Potential for Biodegradation of Contaminants of Emerging Concern in Stream Systems

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Abstract. Over the last decade, the Emerging Contaminants Project of the U.S. Geological Survey Toxics Substances Hydrology Program has employed novel analytical methods and conducted national-scale surveys in order to assess the environmental occurrence of a number of contaminants of "emerging" concern. In light of the apparent widespread occurrence of these "emerging" contaminants, the U.S. Geological Survey research focus has been extended to include their fate and transport in a variety of environmental settings.

For example, the potential for in situ biodegradation of the endocrine disrupting alkylphenol surfactant compounds, 4-nonylphenols, has been investigated in three, hydrologically-distinct, waste-water treatment plant impacted streams in the United States. Biodegradation of 4-nonylphenol was assessed in water and sediment microcosm mineralization studies using [U-ring-^{14}C] 4-*n*-nonylphenol as a model substrate. Microcosms prepared with sediment collected upstream of the wastewater treatment plant outfalls, and incubated under oxic conditions showed rapid and complete mineralization of [U-ring-^{14}C] 4-*n*-nonylphenol to $^{14}CO_2$ in all three systems. In contrast, no mineralization of [U-ring-^{14}C] 4-*n*-nonylphenol was observed in these sediments under anoxic (methanogenic) conditions. The initial linear rate of [U-ring-^{14}C] 4-*n*-nonylphenol mineralization in sediments from upstream and downstream of the respective outfalls was inversely correlated with the biochemical oxygen demand of the stream bed sediments. These results suggest that waste-water treatment plant procedures that maximize the delivery of dissolved oxygen while minimizing the biochemical oxygen demand in stream receptors, favor efficient biodegradation of 4-NP contaminants in waste-water-impacted stream environments.

Results of this and other U.S. Geological Survey studies indicate that emerging contaminants can undergo significant biodegradation in effluent-impacted surface waters and, consequently, that in situ biodegradation can be an important component of the management of these contaminants in the environment. However, the efficiency and circumstances of biodegradation can vary significantly between stream systems and between compound classes. Likewise, the potentials for *in situ* biodegradation of a large number of emerging contaminant compound classes remain untested. Continued assessment of the potential for emerging contaminant biodegradation is required to develop best management practices for individual surface-water systems and for specific compound classes. A strong understanding of the "assimilative capacity" of surface-water receptors for effluent-derived emerging contaminants will provide a scientific foundation for "up-front technical input" during the development of regulatory contaminant criteria and maximum load polices.

Introduction

The capacity of surface-water systems to assimilate and degrade a wide variety of historical waste-water contaminant compounds is well established. Utilization of this capacity in a manner that protects human health and the environment requires a fundamental understanding of the relevant conditions, rates and extent of assimilation, and degradation in individual surface-water receptors. With respect to the "emerging" waste-water contaminants, which have been detected only recently in surface-water systems as the result of improved environmental analytics, the potential for assimilation and degradation in surface-water receptors is unknown and must be addressed. As part of the U.S. Geological Survey (USGS) Toxic Substances Hydrology Program, the South Carolina Water Science Center in Columbia, SC has been investigating the potential for *in situ* biodegradation of emerging contaminant compounds that are detected in U.S. surface-water systems as a result of residential, industrial, and agricultural waste-water releases. For example, a recent study assessed the potential for *in situ* biodegradation of the endocrine-disrupting detergent compound, 4-nonylphenol (4-NP).

The release of endocrine-disrupting chemicals in waste-water treatment plant (WWTP) effluent poses a threat to the ecology of surface water receptors, due to the effects of these contaminants on the hormonal control, sexual development, and reproductive success of aquatic organisms and wildlife. Among the endocrine-

disrupting chemicals commonly reported in WWTP effluent, alkylphenol contaminants, including 4-NP, exhibit less estrogenic reactivity than effluent-associated steroid hormone contaminants. In contrast to the steroid hormone contaminants, however, alkylphenol contaminants are essentially ubiquitous in WWTP effluent, have been reported at concentrations up to 644 µg/L and have been detected in the majority of investigated surface-water systems. In a 1999 to 2000 reconnaissance of emerging contaminants at 139 US surface-water sites, Kolpin et al. (2002) documented the occurrence of 4-NP at more than half of the sites at concentrations up to 40 µg/L. Dissolved concentrations above 5 µg/L are expected to place a large portion of the aquatic community at risk (Vazquez-Duhalt et al., 2005).

The widespread occurrence of 4-NP in stream systems is attributable to direct releases in WWTP effluents and microbial transformation of effluent-associated nonylphenol ethoxylates to 4-NP in anoxic, surface-water sediments. Short-chain nonylphenol ethoxylates and 4-NP are produced within a WWTP from the incomplete biodegradation of ubiquitous, nonylphenol ethoxylate nonionic surfactants. Due to its hydrophobic character, 4-NP that is released to the stream environment, rapidly and strongly adsorbs to the sediments suspended in the water column and to the bedded sediments. Sediment concentrations of 4-NP are typically highest in the proximity of WWTP outfalls.

Little is known about the potential for or the environmental conditions that may affect the microbial degradation of 4-NP in stream sediments. Consequently, the potential for microbial degradation of 4-NP in stream systems impacted by WWTP effluent was assessed in a series of microcosm studies conducted with the single isomer [U-ring-^{14}C] 4-*n*-NP and sediments collected from three effluent-impacted streams upstream and downstream of the respective WWTP outfalls.

Background

"Contaminants of Emerging Concern," also termed "Emerging Contaminants," is a catch-all phrase that refers to a wide range of contaminant compounds, which occurrence in and potential impact on the environment have long been suspected but only recently validated with the advent of suitably sensitive and reliably quantitative modern analytical capabilities. The US Environmental Protection Agency (EPA) defines "contaminants of emerging concern" as chemicals and other substances that have no regulatory standard, have been recently "discovered" in natural streams (often because of improved analytical chemistry detection levels), and potentially cause deleterious effects in aquatic life at environmentally relevant concentrations. The "contaminants of emerging concern" umbrella covers several broad classes of contaminant compounds that are loosely categorized according to source, original intended use, and/or primary mode of ecological impact and which include: pharmaceuticals and personal care products, organic waste-water compounds, antimicrobials, antibiotics, animal and human hormones, endocrine disrupting compounds, as well as domestic and industrial detergents.

The USGS Toxics Substances Hydrology Program (http://toxics.usgs.gov/regional/emc/index.html) has developed quantitative analytical methods for a range of "emerging contaminants" in a variety of environmental matrices (groundwater, surface water, soil, sediment, sludge, and biosolids) and has conducted nationwide environmental surveys which document the widespread occurrence of these compounds in groundwater and surface-water systems throughout the US (e.g. Kolpin et al., 2002). Owing to the fact that many so-called "emerging contaminants" are anthropogenic compounds selected for use and function in the human population (e.g. human hormones, pharmaceuticals, antibiotics, etc.), the well-publicized documentation of their wide-spread environmental occurrence has prompted a strong public response and increased calls for regulation of the recognized sources of these contaminants. Numerous potential sources of emerging contaminants to the environment have been documented, including: industry and manufacturing, land application of municipal biosolids, landfills and associated leachates, aquaculture operations, domestic septic systems, and municipal and industrial wastewater treatment systems. Among these, however, municipal waste-water treatment plants are perhaps the most publicly recognized and, consequently, are under particular pressure to proactively address the issue of emerging contaminants releases to the environment.

By definition, no regulatory standards or regulatory guidance currently exist for the "contaminants of emerging concern." However, regulation of these contaminants under the Ambient Water Quality Criteria for aquatic life (aquatic life criteria, ALC) as designated in section 304(a) of the United States Clean Water Act (CWA) (33 U.S.C. Sections 1251-1387) has been proposed. Analytical techniques for environmental detection and reliable quantification of the emerging contaminants continue to evolve. Consequently, standardized methods required for routine environmental assessment have not yet been established. Likewise, although the potentially catastrophic ecological and human health threats associated with several classes of emerging contaminant compounds are increasingly apparent (e.g. endocrine disrupting compounds, antibiotics…), the environmental thresholds for such deleterious effects have not been determined, in most cases. In the absence of standardized analytical methods or a clear regulatory guidance, the appropriate approach

to and the consequences of conducting site characterizations for emerging contaminants remain uncertain.

A proactive and practical strategy for addressing the release of emerging contaminant compounds in waste-water effluent is to begin assessing the innate capacity of surface-water receptors to assimilate and attenuate these classes of contaminants. The capacity of surface-water systems to assimilate and degrade a wide variety of historical waste-water contaminant compounds is well established. However, utilization of this capacity in a manner that protects human health and the environment requires a fundamental understanding of the relevant conditions, rates and extent of assimilation, and degradation of the target contaminants in individual surface-water receptors. With respect to the "emerging" waste-water contaminants, the potential for assimilation and degradation of the majority of these compounds in surface-water receptors is unknown and must be addressed in order to develop practicable, cost-efficient, scientifically-defensible, and ecologically-protective management plans.

Approach and Methods

The ability of surface-water microorganisms to degrade [U-ring-^{14}C] 4-*n*-NP was examined in sediment collected from three stream systems: Fourmile Creek near Ankeny (IA), Boulder Creek near Boulder (CO), and the South Platte River near Denver (CO).

Experimental bed sediment microcosms were prepared in quadruplicate and were composed of 10 ml serum vials with 5 ml of saturated sediment and an atmosphere of air (oxic treatments) or nitrogen (anoxic treatments). Triplicate control microcosms were prepared for each sediment and sample location by autoclaving three times for 1 h. All microcosms were amended with approximately 0.027 µCi of [U-ring-^{14}C] 4-*n*-NP to yield initial dissolved concentrations of approximately 0.25 µM (55 µg/L). Microcosms were incubated in the dark at 23 ºC for up to 154 d.

Headspace concentrations of O_2, CH4 and CO_2 as well as $^{14}CH_4$ and $^{14}CO_2$ were monitored by analyzing 0.5 ml of headspace using gas chromatography with thermal conductivity detection and radiometric detection, respectively. The sediment biochemical oxygen demand (BOD) was estimated from the difference in the microcosm oxygen decline between experimental and autoclaved control microcosms over the initial 20 d of incubation.

Results and Discussion

Sediments collected upstream of the WWTP outfall in all three surface-water systems demonstrated complete mineralization of [U-ring-^{14}C] 4-*n*-NP to $^{14}CO_2$ when incubated under oxic conditions. Upstream sediments

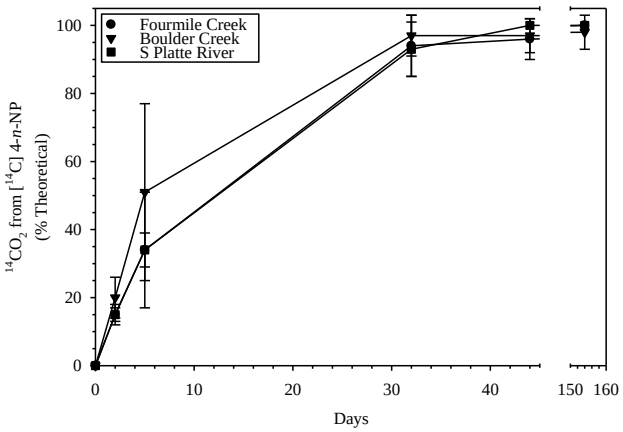

Figure 1. Percentage mineralization of [U-ring-^{14}C] 4-n-NP to $^{14}CO_2$ in oxic microcosms containing sediments collected upstream of the waste-water treatment plant (WWTP) outfalls in Fourmile Creek, Boulder Creek, and the South Platte River.

were used to assess the 4-*n*-NP degradation potential of stream-sediment microbial communities that were not under the immediate influence of waste-water discharge. However, the possibility of anthropogenic impacts existed at the upstream sample locations, because all three stream systems were in or near urban centers. The initial linear rates of $^{14}CO_2$ recovery under oxic conditions ranged from 7±1 up to 10±3 % d^{-1} (percent of theoretical). In all cases, recovery of [U-ring-^{14}C] 4-*n*-NP radioactivity as $^{14}CO_2$ was greater than 90 % of theoretical within 32 d of incubation. The stoichiometric recovery of [U-ring-^{14}C] 4-*n*-NP activity as $^{14}CO_2$ indicated complete ring destruction and the presumptive loss of estrogenicity and toxicity. The aromatic ring and an extensive alkyl backbone are considered essential to the estrogenic character of 4-NP. In this and all other experiments conducted in this study, the recovery of $^{14}CO_2$ observed in experimental treatments was attributed to biological activity, because no recovery of $^{14}CO_2$ was observed in sterilized control microcosms.

In this study, no accumulation of $^{14}CO_2$ was detected (minimum detection limit = 2 % of theoretical) in any of the collected sediments (both upstream and downstream of the WWTP) when incubated under anoxic conditions (data not shown). Under anoxic conditions, the sediments from both locations in all three streams were methanogenic. The apparent lack of anoxic microbial mineralization of [U-ring-^{14}C] 4-*n*-NP in this study compared to the stoichiometric mineralization observed in the same sediments under oxic conditions is consistent with the complete inhibition of 4-NP biodegradation reported previously for anoxic incubation conditions (Razo-Flores et al., 1996; Hesselsoe et al. 2001). These results suggest that factors affecting the availability of oxygen in the bed sediments may substantially influence the efficiency of microbial

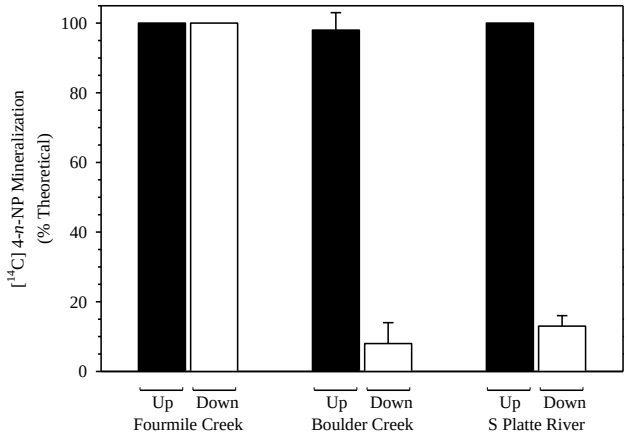

Figure 2. Final recovery of $^{14}CO_2$ from [U-ring-^{14}C] 4-n-nonylphenol (4-n-NP) in oxic microcosms containing sediment upstream (up) and downstream (down) of the WWTP outfalls in Fourmile Creek, Boulder Creek, and S. Platte River.

degradation of 4-NP and, thus, the persistence of 4-NP in effluent-impacted streams.

The efficiency of microbial mineralization of [U-ring-^{14}C] 4-*n*-NP to $^{14}CO_2$ was significantly lower in oxic microcosms prepared with downstream sediments than in oxic microcosms prepared with upstream sediments. The smallest decrease in the efficiency of mineralization between upstream and downstream sediments was observed in Fourmile Creek. Complete recovery of [U-ring-^{14}C] 4-*n*-NP radioactivity as $^{14}CO_2$ was observed in both Fourmile Creek sediment treatments within 154 d of incubation, however, the mean, initial rate of $^{14}CO_2$ production was 70 % lower in downstream sediment microcosms (2.3±0.1 % d^{-1}) than in upstream sediment microcosms (8±2 % d^{-1}). In contrast, the final recoveries of $^{14}CO_2$ in microcosms containing downstream sediment from Boulder Creek and the S. Platte River were only about 10 % of those observed in corresponding upstream microcosms. Further, the mean, initial linear rates of $^{14}CO_2$ production in Boulder Creek and S. Platte River treatments were two orders of magnitude lower in downstream sediment microcosms (circa 0.07 % d^{-1}) than in upstream sediment microcosms (circa 9 % d^{-1}).

The initial rate of [U-ring-^{14}C] 4-*n*-NP mineralization to $^{14}CO_2$ (expressed as % d^{-1}) decreased exponentially with increasing sediment BOD (expressed as the percentage decrease in microcosm oxygen content per day). The statistical relationship between sediment BOD and [U-ring-^{14}C] 4-*n*-NP mineralization was highly significant ($p < 0.01$), with 93 % of the variation in the $\log_{10}$ of the mean, initial rate of [U-ring-^{14}C] 4-*n*-NP mineralization attributable to the variation in BOD in these stream sediments. These microcosm results demonstrated that effluent-associated increases in the sediment BOD have the potential to substantially limit the efficiency of microbial 4-NP degradation, and, consequently, may contribute to the persistence of 4-NP in WWTP-impacted stream systems.

The results of this study indicate that the stability of 4-NP in stream systems, and thus its potential impact on ecosystems, is strongly affected by the supply of dissolved oxygen. Natural or engineered conditions that lead to high dissolved oxygen concentrations in stream-sediment pore waters favor efficient 4-NP biodegradation, and may reasonably be expected to minimize the ecological impacts of 4-NP releases. Conversely, conditions that lead to anoxic conditions in stream bed sediments, such as the release of high-BOD effluents, are not conducive to 4-NP biodegradation and may exacerbate the adverse ecological impacts of this contaminant. In either case, the results of this study suggest that the environmental stability of 4-NP contaminants is sensitive to environmental factors, such as dissolved oxygen status, that are subject to human manipulation.

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