

POTENTIAL FOR 4-*n*-NONYLPHENOL BIODEGRADATION IN STREAM SEDIMENTS

PAUL M. BRADLEY,\*† LARRY B. BARBER,‡ DANA W. KOLPIN,§ PETER B. MCMAHON,|| and FRANCIS H. CHAPPELLE†

†U.S. Geological Survey, 720 Gracern Road, Suite 129, Columbia, South Carolina 29210-7651

‡U.S. Geological Survey, 3215 Marine Street, Boulder, Colorado 80303

§U.S. Geological Survey, 400 S Clinton Street, Iowa City, Iowa 52244

||U.S. Geological Survey, Denver Federal Center, PO Box 25046, Mail Stop 415, Denver, Colorado 80225

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**Abstract**—The potential for in situ biodegradation of 4-nonylphenol (4-NP) was investigated in three hydrologically distinct streams impacted by wastewater treatment plants (WWTPs) in the United States. Microcosms were prepared with sediments from each site and amended with [U-ring-<sup>14</sup>C]4-*n*-nonylphenol (4-*n*-NP) as a model test substrate. Microcosms prepared with sediment collected upstream of the WWTP outfalls and incubated under oxic conditions showed rapid and complete mineralization of [U-ring-<sup>14</sup>C]4-*n*-NP to <sup>14</sup>CO<sub>2</sub> in all three systems. In contrast, no mineralization of [U-ring-<sup>14</sup>C]4-*n*-NP was observed in these sediments under anoxic (methanogenic) conditions. The initial linear rate of [U-ring-<sup>14</sup>C]4-*n*-NP mineralization in sediments from upstream and downstream of the respective WWTP outfalls was inversely correlated with the biochemical oxygen demand (BOD) of the streambed sediments. These results suggest that the net supply of dissolved oxygen to streambed sediments is a key determinant of the rate and extent of 4-NP biodegradation in stream systems. In the stream systems considered by the present study, dissolved oxygen concentrations in the overlying water column (8–10 mg/L) and in the bed sediment pore water (1–3 mg/L at a depth of 10 cm below the sediment–water interface) were consistent with active in situ 4-NP biodegradation. These results suggest WWTP procedures that maximize the delivery of dissolved oxygen while minimizing the release of BOD to stream receptors favor efficient biodegradation of 4-NP contaminants in wastewater-impacted stream environments.

**Keywords**—Biodegradation Endocrine disruption Nonylphenol Oxic Streams

## INTRODUCTION

The release of endocrine-disrupting chemicals in wastewater treatment plant (WWTP) effluent poses a threat to the ecology of surface-water receptors because of the effects of these contaminants on the hormonal control, sexual development, and reproductive success of aquatic organisms and wildlife [1]. Endocrine-disrupting chemicals commonly reported in WWTP effluent include natural estrogens (e.g., estradiol and estrone), synthetic estrogens (e.g., ethynylestradiol), and the surfactant degradation product 4-nonylphenol (4-NP). The estrogens demonstrate a high degree of endocrine reactivity both in vitro and in vivo [2–4], but they typically are found in WWTP effluent at low concentrations (<10 ng/L) [1] and are detected infrequently (frequency of detection, generally <20%) in surface-water receptors [5–7]. In contrast, alkylphenol contaminants, including 4-NP, exhibit less estrogenic reactivity [2–4] but are essentially ubiquitous in WWTP effluent [1,6], have been reported at concentrations up to 644 µg/L [8,9], and have been detected in the majority of investigated surface-water systems [5–7]. The U.S. Environmental Protection Agency (EPA) no-observable-effect concentration for the estrogenic effects of NP in freshwater systems is 8.2 µg/L [10]. Nonylphenol-based compounds are the primary alkylphenol contaminants detected in WWTP-impacted stream systems [6], because nonylphenol ethoxylates constitute ap-

proximately 82% of the world production of alkylphenol ethoxylate [1].

Understanding the fate of 4-NP in stream systems is an environmental priority not only because of its demonstrated endocrine-disrupting character but also because of its innate toxicity, bioaccumulation potential, widespread occurrence, and apparent persistence in wastewater-impacted streams [1,3,4,6,9–17] (M.A. Bertin, 2004, Master's thesis, University of Cincinnati, Cincinnati, OH, USA). For example, in a 1999-to-2000 reconnaissance of emerging contaminants at 139 U.S. surface-water sites, Kolpin et al. [6] documented the occurrence of 4-NP at more than half the sites at concentrations up to 40 µg/L. Dissolved concentrations greater than 5 µg/L are expected to place a large portion of the aquatic community at risk [17]. The U.S. EPA water-quality criteria for 4-NP in freshwater systems include a final acute value for toxicity of 55.7 µg/L and a chronic toxicity no-observable-effect concentration of 7.4 µg/L [10]. 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 [17]. Short-chain nonylphenol ethoxylates and 4-NP are produced within a WWTP from the incomplete biodegradation of ubiquitous, nonylphenol ethoxylate nonionic surfactants [17]. Because of 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 [9,17]. Sediment concentrations of 4-NP typically are highest in the proximity of WWTP outfalls [12–14,18].

Little is known about the potential for, or the environmental

\* To whom correspondence may be addressed (pbradley@usgs.gov).

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conditions that may affect, the microbial degradation of 4-NP in stream sediments. Reports of significant biodegradation of 4-NP under oxic conditions in seawater and sediment [19], in soil [20], in cultures derived from sewage sludge [21–25], and in river sediments in Taiwan [26] suggest that biodegradation of 4-NP by stream-sediment microorganisms may be substantial. A number of studies concluding that 4-NP generally is recalcitrant in soil and sediments [9,13,16], however, suggest that efficient 4-NP biodegradation is not ubiquitous and is dependent on favorable environmental conditions. The efficiency of 4-NP biodegradation in sludge aggregate, soil, and sediment appears to be particularly sensitive to extant oxygen conditions, with complete inhibition of 4-NP biodegradation reported to occur under anoxic incubation conditions [15,23]. Extrapolating these findings to stream systems suggests that the elevated sediment oxygen demand generally associated with organic-enriched WWTP effluent may substantially limit the potential for 4-NP biodegradation in the sediments of wastewater-impacted streams.

The purpose of the present study was to assess the potential for microbial degradation of 4-NP in stream systems impacted by WWTP effluent. A series of microcosm studies was conducted with a single isomer, [U-ring- $^{14}\text{C}$ ]-radiolabeled 4-*n*-nonylphenol (4-*n*-NP), and sediments collected from three effluent-impacted streams upstream and downstream of WWTP outfalls to assess the ability of stream-sediment microorganisms to mineralize 4-*n*-NP, the impact of water column and sediment oxygen availability on the relative efficiency of 4-*n*-NP biodegradation in stream sediments, and the impact of WWTP effluent-associated increases in oxygen demand on 4-*n*-NP biodegradation in wastewater-impacted streams.

## MATERIAL AND METHODS

### Chemicals

In theory, technical 4-NP presents more than 200 isomeric forms, which differ in the degree of branching of the nonyl group [27]. More than 22 isomers have been identified in the commercial 4-NP technical mixture, an intermediate in the production of the nonylphenol ethoxylate surfactants that are employed worldwide in domestic and industrial detergents [28]. Thus, the 4-NP produced in WWTPs and found in the environment as the result of microbial degradation of waste nonylphenol ethoxylate surfactants is expected to exhibit an isomeric complexity similar to that of the 4-NP technical mixture. The estrogenicity of alkylphenol compounds reportedly varies by a factor of 10 depending on the structure of the alkyl chain, with increased branching in the benzylic position being associated with increased estrogenicity [29,30]. Likewise, the biodegradability of 4-NP is reported to decrease with increasing nonyl group branching [21,31,32]. In contrast, efficient biodegradation has been reported for the linear-chain isomer (i.e., 4-*n*-NP) [21,31,32].

In light of the expected isomeric complexity (and the putative range in biodegradability and estrogenicity) of 4-NP contaminants in effluent-impacted stream systems, the present study focused on the potential for biodegradation of the isomer reported to be the most easily biodegraded, 4-*n*-NP, to approximate an upper limit for 4-NP biodegradation in stream environments and to assess the importance of sediment oxygen availability and sediment oxygen demand on the potential biodegradation of 4-NP. The potential for in situ biodegradation of 4-*n*-NP contaminants was investigated using an aqueous solution of uniformly ring-labeled [U-ring- $^{14}\text{C}$ ]-4-*n*-NP (52

$\mu\text{Ci}/\mu\text{mol}$ ; American Radiolabeled Chemicals, St. Louis, MO, USA). The radiochemical purity of the [U-ring- $^{14}\text{C}$ ]-4-*n*-NP was determined, using radiometric detection high-performance liquid chromatography, to be greater than 99%. Both  $\text{H}^{14}\text{CO}_3^-$  (Sigma Biochemicals, St. Louis, MO, USA) and  $^{14}\text{CH}_4$  (PerkinElmer Life Sciences, Boston, MA, USA) were used as radiolabeled standards for calibration and methods development. Both had radiochemical purities of greater than 98%.

### Study sites

The ability of surface-water microorganisms to degrade [U-ring- $^{14}\text{C}$ ]-4-*n*-NP was examined in sediment collected from three stream systems described previously [33]: Fourmile Creek near Ankeny (IA, USA) [34], Boulder Creek near Boulder (CO, USA) [35], and South Platte River near Denver (CO, USA) [36,37]. Sediments consisting of fine grain sands were collected from Fourmile Creek at locations approximately 30 m upstream and 30 m downstream of the Ankeny WWTP outfall. Sediments consisting of coarse sands and gravels were collected from Boulder Creek approximately 200 m upstream and 20 m downstream of the Boulder 75th Street WWTP outfall. South Platte River bed sediments consisting of coarse sands and gravels were collected from locations approximately 100 m upstream and 100 m downstream of the Metro Wastewater Reclamation District outfall downstream of Denver.

### Microcosm studies

Experimental bed sediment microcosms were prepared in quadruplicate as described previously [33,38,39] 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 each time. All microcosms were amended with approximately 0.027  $\mu\text{Ci}$  of [U-ring- $^{14}\text{C}$ ]-4-*n*-NP to yield initial dissolved concentrations of approximately 0.25  $\mu\text{M}$  (55  $\mu\text{g}/\text{L}$ ). Microcosms were incubated in the dark at 23°C for up to 154 d. Microcosms were incubated under static conditions to assess the impacts of sediment oxygen supply and sediment oxygen demand on the efficiency of 4-*n*-NP mineralization in stream sediments.

### Microcosm analytical methods

Headspace concentrations of  $\text{CH}_4$  and  $\text{CO}_2$  as well as of  $^{14}\text{CH}_4$  and  $^{14}\text{CO}_2$  were monitored by analyzing 0.5 ml of headspace using gas chromatography with thermal conductivity detection and radiometric detection, respectively. Compound separation was achieved by isocratic (80°C), packed-column (3 m of 13 $\times$  molecular sieve) gas chromatography using a Hewlett-Packard 5890 Series II Plus GC (Agilent Technologies, Santa Clara, CA, USA). The headspace sample volumes were replaced with pure oxygen (oxic treatments) or nitrogen (anoxic treatments). Dissolved-phase concentrations of  $^{14}\text{CH}_4$  and  $^{14}\text{CO}_2$  were estimated based on Henry's law partition coefficients that were determined experimentally as described previously in detail [33,38,39]. The radiometric detector output was calibrated by liquid scintillation counting using  $\text{H}^{14}\text{CO}_3^-$ . To confirm the maintenance of oxic conditions (headspace  $[\text{O}_2]$ , 2–21% by volume) in oxic treatments or the absence of oxygen (headspace  $[\text{O}_2]$  minimum detection limit, 0.2 ppt by volume or 10  $\mu\text{mol}/\text{L}$ ) in anoxic treatments, headspace concentrations of  $\text{O}_2$  were monitored throughout the present study using gas chromatography with thermal conductivity detection.

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.

### Stream oxygen analyses

A one-time assessment of in-stream oxygen concentration profiles was performed in all three systems to provide an environmental context for the interpretation of the microcosm results and to assess the extent to which routine WWTP operational procedures supply oxygen to the downstream water column and sediments. Water-column and bed sediment oxygen concentrations were measured at two transects upstream and two to four transects downstream of the WWTP outfalls in each stream system. Each transect included four water-column and four sediment measurements spaced evenly across the stream, oriented perpendicular to stream flow, and beginning and ending 2 to 3 m from the stream banks.

Water-column dissolved oxygen concentrations were measured using a Model 556 YSI multiparameter instrument (YSI, Yellow Springs, OH, USA) that was corrected for barometric pressure and calibrated to water-saturated air immediately before oxygen profiling. For all three stream systems, the depth of the water column within the study reach ranged from 10 to 75 cm. No significant variation in dissolved oxygen concentration was observed with depth in the water column. The dissolved oxygen concentration of the bed sediment interstitial water was assessed using commercially available colorimetric vacuum ampoules (Chemetrics, Calverton, VA, USA) based on rhodazine D chemistry for the quantification of dissolved oxygen in the range of 0.05 to 1.0 mg/L and on indigo carmine chemistry for the quantification of dissolved oxygen in the range of 1 to 12 mg/L. Ampoules were inserted into the bed sediment to a depth of 10 cm below the sediment–water interface, equilibrated for 1 min, and filled with interstitial water (sample volume, ~1 ml) by breaking the point of the ampoule against the underlying substrate. Sediment oxygen concentrations were determined by visual comparison with colorimetric standards provided in the test kits. Oxygen concentration estimates obtained using the different analytical methods were compared in duplicate water-column measurements at three locations in each system and found to be equivalent.

## RESULTS AND DISCUSSION

### Mineralization under oxic conditions

Sediments collected upstream of the WWTP outfall in all three surface-water systems demonstrated complete mineralization of [U-ring- $^{14}\text{C}$ ]4-*n*-NP to  $^{14}\text{CO}_2$  when incubated under oxic conditions (Fig. 1). Upstream sediments were used to assess the 4-*n*-NP degradation potential of stream sediment microbial communities that were not under the immediate influence of wastewater discharge. 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  $^{14}\text{CO}_2$  recovery under oxic conditions ranged from  $7\% \pm 1\%$  to  $10\% \pm 3\%/d$  (percentage of theoretical). In all cases, recovery of [U-ring- $^{14}\text{C}$ ]4-*n*-NP radioactivity as  $^{14}\text{CO}_2$  was greater than 90% of theoretical within 32 d of incubation. The stoichiometric recovery of [U-ring- $^{14}\text{C}$ ]4-*n*-NP activity as  $^{14}\text{CO}_2$  indicated complete ring destruction and the presumptive loss of estrogenicity and toxicity. The aromatic ring and an extensive alkyl backbone are con-

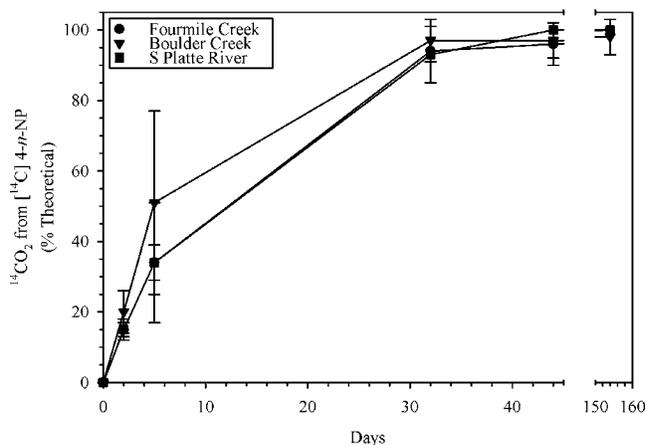


Fig. 1. Percentage mineralization of [U-ring- $^{14}\text{C}$ ]4-*n*-nonylphenol (4-*n*-NP) to  $^{14}\text{CO}_2$  in oxic microcosms containing sediments collected upstream of the wastewater treatment plant outfalls in Fourmile Creek (Ankeny, IA, USA), Boulder Creek (Boulder, CO, USA), and South Platte River (Denver, CO, USA). Data are presented as the mean  $\pm$  standard deviation for quadruplicate experimental microcosms. No significant recovery of  $^{14}\text{CO}_2$  or  $^{14}\text{CH}_4$  was observed in autoclaved or sediment-free control microcosms.

sidered to be essential to the estrogenic character of 4-NP [1,10,17]. In this and all other experiments conducted during the present study, the recovery of  $^{14}\text{CO}_2$  observed in experimental treatments was attributed to biological activity, because no recovery of  $^{14}\text{CO}_2$  was observed in sterilized control microcosms.

The results of the present study are consistent with previous reports of significant biodegradation of 4-NP under oxic conditions in seawater and sediment [19], in soil [20], and in cultures derived from sewage sludge [21–25]. Significant biodegradation of 4-NP was reported in sediments collected from a river in Taiwan and incubated under oxic conditions [26]. The fact that efficient oxic microbial degradation of 4-*n*-NP has been observed in river sediments from such geographically separate sites [26] (present study) suggests that the potential for aerobic 4-*n*-NP biodegradation may be widespread in stream systems.

### No mineralization under anoxic conditions

In the present study, no accumulation of  $^{14}\text{CO}_2$  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- $^{14}\text{C}$ ]4-*n*-NP in the present study compared to the stoichiometric mineralization observed in the same sediments under oxic conditions is consistent with the complete inhibition of 4-NP biodegradation as reported previously for anoxic incubation conditions [15,23]. These results suggest that factors affecting the availability of oxygen in the bed sediments may substantially influence the efficiency of microbial degradation of 4-NP and, thus, the persistence of 4-NP in effluent-impacted streams. The availability of oxygen in streambed sediments reflects the dynamic balance between sediment processes that consume oxygen (primarily the biological and chemical oxygen demand of the sediment) and processes that supply oxygen to the sediment environment (primarily the infiltration and circulation

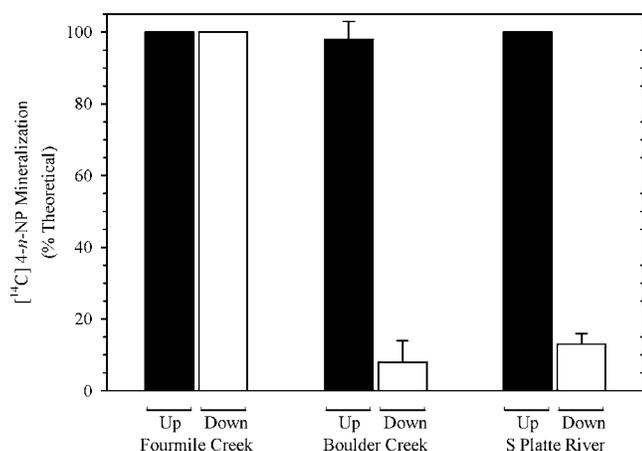


Fig. 2. Final recovery of  $^{14}\text{CO}_2$  from [U-ring- $^{14}\text{C}$ ]4-*n*-nonylphenol (4-*n*-NP) in oxic microcosms containing sediment upstream (up) and downstream (down) of the wastewater treatment plant outfalls in Fourmile Creek (Ankeny, IA, USA), Boulder Creek (Boulder, CO, USA), and South Platte River (Denver, CO, USA). Data are reported as the mean  $\pm$  standard deviation for quadruplicate experimental microcosms after 154 d of incubation. No significant recovery of  $^{14}\text{CO}_2$  or  $^{14}\text{CH}_4$  was observed in autoclaved or sediment-free control microcosms.

of oxygenated water from the overlying water column and/or from the discharge of oxygenated groundwater).

#### Impact of effluent-associated BOD on 4-*n*-NP mineralization

To assess the potential impact of effluent-associated loading of organic material on the sediment oxygen demand and, consequently, on the efficiency of 4-*n*-NP biodegradation in stream sediments, the potential for oxic microbial mineralization of [U-ring- $^{14}\text{C}$ ]4-*n*-NP was compared between sediments collected upstream and downstream of the WWTP outfalls in each system. The static conditions, under which all treatments in the present study were incubated, effectively limited the mechanism of oxygen supply to diffusion and exaggerated the potential impact of sediment BOD on oxygen availability. Thus, the sensitivity of the relationship between rates of [U-ring- $^{14}\text{C}$ ]4-*n*-NP mineralization in sediments and the sediment BOD could be readily assessed.

The efficiency of microbial mineralization of [U-ring- $^{14}\text{C}$ ]4-*n*-NP to  $^{14}\text{CO}_2$  under static conditions was significantly lower in oxic microcosms prepared with downstream sediments than in oxic microcosms prepared with upstream sediments (Fig. 2). The smallest decrease in the efficiency of mineralization between upstream and downstream sediments was observed in Fourmile Creek. Complete recovery of [U-ring- $^{14}\text{C}$ ]4-*n*-NP radioactivity as  $^{14}\text{CO}_2$  was observed in both Fourmile Creek sediment treatments within 154 d of incubation; however, the mean initial linear rate of  $^{14}\text{CO}_2$  production was 70% lower in downstream sediment microcosms ( $2.3\% \pm 0.1\%/d$ ) than in upstream sediment microcosms ( $8\% \pm 2\%/d$ ). In contrast, the final recoveries of  $^{14}\text{CO}_2$  in microcosms containing downstream sediment from Boulder Creek and the South Platte River were only approximately 10% of those observed in corresponding upstream microcosms. Furthermore, the mean initial linear rates of  $^{14}\text{CO}_2$  production in Boulder Creek and South Platte River treatments were two orders of magnitude lower in downstream sediment microcosms ( $\sim 0.07\%/d$ ) than in upstream sediment microcosms ( $\sim 9\%/d$ ). These results suggest

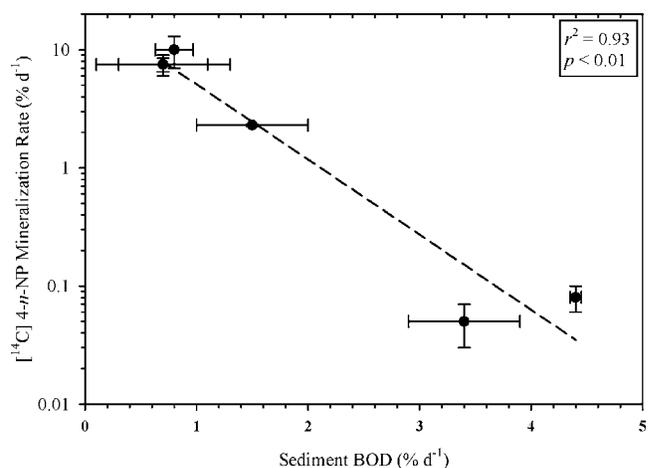


Fig. 3. Correlation between the initial linear rate of mineralization of [U-ring- $^{14}\text{C}$ ]4-*n*-nonylphenol (4-*n*-NP) to  $^{14}\text{CO}_2$  (%/d) and the sediment biochemical oxygen demand (BOD; %/d) for sediments collected from Fourmile Creek (Ankeny, IA, USA), Boulder Creek (Boulder, CO, USA), and South Platte River (Denver, CO, USA). Data are presented as the mean  $\pm$  standard deviation for quadruplicate experimental microcosms.

that the bed sediment biogeochemical changes associated with WWTP effluent loading may potentially limit the efficiency of 4-*n*-NP biodegradation in all three stream systems.

The decreasing efficiency of 4-*n*-NP mineralization observed in downstream sediments corresponded to significant increases in sediment BOD downstream of the WWTP outfall in each stream system (Fig. 3). The mean initial linear rate of [U-ring- $^{14}\text{C}$ ]4-*n*-NP mineralization to  $^{14}\text{CO}_2$  (expressed as the percentage per day) 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- $^{14}\text{C}$ ]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- $^{14}\text{C}$ ]4-*n*-NP mineralization being 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-*n*-NP degradation and, consequently, may contribute to the persistence of 4-*n*-NP in WWTP-impacted stream systems.

#### In situ oxygen supply

The results of the microcosm study suggest that a sustained supply of oxygen to the downstream sediment environment is necessary to ensure efficient in situ biodegradation of 4-*n*-NP in WWTP-impacted stream systems. For experimental purposes, the microcosm studies were conducted under static conditions designed to minimize the advective oxygen supply and maximize the potential impact of the sediment BOD on 4-*n*-NP biodegradation. The potential negative effects of effluent-associated sediment BOD on the biodegradation of 4-*n*-NP may be prevented, however, if WWTP operational procedures are sufficient to maintain an oxygen-saturated water column and if the infiltration and recirculation of oxygenated surface water is sufficient to offset the sediment BOD and maintain oxic conditions in the shallow bed sediment environment.

The hydrogeology of the three study reaches suggests that the infiltration of oxygenated surface water (and, thus, the occurrence of oxic conditions) is extensive in the shallow bed

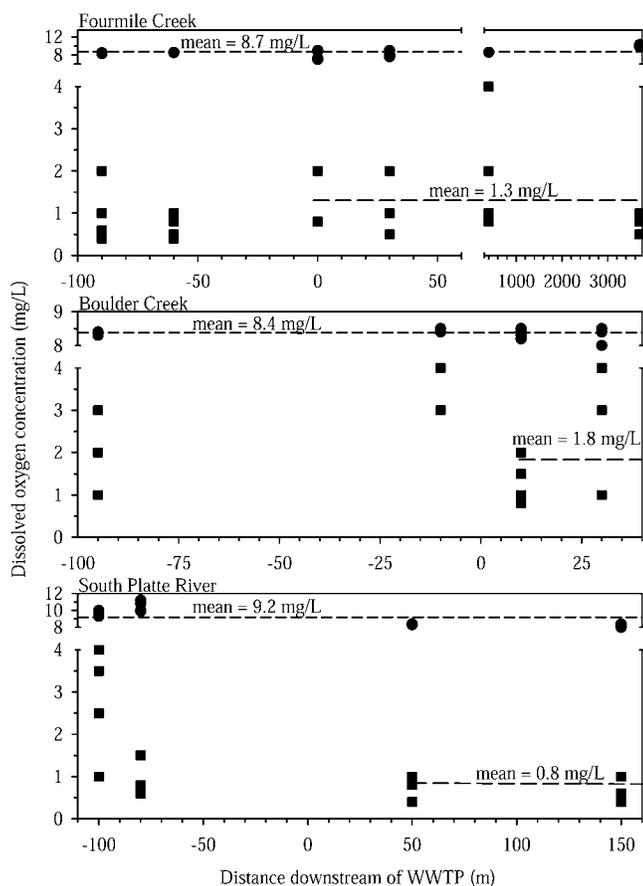


Fig. 4. Distribution of dissolved oxygen concentrations (mg/L) observed in the water column (●) and in bed sediments at a depth of 10 cm below the sediment–water interface (■) in Fourmile Creek (Ankeny, IA, USA), Boulder Creek (Boulder, CO, USA), and South Platte River (Denver, CO, USA). Dashed lines denote the mean dissolved oxygen concentration observed in the water column or in the sediment for the indicated range of sample locations. A downstream distance of zero indicates the location of the wastewater treatment plant (WWTP) outfall.

sediment environment. The bed sediments in all three systems consist of coarse-grained deposits that facilitate the infiltration and recirculation of oxygenated surface water. In all three systems, WWTP effluent substantially increases downstream flow, with from 20% to greater than 90% of downstream flow being attributable to effluent discharge. Consequently, in each of these systems, WWTP discharge creates a localized increase in the hydraulic head and a vertical gradient from the water column into the sediment environment immediately downstream of the WWTP outfall. This vertical gradient may reasonably be expected to drive infiltration of oxygenated surface water into the coarse-grained bed sediments and, consequently, to promote the development and maintenance of an oxic bed sediment environment capable of supporting efficient microbial mineralization of 4-NP.

In situ dissolved oxygen concentration data from all three study systems confirmed that routine WWTP effluent oxygenation procedures were sufficient to maintain dissolved oxygen levels in the water column downstream of the respective WWTP outfalls at concentrations near or above saturation (Fig. 4). The mean water-column dissolved oxygen concentrations in Fourmile Creek and Boulder Creek were  $8.7 \pm 0.8$  and  $8.4 \pm 0.1$  mg/L, respectively, and did not vary significantly along the study reach. A statistically significant downstream change

in water-column dissolved oxygen concentrations was observed in the South Platte River. Water-column dissolved oxygen concentrations in the South Platte River study reach during the sampling period decreased by 17% from a supersaturated mean concentration of  $10 \pm 1$  mg/L upstream to a mean of  $8.3 \pm 0.1$  mg/L downstream of the WWTP outfall. In all cases, the dissolved oxygen concentrations in the water column of each stream remained high and, thus, represented a substantial potential source of oxygen to the bed sediment environment.

Dissolved oxygen also was detected in the bed sediments at a depth of 10 cm below the sediment–water interface at all sediment sampling locations examined in the present study (Fig. 4). Sediment interstitial dissolved oxygen concentrations at a depth of 10 cm ranged from 0.4 to 4 mg/L. In the Fourmile Creek and Boulder Creek study reaches, the mean dissolved oxygen concentrations observed in downstream sediments at a depth of 10 cm were  $1.3 \pm 0.9$  and  $1.8 \pm 1.1$  mg/L, respectively, and did not differ significantly from the dissolved oxygen concentrations observed in upstream sediments. Likewise, interstitial dissolved oxygen concentrations observed at a depth of 10 cm in downstream South Platte River sediments averaged  $0.8 \pm 0.3$  mg/L and did not differ significantly from the mean concentration of  $1.1 \pm 0.5$  mg/L observed in stream-bed sediments 80 m upstream of the WWTP outfall. Sediments located 100 m upstream of the Denver WWTP outfall were characterized by substantially larger grain size (small cobbles and gravels rather than the coarse-grained sands observed farther downstream) and interstitial dissolved oxygen concentrations of  $2.8 \pm 1.3$  mg/L at a depth of 10 cm below the sediment–water interface. The dissolved oxygen concentrations observed in South Platte River sediments in the present study are consistent with a previous report of interstitial dissolved oxygen concentrations up to 1.7 mg/L at a depth of 30 cm below the sediment–water interface along a South Platte River study reach that extended from 300 m upstream to 40 km downstream of the Metro WWTP outfall [37]. The sediment pore-water oxygen concentrations observed in the present study indicated that the combination of WWTP treatment processes and effluent oxygenation practices, the substantial impact of WWTP discharge on downstream flows and hydraulic gradients, and the hydrogeology of the bed sediment environments were sufficient to maintain oxic conditions in the downstream bed sediments of all three stream systems.

#### Implications for WWTP-impacted stream systems

The release of nonylphenol-based contaminants to surface-water systems as the result of WWTP operations may affect the hormonal control, sexual development, and reproductive success of aquatic organisms and wildlife [1]. The results of the present 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 streambed 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 the present study suggest that the environmental stability of 4-NP contaminants is sensitive to environmental fac-

tors, such as dissolved oxygen status, that are subject to human manipulation. For experimental reasons, the present study was conducted with the labile 4-*n*-NP isomer. By comparison, the branched isomers of 4-NP are expected to exhibit lower degradation rates, greater environmental recalcitrance, and possibly, greater sensitivity to sediment interstitial oxygen conditions.

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