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Biodegradation Residual of 4-Octylphenoxyacetic Acid in Laboratory Columns under Groundwater Recharge Conditions

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The biodegradation of 4-octylphenoxyacetic acid (OP1EC) was studied in laboratory columns to determine the residual concentration that can persist during groundwater recharge or transport. Biofilm models predict residual concentrations are independent of the initial concentration and residence time. Two column trains, constructed with glass beads and providing a residence time of 280 min were fed continuously with lake water (LW) containing biodegradable dissolved organic carbon (DOC) and groundwater (GW) containing no biodegradable DOC. The feedwater was amended with OP1EC first at high (1 mg/L) and then at low (50 μg L^{-1}) concentration. To simulate mixing of recharged LW and regional GW, the effluents of the GW and LW train were blended and fed to a column (BW) with 114 min residence time. When the influent OP1EC concentration was 1000 μg/L the residual concentrations in the LW and GW trains ranged from 0.3 to 3 and 0.8 to 3 μg L^{-1}, respectively. When the feed concentrations were decreased to 50 μg L^{-1}, the residual concentration in the LW decreased to below the detection limit (<0.1 μg L^{-1}) but stayed above 0.2 μg L^{-1} in the GW train effluent. Mixing the two LW and GW column effluents stimulated additional (up to 11%) dissolved organic carbon (DOC) removal but no additional OP1EC degradation.

Introduction

The practice of replenishing potable groundwater supplies by artificially recharging aquifers with treated wastewater is relatively new and associated with significant scientific and regulatory uncertainty, especially with regard to residual organic contaminants. Residuals of some organic contaminants, such as alkylphenol polyethoxycarboxylate (APEC) compounds, can persist at or below the μg/L level during groundwater recharge (*1*−*3*) even though these compounds have been reported to be biodegradable in batch tests (*4*). Persistence of contaminant residuals could be important to consider when designing and regulating water reuse operations and minimizing ecological impacts. Endocrine disrupting substances, for example, can be active at levels that are significantly below the maximum contaminant levels specified for most other regulated substances and 3−6 orders of magnitude lower than those associated with classic toxic effects (*5, 6*).

In this study, we investigated the biodegradation residual of OP1EC (4-($CH_3C(CH_3)_2CH_2C(CH_3)_2$)$C_6H_4OCH_2COOH$) under simulated groundwater recharge conditions. OP1EC was used as a model compound for APECs (octyl and nonyl) because these compounds are frequently detected in wastewater effluents and effluent-impacted surface and groundwaters (*3, 7*−*9*). APECs are relatively refractory biological metabolites of alkylphenol polyethoxylates (APEs), a class of nonionic surfactants (*4, 10*). APECs and APEs are suspected endocrine disrupting substances and are of potential ecotoxicological significance (*11*−*13*).

Biodegradation residuals are not observed in routine batch tests that typically use high (mg/L) initial concentrations. Therefore, data of such tests are difficult to extrapolate to environmental conditions that are characterized by low concentrations (μg/L or lower) (*14*). The minimum substrate concentration threshold below which biodegradation does not occur is sometimes referred to as S_{min} (*15*). At or below S_{min} no net growth of organisms (growth minus decay processes) occurs because the available substrate just compensates for the losses due to maintenance and endogenous respiration. S_{min} has been found to be in the range of 1−100 μg L^{-1} for pure cultures with single-carbon substrates (*16*).

Although simple in concept, it is recognized that S_{min} determinations can depend on many factors. For example, under low substrate conditions, microorganisms can reduce their metabolic activity and survive by assuming a dormant state (*17*); no substrate is utilized under these conditions. Alternatively, microorganisms can adapt by utilizing the available energy more efficiently, thereby lowering S_{min} (*16, 18, 19*) or by inducing enzymes for concurrent utilization of two or more substrates. Concurrent utilization is in contrast to the sequential degradation (diauxic growth) that is typically observed at high substrate concentrations (*20*−*24*). By utilizing two or more substrates concurrently, organisms can grow even if individual substrate concentrations are below S_{min} (*16, 25, 26*).

The objective of this study was to quantify the biodegradation residual of OP1EC in laboratory columns under groundwater recharge and transport conditions and to test whether the observed concentrations are comparable with those observed in field studies (*1, 2, 27*). The experiment was designed to evaluate high and low substrate concentrations (1000 and 50 μg L^{-1}, respectively), residence times ranging from 3 min to 1 month, the presence or absence of biodegradable DOC, and mixing of recharge water with regional groundwater. Assuming that only attached biomass contributes to biotransformation biofilm models predict that residual concentrations are independent of the initial concentration and residence time (*15*). Lower residuals would be expected if the biodegradable DOC supports growth of bacteria that fortuitously degrade (cometabolize) OP1EC. Residual concentrations could be affected by nutrient limitations. To test whether blending of LW and GW provides a better growth medium than either water individually, the LW and GW effluents were blended and fed to the blended-water column (BW) with 114 min residence time. The effluent of BW was analyzed for additional OP1EC and DOC removal.

Experimental Design and Analytical Methods

Experimental Design. Anaheim LW and GW from an adjacent well were augmented with OP1EC and fed to two identical trains of five columns. The column specifications are given in Table 1. The five columns were packed with 1, 2, or 4 mm glass beads to support biofilm development (Figure 1) and in combination provided a total residence time of 285 min. The effluents of LW5 and GW5 were blended and fed to the BW5 column with a residence time of 114 min. Of the LW5,

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TABLE 1. Characteristics of Individual Columns

column	∅ cm	*L* mm	packing ∅ mm	porosity %	$\bar{t}$[a] min	$D/(\mu L) \times 10^{-3}$
1	1.0	79	1	46	3.4	12
2	1.0	156	1	48	6.4	6.4
3	1.0	276	1	49	9.9	3.2
4	2.5	169	2	35	37.3	2.6
5[b]	4.8	299	4	41	225	0.0.9
6 (reactor)	10	150	R-rings[c]	77	1 mo	n/a

[a] Mean residence times at flow rates: 1 mL/min in sectors LW and GW 1−5, and 25 mL/day in the 1-month reactors 6. [b] Except for BW5 which was 126 at 2 mL/min. [c] Raschig rings (10 mm).

GW5, and BW5 effluents, a total of 25 mL d^{-1} was diverted and fed to 1-L bottle reactors (LW6, GW6, and BW6, respectively), using a timer-controlled magnetic valve. The 1-L bottle reactors were filled with 10-mm Raschig rings and exhibited flow characteristics of a continuously stirred tank reactor (CSTR) with a mean detention time of 1 month.

Anaheim Lake is a percolation basin located adjacent to the Santa Ana River (Orange County, CA); it is 10−15 m deep and was used as a sand and gravel quarry. Anaheim Lake is filled primarily with Santa Ana River water but occasionally with surface water from other sources (California State Project or Colorado River water). The Santa Ana River flow contains seasonally variable portions of storm runoff and tertiary treated municipal effluent (up to 100% during the dry season). Storm runoff is captured by an upstream reservoir and released slowly during winter and spring for maximizing groundwater recharge. Storm runoff and Prado Dam effluent is generally higher in DOC (as high as 10 mg/L) than the tertiary treated effluents (2−5 mg/L) (*28*).

For the groundwater train, waters from three different wells (W1, W2, and W3) were tested. W1 and W2 wasters were characterized by relatively high TDS (805 and 704 mg/L, respectively) and calcium concentrations (129 and 104 mg/L, respectively). Using W1 and W2 water led to precipitation and clogging problems at the inlet of the high pH OP1EC feed. From day 48 forward water from well W3 was used and clogging stopped. Water was collected monthly by Orange County Water District personnel in high-density polyethylene containers and shipped overnight to Stanford University. The water was filtered using 25 μm disposable, prerinsed Nylon filters, and stored in 20 l serum bottles that were immersed in 4 °C waterbaths. Peristaltic pumps delivered feedwater through an inline membrane filter (12 μm, polycarbonate) to the two column trains at a flow rate of 1 mL min^{-1}. The OP1EC influent concentration was 2000 $\mu g\ L^{-1}$, initially for 15 days, then 1000 $\mu g\ L^{-1}$ for 4 months, and finally 50 $\mu g\ L^{-1}$ for 4 months. The 1000 and 50 μg/L OP1EC feed concentrations were obtained by adding a 20 l and 20.1 mg/L OP1EC solution at a rate of 0.3 and 0.15 mL/h, respectively, to the 1 mL/min LW- or GW-flow. The feedwater warmed to ambient temperature (20−22 °C) by the time it reached the inlet of the column trains. At the inlet, dissolved oxygen (DO) was present near saturation levels.

All parts downstream of the OP1EC addition including tubing, fittings, columns, the removable column caps, and reactors were made of glass, Teflon, or stainless steel. Glass bottles sealed with Teflon stoppers served as long-term (1 month) bioreactors (LW6, GW6, BW6), and three-way-valves were used as sampling ports. Transparent parts were covered with aluminum foil, and the entire system was covered with a Mylar blanket to prevent algae growth.

The residence time of the individual columns were determined by evaluating a step change in nitrate concentrations at a flow rate of 1 mL min^{-1}. The low $D/(\mu L)$ values indicated plug flow characteristics (*29*). Impermeability for oxygen was tested by stripping DO from the feedwater using nitrogen gas and measuring DO at various stages. The DO concentration increased in the Tygon tubing of the peristaltic pump from 0 to approximately 3 mg L^{-1} and then remained stable (data not shown).

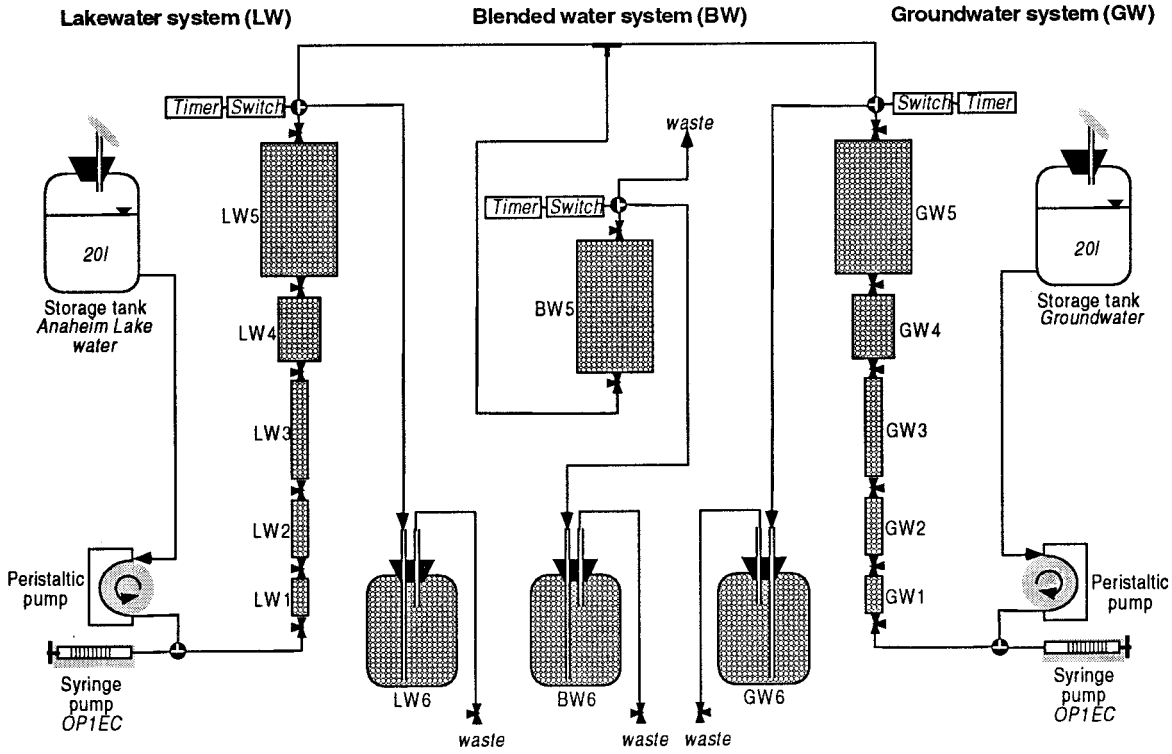

FIGURE 1. Flow scheme for the aquifer model system. LW1-LW5 and GW1-GW5 are the column trains for lake water and groundwater, respectively; BW is the column receiving the 50:50 blend of columns LW5 and GW5; LW6, GW6, and BW6 are the 1-month bioreactors. Column dimensions, packing, and flow characteristics are summarized in Table 1.

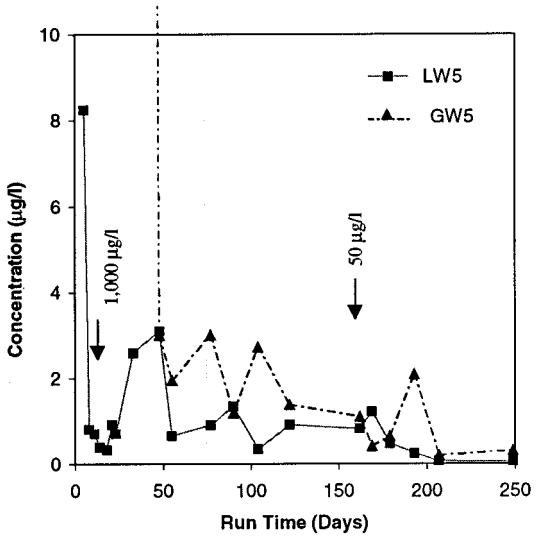

FIGURE 2. OP1EC residual concentrations after 280 min residence time (in LW5 and GW5 effluent). The OP1EC feed concentrations were 2000 μg L^{-1} through day 14, 1000 μg L^{-1} through day 162, and 50 μg L^{-1} through day 249.

TABLE 2. Degradation of Background DOC[a] and PS in the Lake Water Train

run time (days)	23	61	122	207	249
month	Apr	May	Jul	Oct	Nov
DOC in(mg/l)	9.2	6.4	4.8	3.8	2.9
DOC out(mg/l)	8.2	5.5	4.1	3.4	2.7
% DOC removed	11	13	14	10	9.6
$\Delta O_2/\Delta DOC$ (mg/mg)	1.5	3.2	4.3	5.9	5.9
PS in (mg/L)	n/a	2.1	1.0	0.42	0.48
PS out (mg/L)	n/a	1.8	0.86	0.32	0.35
% PS removed (−)	n/a	16	17	24	27

[a] DOC concentrations do not include OP1EC contribution.

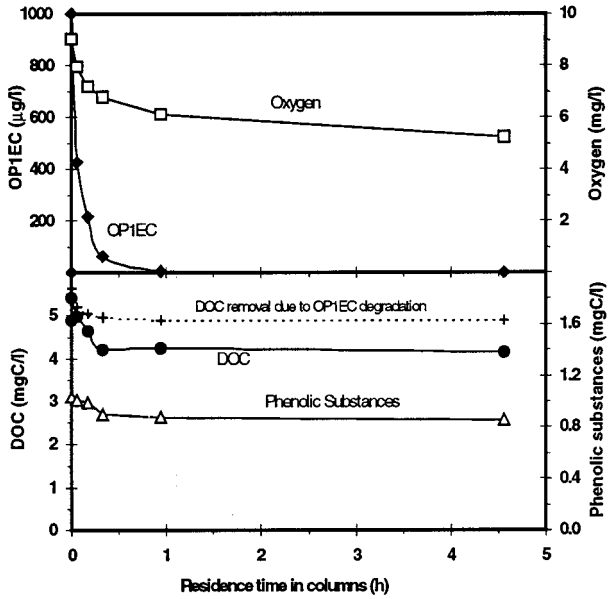

FIGURE 3. Concentration profiles in lake water system, 7/26/98 (122 days after startup), influent concentration: 1000 μg OP1EC L^{-1}. The dotted line indicates the DOC removal due to OP1EC transformation. Note: the calculated DOC increase of 1000 μg L^{-1} OP1EC is 0.73 mg L^{-1}.

To ensure rapid development of steady-state conditions, the first column in each train and 1-month reactors were first inoculated for 6 days with fluid of an enrichment culture

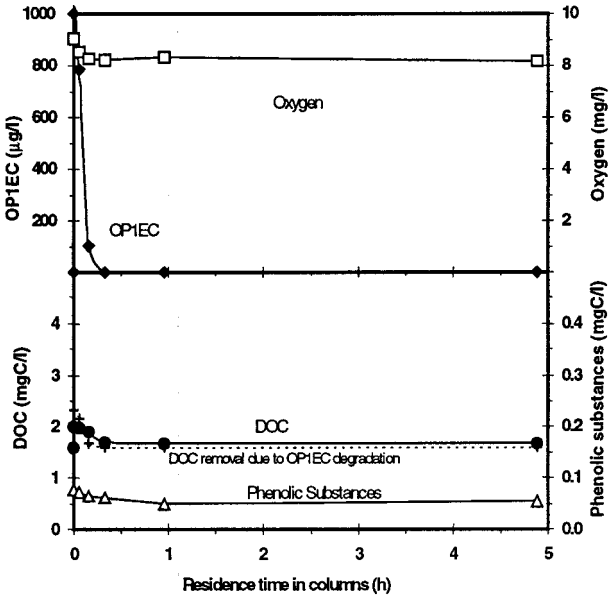

FIGURE 4. Concentration profiles in groundwater system, 7/26/98 (122 days after startup). Influent concentration: 1000 μg OP1EC L^{-1}. Note: the calculated DOC increase of 1000 μg L^{-1} OP1EC is 0.73 mg L^{-1}.

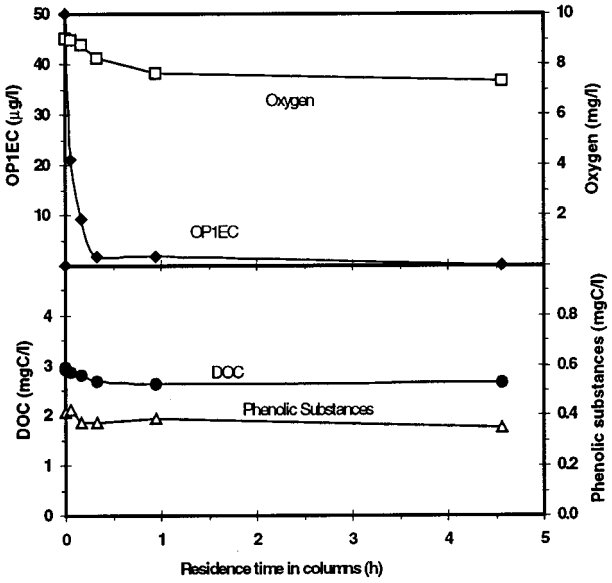

FIGURE 5. Concentration profiles in lake water system, 11/30/98 (249 days after startup). Influent concentration: 50 μg OP1EC L^{-1}. Note: 50 μg L^{-1} of OP1EC that was added to the influent increased the DOC by 0.036 mg L^{-1}.

(estimated density 10^5−10^6 cell/mL) (*30*) containing 8.6 mg L^{-1} OP1EC. Then columns were inoculated with LW and GW bacteria by the continuous flow of LW and GW. The approach to steady-state residual OP1EC concentrations was observed by measuring OP1EC in the effluents of column LW5 and GW5.

At the end of the column study, batch experiments were conducted with biomass that was removed from glass beads of LW1 and GW1 to compare column and batch results. Biomass was collected by vigorously shaking glass beads in Milli-Q water (three times), rinsed with Milli-Q water, and added to flasks containing 200 mL of lake water or groundwater. Bottles were then amended with 10 000 μg L^{-1} OP1EC and incubated for 53 days at 20 °C under continuous shaking.

Sampling and Analyses. Sampling was done by attaching a 0.22 μm Teflon membrane-filter to the valve and then

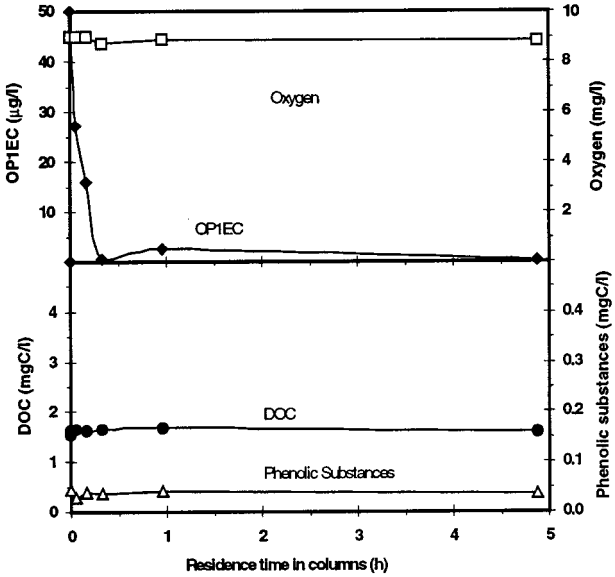

FIGURE 6. Concentration profiles in groundwater system, 11/30/98 (249 days after startup). Influent concentration: 50 μg OP1EC L^{-1}. Note: 50 μg L^{-1} of OP1EC that was added to the influent increased the DOC by 0.036 mg L^{-1}.

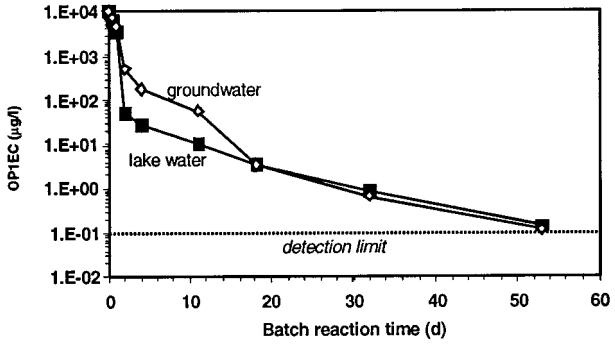

FIGURE 7. Batch experiments with glass beads from column section LW1 in lake water and from section GW1 in groundwater. Initial OP1EC concentration: 10 000 μg L^{-1}.

TABLE 3. Degradation of DOC[a] in the LW/GW Blend Column (BW5)

run time (months)	2	4	7	8
sampling date	May	Jul	Oct	Nov
DOC in (mg/L)	4.2	3.0	2.5	2.1
DOC out (mg/L)	3.8	2.6	2.4	2.1
% DOC removed	9	13	5	1.4

[a] OP1EC contribution to DOC is negligible.

allowing water to flow out under pressure directly into a syringe or a vial. Samples were stored at 4 °C up to 3−4 days until analyzed. For OP1EC analysis, the GC-MS method of Ding et al. (*3*) was adapted with few modifications. Briefly, 20-mL samples were concentrated to dryness and derivatized. A 1-μL sample was injected, and the m/z 235 ion of OP1EC was used for external quantification. The quantification limit was 0.1 μg L^{-1}. OP1EC was prepared using a method reported previously (*30*, *31*). Compound identity and purity were verified using ^{1}H NMR and elemental analysis.

DO was analyzed by connecting a 2 mL vial to a sampling port and using an oxygen electrode with a stirred reaction chamber (Hansatech Instruments Ltd., Kings Lynn, England). DOC was measured as nonpurgeable organic carbon (NPOC) after filtration with a 0.22 μm-nylon filter and adjustment to pH 2 using an automated Shimadzu TOC-5050 analyzer (Shimadzu Scientific Instruments Inc., Columbia, MD). DOC was further characterized using a presumptive test for phenolic substances (PS) (*32*). The method is based on the assumption that the aromatic hydroxyl groups of DOC reduce Cu^{2+} to Cu^{1+} and provides a relative measure of the content of phenolic hydroxyl groups in DOC. The formed Cu^{1+} is quantified colorimetrically as the bicinchoninic acid−Cu^{1+} complex (Micro BCA Protein Assay Reagent Kit no. 23235, Pierce, Rockford, IL). Phenol was used as the standard, and concentrations were expressed in units of mg phenol-C L^{-1} (i.e., each presumptive phenolic hydroxyl group counts as six carbon atoms).

Results and Discussion

OP1EC Residual Concentrations as a Function of Run Time and Influent Concentration. The OP1EC residual concentrations were measured after passing through columns 1−5, i.e., after 280 min of residence time (Figure 2). In the LW train, concentrations decreased from 2000 to 0.8 μg L^{-1} within 8 days. From day 14 through day 162, when the feed concentration was maintained at 1000 μg L^{-1}, the OP1EC residual varied between 0.3 and 3 μg L^{-1}. No clear upward or downward trend was observed. After decreasing the feed concentration to 50 μg L^{-1} on day 162, the residual in the LW train slowly decreased to below the 0.1 μg L^{-1} detection limit. In the GW train, efficient removal was only achieved after day 48 when well W3 water was used as feed and clogging stopped. During days 48−122 the OP1EC feed was 1000 μg/L and the residual ranged from 0.8 to 3 μg L^{-1}. When the feed concentration was lowered to 50 μg L^{-1} in the GW train, the residual decreased also but stayed above 0.2 μg L^{-1}. Although data are too variable to make firm conclusions, Figure 2 indicates that residual concentrations in the GW were generally higher than in LW, perhaps because cometabolism during DOC degradation contributed to OP1EC removal.

DOC Removal. The background DOC (defined as DOC present prior to OP1EC addition) concentrations decreased from April through November from 9.2 to 2.9 mg/L (Table 2). This decrease is attributed to the seasonal decrease in the storm runoff contribution to the Santa Ana River flow that is used to recharge Anaheim Lake and the increasing relative contribution of tertiary treated effluents. Removals decreased nearly in proportion from 1 mg L^{-1} to 0.2 mg/L, 11% (s = 1.8%) on average. Consistent with previous field data, the removal of PS exceeded that of DOC (by 3−13%) (*32*). DO was always present in excess (>5 mg L^{-1}). Oxygen utilization per mg DOC removed increased from 1.5 to 5.8 mg/mg. The higher ratios were observed while feeding the low (50 μg/L) OP1EC and were attributed to the oxidation of biomass that accumulated feeding high OP1EC concentrations. Nitrate concentrations were constant, and nitrification was excluded as an oxygen consuming process (data not shown).

Removals as a Function of Residence Time. The concentration−time profiles for OP1EC, oxygen, DOC, and phenolic substances as a function of residence time are shown in Figures 3−6. The 1000 and the 50 μg/L profiles were obtained after 122 and 249 days of run time, respectively. The profiles obtained after 23 and 61 days run time were very similar (data not shown). In all cases, OP1EC concentrations decreased to residual concentrations within 20−30 min with the possible exception of LW at 1000 μg/L where it might have been as long as 60 min. The biodegradable DOC and PS of LW were removed concurrently with OP1EC. Further removal of DOC and a decrease in PS after 60 min cannot be discerned.

To calculate biodegradation of background DOC, it was assumed that OP1EC degradation was complete, i.e., that no intermediates were formed which contributed to the DOC pool. Previous batch studies indicated the formation of 2,4,4-

trimethyl-2-pentanol as a stable intermediate of OP1EC degradation (*30*). Samples were analyzed for this compound and other potential intermediates but none were detected.

In the LW train at high concentrations, DOC removal due to OP1EC degradation accounted for approximately half of the biodegraded DOC; in the GW train the entire DOC removal was attributed to OP1EC degradation. At 50 μg/L OP1EC feed concentration, DOC data reflect the behavior of background DOC. The data in Figures 5 and 6 show that in the LW train approximately 0.2 mg/L of LW DOC were removed within less than 30 min, whereas the GW DOC remained stable, as expected. The GW data indicates for OP1EC biodegradation a stoichiometry of 1 mg/L oxygen used per mg/L OP1EC removed. Complete mineralization would require approximately twice the amount of oxygen suggesting a high yield of biomass.

The effluent of the 1-month reactors was analyzed for OP1EC and DOC (data not shown) on days 16, 77, 162, and 204. The approach to residual concentrations was slow. On days 16 and 77 in LW6 and GW6 effluents, OP1EC concentrations still ranged from 3 to 7 μg/L but were lower (<0.1 to 0.3 μg/L) thereafter. In all BW6 samples, the residual concentrations ranged from <0.1 to 1 μg/L. More data are needed to establish a time trend, but the limited data suggests that residuals can persist even during an additional month of residence time.

The Effect of Blending LW and GW on DOC and OP1EC. Comparing influent and effluent of BW5 allows evaluation of biotransformation enhancement as a consequence of mixing biologically stable native and recharge water (after passing through the biologically active zone). Blending (data not shown) did not stimulate OP1EC removal. DOC removal in BW5 was small but significant and ranged from 0.03 to 0.4 mg L^{-1} or 1.4−13.5% (Table 3). Consistent with the DOC removal, a brownish-yellowish deposit formed near the inlet, suggesting the formation of biofilm formation. This observation suggests that simply blending the recharge water and groundwater could further stimulate DOC removal, perhaps, because mixing of two waters eliminates nutrient limitations that may exist in one or the other water.

Batch Biodegradation Experiments. The results of the batch biotransformation experiments are shown in Figure 7. They indicate that the organisms were able to degrade the main portion of the available substrate within a few days in both cases. However, the rate slowed considerably when concentrations were in the low μg L^{-1} range. After 2 months, the concentrations decreased to the quantification limit of 0.1 μg L^{-1} in both cases. To determine the dry weight of the biofilm, the pH in the batch reactors was adjusted to 3 with HCl, the biomass was scraped from the glass beads, rinsed with water on a 0.22 μm nylon membrane filter, and dried overnight at 70 °C. The mass of total dried solids removed from the glass beads from the first LW column was 27 mg after the batch experiment; for the GW system 1.5 mg of dried solids was measured. The higher biomass in the LW system is attributed to growth stimulated by the biodegradable background DOC. The OP1EC transformation rate did not differ significantly in the LW and GW bioreactors (Figure 7) suggesting that similar populations of OP1EC degrading organisms were present.

Taken together, the results of this study indicate that during groundwater recharge residuals of organic compounds such as OP1EC may persist (up to several μg l^{-1}) even though conventional batch biodegradation tests may suggest complete removal. This finding is consistent with field data that document trace levels of APEC compounds in groundwater (*2*, *27*). The fact that generally higher residuals were observed in GW than in LW is perhaps a result of DOC-stimulated cometabolic OP1EC removal. Further work using sediment columns is needed to extrapolate these findings to long-term field conditions.

Acknowledgments

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