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Fate of malathion and a phosphonic acid in activated sludge with varying solids retention times

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ABSTRACT

This study examined the ability of activated sludge (AS) to sorb and biodegrade ethylmethylphosphonic acid (EMPA) and malathion, a degradation product and surrogate, respectively, for an organophosphate chemical warfare agent. Sorption equilibrium isotherm experiments indicate that sorption of EMPA and malathion to AS is negligible. EMPA at a concentration of 1 mg L^{-1} degraded by approximately 30% with apparent first-order kinetics, possibly via co-metabolism from nitrification. Heterotrophic bacteria and abiotic mechanisms, however, are largely responsible for malathion degradation also with apparent first-order kinetics. EMPA did not inhibit chemical oxygen demand (COD) oxidation or nitrification activity, although malathion did appear to induce a stress response resulting in inhibition of COD oxidation. The study also included a 30-day experiment in which malathion, at a concentration of 5 mg L^{-1}, was repeatedly fed to AS in bench-scale sequencing batch reactors (SBRs) operating at different solids retention times (SRTs). Peak malathion concentrations occurred at day 4.5, with the longer SRTs yielding greater peak malathion concentrations. The AS reduced the malathion concentrations to nearly zero by day 10 for all SRTs, even when the malathion concentration in the influent increased to 20.8 mg L^{-1}. The data suggest a biodegradation pathway for malathion involving an oxygenase. Phylogenetic analyses revealed that all samples had an abundance of *Zoogloea*, though there was greater bacterial diversity in the SBR with the SRT of 50 days. The SBR with an SRT of 9.5 days had an apparent reduction in the diversity of the bacterial community.

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1. Introduction

Organophosphates (OPs) have frequently been used in agriculture as insecticides. They have also been used as chemical warfare agents (CWAs), specifically nerve agents, since the 1930s (Salem et al., 2008). The toxicity of this class of CWAs is attributed to binding of the compound to the active site of acetylcholinesterase (AChE) and the resulting deactivation of the enzyme (Sidell et al., 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 use, such as the 1925 Geneva Protocol and the current Chemical Weapons Convention (CWC, 2013), a determined organization may yet succeed in obtaining and weaponizing these compounds to kill or terrorize innocent lives. For example, a terrorist group used a nerve agent in the Japanese subway system attack in 1995 (Seto, 2001), and these compounds have recently been used in Syria (Charbonneau and Nichols, 2013).

Should a nerve agent be employed against civilian or military personnel or infrastructure, standard operating procedures typically include decontamination with copious amounts of water, coupled with soap or a hypochlorite bleach solution (USAMRICD, 2007). Some of this water/CWA solution may enter the wastewater collection system and reach a local municipal wastewater treatment plant using activated sludge (AS) for treatment. Although few studies have been conducted to determine the effectiveness of wastewater facilities to degrade these compounds, potential mechanisms of removal (if applicable at a particular facility) could be biological degradation, sorption to sludge, or air stripping (US EPA, 2013). CWA biological degradation in AS could be conducted by either direct metabolism via heterotrophs as a carbon source or by co-metabolism via litho-autotrophic nitrification. The ammonia monooxygenase enzyme involved with nitrification is known to degrade organic compounds via co-metabolism (Ren et al., 2007a; Vader et al., 2000; Shi et al., 2004). Furthermore, because these compounds are typically hydrophobic, they could sorb onto the AS biomass (Bondarenko and Gan, 2004). The OP compounds could either be transformed into less harmful compounds via biodegradation, removed with the waste AS via sorption, or leave with the effluent if not degraded or sorbed. Air stripping is related to the Henry's Law constant of the OP compound of interest. The compounds used in this study have low volatility, making air stripping a negligible removal mechanism relative to the other mechanisms.

VX, the nerve agent of interest for this study, has physical characteristics that make VX of more interest than the G-agent counterparts for some types of CWA release and decontamination scenarios. First, VX has a LD_{50} of 5 mg for a 70 kg person making VX the most lethal of the nerve agents (Lillie et al., 2005). Second, VX is more persistent than G-agents with a hydrolysis rate of 1000 h and a low volatility of 10.5 mg m^{-3} and comparatively lower Henry's constant, making VX the least likely of the nerve agents to enter the vapor form (Munro et al., 1999). Because testing of VX was outside the capacity of our laboratory, one surrogate and one degradation compound were used instead. One compound used in testing was ethylmethylphosphonic acid (EMPA), a comparatively nontoxic hydrolysis product of VX, which will form at a pH of less than 6 or greater than 10 (Munro et al., 1999). Given that decontamination procedures may include the use of bleach, it is reasonable to expect that under those circumstances VX may hydrolyze to EMPA.

Biodegradation of EMPA has been reported for hydrolysates produced during the destruction of the VX stockpile (Irvin et al., 2004), which demonstrated that EMPA degradation occurred "easily". However, specially selected microbial consortiums were utilized, and it is unclear how those consortiums compare with those found in AS from municipal wastewater plants.

The other surrogate compound used was malathion, a commonly used OP insecticide. Malathion was chosen because it has several characteristics similar to VX such as a chemical structure similar to the structure of VX, but malathion is far less toxic and safer to handle (Bartelt-Hunt et al., 2008). Malathion also has an octanol–water partition coefficient close to the partition coefficient of VX, which may yield sorption kinetics between the two compounds that are similar. Like VX, malathion has a low vapor pressure, a low Henry's constant, and a slow hydrolysis rate. Consequently, less degradation will occur via volatilization or hydrolysis, making biodegradation the primary means of removal of the malathion. We note that while both VX and malathion are organophosphate compounds and have similarities, their structures are quite different from each other and may have different biodegradation characteristics. Nevertheless, malathion in its own right is recognized for negative effects on male reproductive systems (Choudhary et al., 2008; Espinoza-Navarro et al., 2005) as well as maternal and fetal exposures when mixed with other organophosphates (Yu et al., 2013). Given its toxicity and that it is commonly found in municipal wastewater influent (Campo et al., 2013), surface waters (Gao et al., 2013), groundwater (Karyab et al., 2013), sediments (Ensminger et al., 2013), and market foods (Wang et al., 2013), evaluating the behavior of malathion in activated sludge has significant merit.

In a wastewater treatment facility, the amount of biodegradation can be related to the solids retention times (SRTs) of the AS. SRT is an important design parameter in the AS process because it controls other process parameters such as effluent water quality and oxygen demand (Wu et al., 2011). A low SRT generally provides higher concentrations of substrates to the AS, causing the AS to be less starved and undergo less endogenous decay while still sustaining acceptable nutrient removal. However, higher SRTs create a more starved environment for the AS, impacting the settleability of the AS as well as the amount of AS that can be produced in the system. Some municipal wastewater treatment facilities may favor a higher SRT to allow an increase in the daily processing volume or to make more bacteria available to degrade excess substrate. 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 purpose of this study was to evaluate the extent to which EMPA and malathion sorb onto AS and biologically degrade in AS batch tests. The study was also designed to