finally, areas of future research are identified followed by a summary of the thesis.

Review of Findings

This work demonstrates constant exposure to a CWA similar to VX may pass through an activated sludge wastewater treatment facility largely degraded, yet in detectable quantities regardless of which SRT the plant is being operated under. Consequently, as a result of the influent of malathion to the activated sludge, COD oxidation suffered from what was observed in the absence of malathion. This may suggest either malathion or one of its metabolites exhibits a toxic effect on some of the heterotrophic bacteria present in the activated sludge.

Significance of Research

Should a CWA incident occur, standard operating procedures dictate decontamination to be done with large quantities of water. It is possible for significant quantities of this decontamination water to enter a wastewater treatment facility. This study demonstrates that the majority of malathion, and potentially VX, which enters the facility will be degraded once the community adapts to its presence yet may have negative impacts on both the activated sludge community as well as the effluent quality. This occurs regardless of which SRT the facility is being operated under; however, during the initial several days of exposure, lower SRTs seem to better favor malathion degradation, possibly due to a contact time dependent toxicity to the microbial community. If the degradation characteristics of VX are analogous to this surrogate compound, there may be a significant concern for the efficacy of the facilities operability as well as OP toxicity in the effluent, especially in cases where the effluent is a downstream potable water source.

Limitations

This was a preliminary study to determine the fate of VX, by means of a surrogate compound, against an activated sludge system. This study may therefore be used as an approximation for field conditions concerning degradation studies under different operational parameters, in this case the SRT.

One limitation to this study is the use of the surrogate malathion rather than the live agent. Although malathion has similar chemical and physical properties to VX, the behavior of VX in an activated sludge environment may be more complicated since the molecular structure contains functional groups which allow VX to act basic or acidic as well as exhibit hydrophobic properties (Love, 2004). Also, VX has no strong electron-withdrawing leaving group to promote degradation, a fact that is not shared by malathion or other VX surrogates such as glyphosate, dimethyl methylphosphonate, or diisopropyl methylphosphonate that have been used in other VX related studies (Love, 2004). As a result, it must be realized that the conclusions derived from the use of malathion be limited when using them for predicting the behavior of VX under similar conditions.

Future Research

One area of future research is to manipulate other physical parameters of a municipal wastewater treatment plant to better maximize the degradation of malathion. One such area to consider is the effects of temperature on the microbial communities' capacity to degrade malathion. During winter seasons where the temperature is lower, the bacteria may be less active and consequently less efficient in degrading malathion. If the temperature of the activated sludge shows to be an important factor in malathion degradation, a critical temperature for adequate malathion degradation can be determined. Also, if there is a temperature dependence, there may be seasonal variations in removal during summer versus winter months. Furthermore, should such a dependency exist, sufficient degradation of malathion may only occur in areas which regularly operate at or above some critical temperature.

In addition, other surrogates should be tested in the same manner as malathion was in this study as well as in studies by Walters (2013) and Schuldt (2012). By examining multiple surrogates, further evidence may arise in showing similarities in surrogate compounds which may strongly support a theory of VX behaving similarly. Other degradation products of VX may be key factors to be tested as well as the structurally similar surrogates. EA 2192 is an example of such a degradation product of interest since its variation is from VX is very minimal, although highly toxic as well (Munro, 1999). In addition, being a degradation route for VX, it is plausible to assume that some EA 2192 may be produced during the decontamination of VX over the more preferred EMPA route and thus will provide a more complete insight to the fate of VX in activated sludge. Both degradation products found throughout this study would also be excellent candidates meriting further investigation. The presence of the phosphorodithioic acids was not quantified and thus it is unclear if it can be degraded via heterotrophic metabolism or nitrifying bacteria co-metabolism. If it can be degraded in an activated sludge system, knowing which bacteria may be responsible could act as a key element in complete degradation of VX, especially if such bacteria were capable of severing the P-S bond both VX and the phosphorodithioic acids possess.

Lastly, this study was able to conclude there is at least one, possibly several, bacteria in the activated sludge community that are capable of metabolizing or removing malathion and surviving within the system. Genomic DNA was extracted from samples of the activated sludge periodically throughout the experiment. Metagenomic analyses can be conducted on these DNA samples to determine which bacteria were being phased out and which remained and thrived in the presence of malathion. Should an OP enter a waste water treatment plant as a result of decontamination efforts or otherwise, knowing what bacteria are capable of surviving in this malathion environment and supplementing more of these CWA degrading bacteria over a time period may further enhance removal until the threat is neutralized. Further monitoring of the bacterial composition as well as the COD, NH_3-N, and NO_3-N concentrations was conducted to determine if and how long it may take for the activated sludge to produce acceptable effluent conditions after the CWA presence ceases. These results will be covered along with the metagenomic analysis separate from this work.

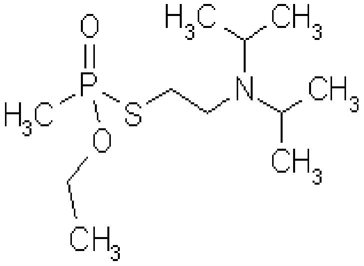
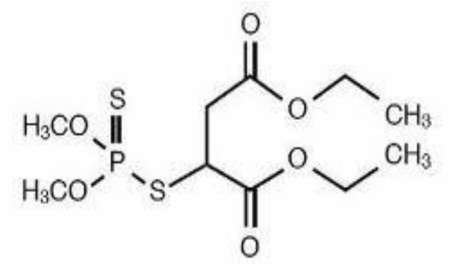
The possibility of EPS trapping the malathion deserves further investigation as well as determining which bacteria are releasing the EPS. If an EPS is responsible for trapping malathion, it is important to identify and collect it, along with the malathion in its matrix, to prevent potential downstream toxicity or toxicity associated with sludge disposal. It is unlikely for a single type of bacteria to maximize organophosphate degradation; but rather, a community of varying bacteria may be best suited for organophosphate degradation while maintaining acceptable WWTP operation. It is thus beneficial to conduct tests with multiple combinations of such bacteria to find an optimal community composition.

Summary

This study examined the fate of malathion, a surrogate for VX, in a municipal wastewater treatment facility utilizing an activated sludge system operated under different SRT conditions. The intent of this study was to determine the fate of a regular influent of malathion in the activated sludge and which SRT would provide optimal degradation. The research methodology involved three bench scale SBRs seeded with activated sludge from the Fairborn Water Reclamation Facility, each of which operated under different SRTs. Results showed no variation in malathion removal with different SRTs until an apparent stress response occurred where nitrifying and malathion metabolizing bacteria began to thrive. During the transition phase, lower SRTs appear to provide the most efficient degradation capacity, possibly due to a lower exposure time to the malathion environment from the larger volume of mixed liquor being wasted on a daily basis. Throughout such time of exposure to significant quantities of malathion, and possibly VX, COD oxidation is lessened, adding to the toxicity of the leaving effluent. Overall, this research further supports the notion stated in Schuldt (2012) that risks associated with an OP attack will not be limited to the incident site under current emergency planning procedures.

Appendix A. Comparison of VX and Malathion

Table 2: Physical and Chemical Properties of VX and Malathion

Property/Parameter	**VX**	**Malathion**
Chemical Structure		
CAS #	50782-69-9	121-75-5
Molecular Weight	267.4	330.358
Physical State	Oily liquid	liquid
Color	Light amber/amber	yellow-amber
Melting point (^oC)	-39	2.9
Boiling point (^oC)	298	148.9
Density liquid (g/ml)	1.008 @ 20^{o}C	1.231
Vapor pressure (mmHg 20 or 25 ^{o}C)	0.0007	1.78E-04
Vapor density (air =1)	9.2	11.4
Water solubility (g/L)	30	0.145
Hydrolysis rate (half-life)	1,000 hr (pH 7)	17.4 days (ph 6.0)
Henry's Law constant (H, atm X m^3/mol)	3.5 X 10-9	4.9 X 10-9
Log Kow	2.09	2.75
Log Koc	2.5	30

Appendix B. Daily NH_3-N and NO_3-N Concentrations in Each SBR Being Fed a Constant Influent of Malathion

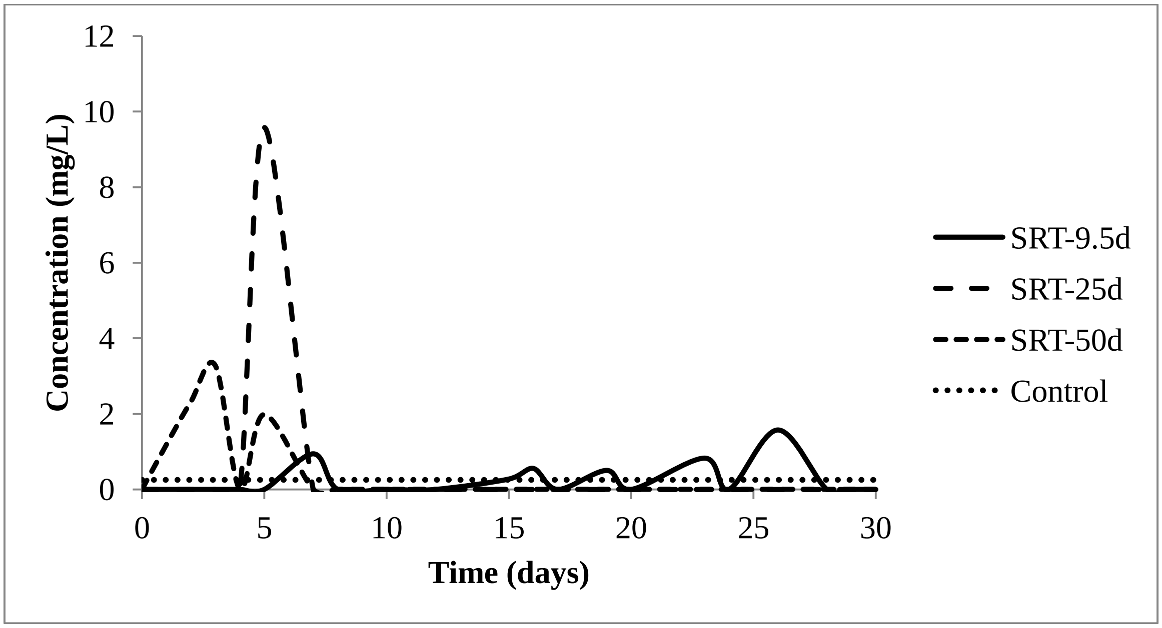


Figure 9: Concentration of NH_3-N (mg/L) over time (days) in each SBR being fed malathion to a final concentration of 5 mg/L (days 0-15) and 20.8 mg/L (days 16-30) plus any residing malathion from previous cycles.

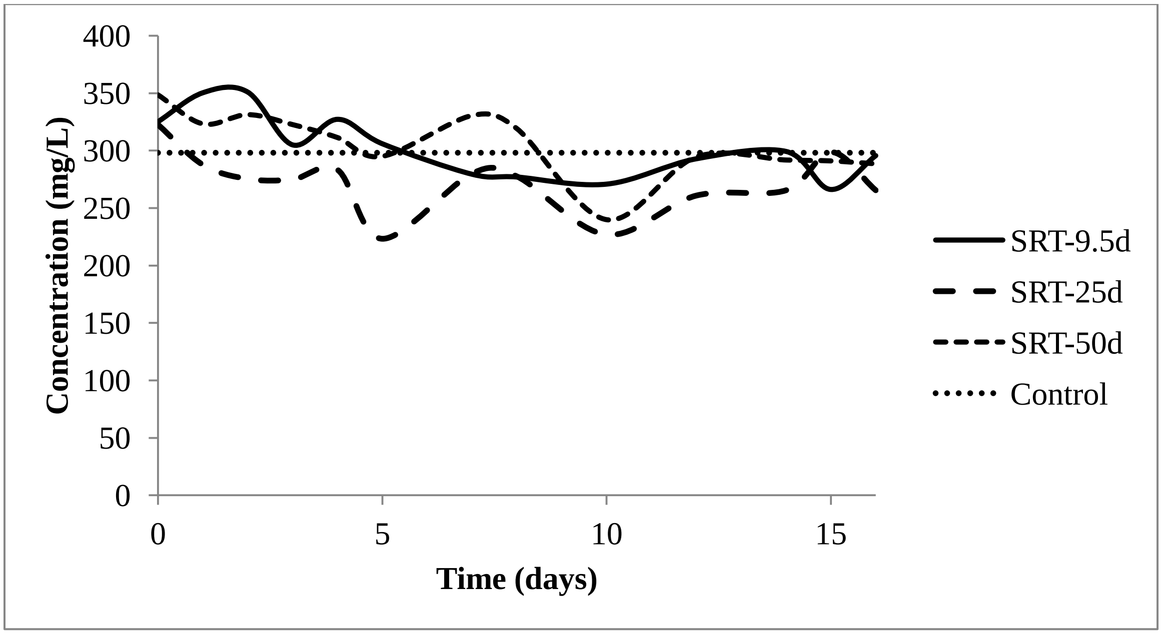


Figure 10: Concentration of NO_3-N (mg/L) over time (days) in each SBR being fed malathion to a final concentration of 5mg/L plus any residing malathion from previous cycles.

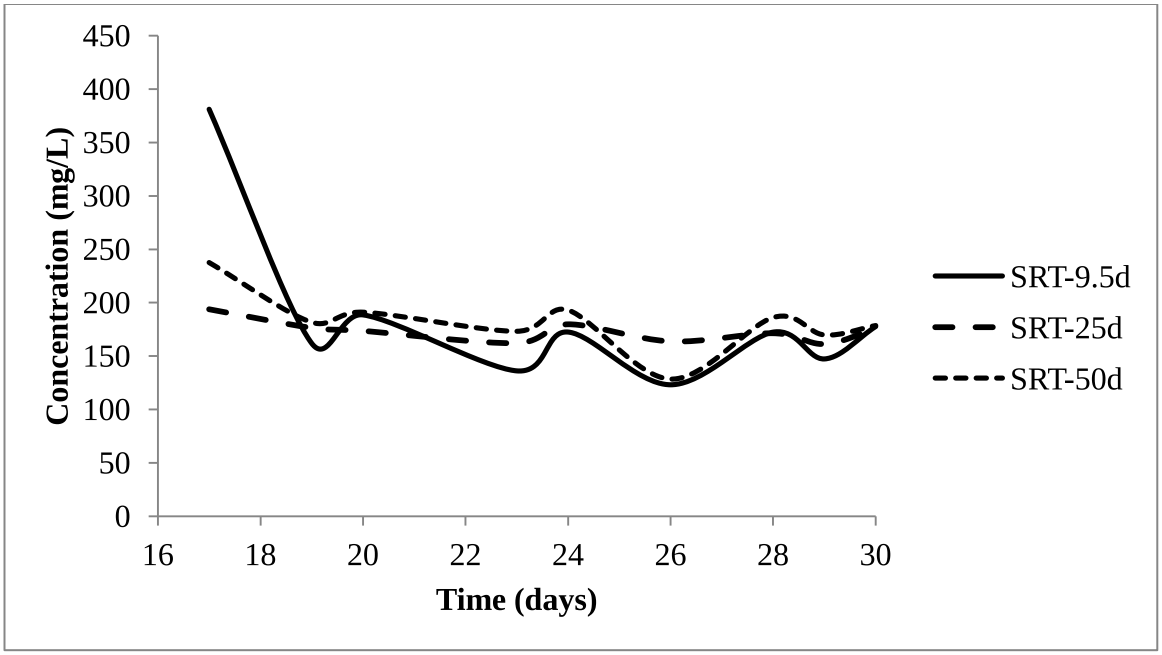


Figure 11: Concentration of NO_3-N (mg/L) over time (days) in each SBR being fed malathion to a final concentration of 20.8 mg/L plus any residing malathion from previous cycles.

Appendix C. Sludge Content in Each SBR

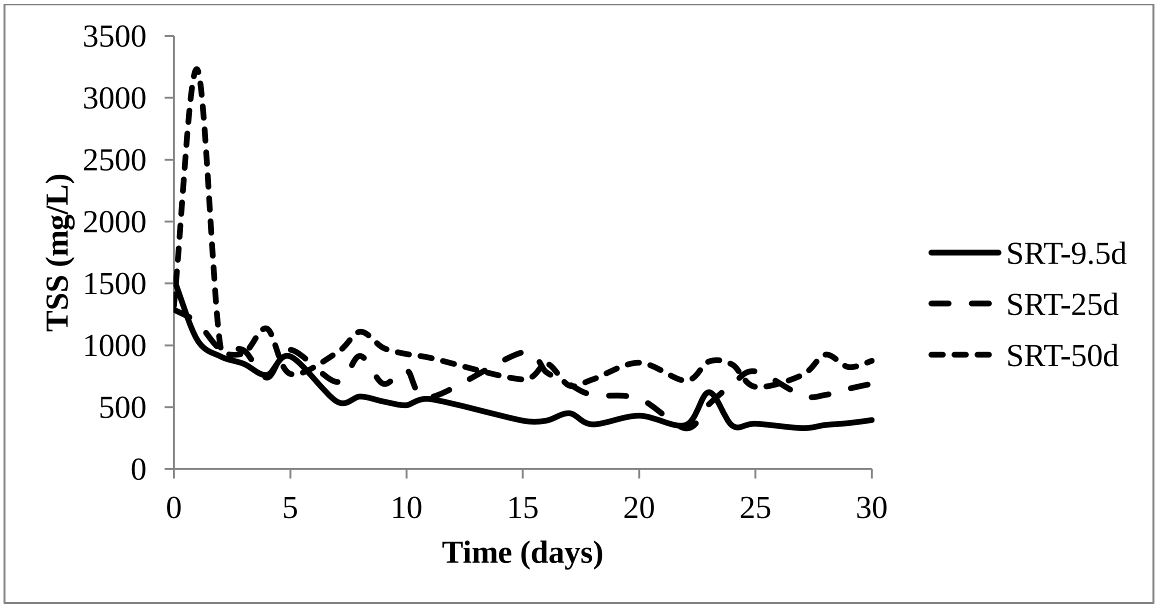


Figure 12: Total Suspended Solids (mg/L) in each SBR over time (days).

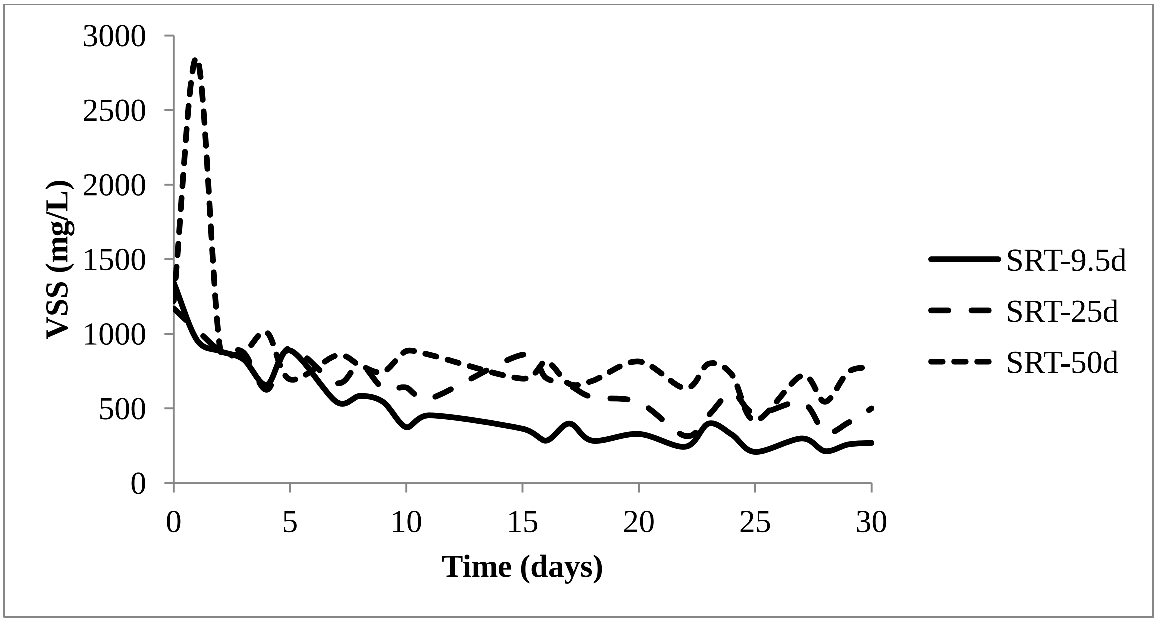


Figure 13: Volatile Suspended Solids (mg/L) in each SBR over time (days).

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14. ABSTRACT

This study examined the fate of malathion, a surrogate compound for VX studies, in bench-scale sequencing batch bioreactors being operated under varied solid retention times (SRTs) to simulate the activated sludge process in wastewater treatment facilities. Results show that a constant influent of the malathion will largely be removed. However, the heterotrophic bacteria undergo a stress response which temporarily inhibits malathion removal, the extent of which increases with increasing SRTs. After this stress response takes place, the activated sludge is able to resume a high degree of malathion removal, even with increased influent concentration; however, this is done so at the expense of acceptable COD removal in the effluent, suggesting malathion may be toxic to the heterotrophic bacteria in activated sludge.

15. SUBJECT TERMS

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