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Weathering Effects on Biodegradation and Toxicity of Hydrocarbons in Groundwater

Marie G. Dreyer, Yarrow M. Nelson (ynelson@calpoly.edu) and Christopher Kitts
(California Polytechnic State University, San Luis Obispo, CA, USA)
Paul Lundegard and Gonzalo Garcia (Unocal, CA, USA)

ABSTRACT: This study examined the effect of weathering on hydrocarbon biodegradation and toxicity at a former oil field near Guadalupe, California. Soil and groundwater at this site contains residual diesel-range hydrocarbons formerly used to dilute the viscous crude oil to facilitate pumping (Lundegard and Garcia, 2001). Natural attenuation is being considered at this site as a means of remediating residual hydrocarbons in soil and groundwater. To provide the lines of evidence required for use of natural attenuation at this site, this research was undertaken to determine if the hydrocarbons continue to be biodegradable after extensive weathering in the field. Observed hydrocarbon biodegradation rates were directly proportional to initial total petroleum hydrocarbon (TPH) concentration, suggesting first-order kinetics. Highly weathered hydrocarbons most distant downgradient from the source zones exhibited slightly lower biodegradation rate constants. Microtox® toxicity decreased rapidly during 20 days of biodegradation in laboratory microcosms.

INTRODUCTION

The effect of weathering on hydrocarbon biodegradation and toxicity in groundwater was investigated by measuring laboratory biodegradation rates and toxicity for groundwater samples from a former oil field near Guadalupe, California, USA. The diluent used at this site was a complex mixture of hydrocarbons with equivalent carbon chain lengths from C10 to C30. The majority (90%) of the hydrocarbons are within the range C14 to C30. These ranges are consistent with diesel and kerosene hydrocarbons, although the hydrocarbons at this site have weathered for over 20 years. Other research at the site have shown initial rapid biodegradation following first-order kinetics, followed by an asymptotic curve of reduced activity roughly following zero-order kinetics (Cunningham, 2004; Kaplan and Kitts 2004). Therefore, a key area of research is to explore possible reasons behind this pattern by examining the trends of biodegradation and toxicity of hydrocarbons in groundwater samples as a function of weathering in the field. A total of thirty-four groundwater samples were collected from across the Guadalupe site with a range of total petroleum hydrocarbon (TPH) concentrations. These monitoring wells were chosen on the basis of historical TPH concentrations and their locations relative to source zones. It was assumed that samples with low TPH concentrations were more weathered than samples with high TPH concentrations. Monitoring wells were also chosen along transects downgradient from source zones. The biodegradability of TPH in each sample was determined by measuring respiration rates (CO_2 production) and 20-day TPH degradation rates in the laboratory. Changes in TPH composition with weathering were evaluated using gas chromatography (GC) with simulated distillation (SIMDIS) to determine equivalent carbon chain length. Total organic carbon (TOC) analysis was used to determine

concentrations of total organic material in the groundwater samples. Finally, Microtox® toxicity was measured to determine the effect of weathering of the hydrocarbon mixtures on toxicity.

METHODS AND MATERIALS

Respirometry Methods. Respirometry was used to measure CO_2 production for 21 of the groundwater samples. Microorganism respiration was measured using a Micro-Oxymax open-cell respirometer (Columbus Instruments, Columbus, Ohio). CO_2 production was monitored for a period of six days. To keep the samples well oxygenated and promote microbial degradation, magnetic stirrers continuously stirred the bottles. To match field conditions, the samples were kept in a water bath regulated at a constant temperature of 19°C. No nutrients were added to any of the samples. A deionized water blank was run as a control.

Twenty-Day Biodegradation Rate Measurement. To quantify biodegradation rates, TPH measurements were made in duplicate before and after 20 days of incubation at 19°C. All samples were constantly stirred during incubation. No nutrients or inoculum were added to any of the samples for the biodegradation experiments. TPH concentrations were determined by Zymax Envirothechnology, San Luis Obispo, CA by extraction into methylene chloride and gas chromatography.

Microtox® Toxicity Measurement. Toxicity measurements were made using the Microtox® method which measures toxicity to the bioluminescent bacterium *Vibrio fisheri*. The Microtox 500 Analyzer by Strategic Diagnostics, Inc. (Newark, DE) was used for all toxicity measurements. For each Microtox® analysis, 2.5 mL of sample was transferred to a vial and dilutions were made in accordance to the "Basic Test" and performed manually in test vials set within the analyzer. Tests were performed on all samples and run in duplicate.

Total Organic Carbon (TOC) Measurement. TOC measurements were made to identify the presence of organic carbon in case of interference with respirometry measurements. This method varies from the respirometer by measuring all organic carbon, rather than just biodegradable carbon. TOC measurements were made using a Shimadzu TOC-5000A analyzer. TOC was measured for all samples in duplicate.

RESULTS AND DISCUSSION

Respirometry. CO_2 production was observed for all groundwater samples, indicating microbial activity. The distilled-water controls did not produce any measurable CO_2 over the same time period. An initial phase of constant microbial activity was seen within the first 36 hours of measurements. This phase was followed by a second phase of declined activity by most samples. In general, increased respiration was observed with increasing TPH concentration. Average CO_2 respiration rates were calculated by dividing the final accumulated CO_2 respiration value by total time (141 hours). Although CO_2 production rates varied over the 6-day period, average respiration rates were used for this correlation. CO_2 respiration rates increased linearly with initial TPH concentrations, up to 7,000 μg/L

(R^2 = 0.873). Above 7,000 µg/L TPH, the respiration rates did not increase with increasing TPH, but rather a leveling off was seen.

TOC Results. TOC concentrations ranged from 13 to 42 mg organic carbon/L of sample. No correlation was found between initial TOC and TPH concentration (R^2 = 0.156). Samples with very low TPH concentrations exhibited a wide range of TOC concentrations from 10 to 35 mg/L, indicating groundwater samples vary in natural organic matter content. TOC levels in the samples may have influenced respiration rates measured for some of the samples.

Twenty-Day Biodegradation Rates. Significant reductions in TPH concentration were observed for all samples with measurable initial TPH concentrations. Biodegradation rates were calculated by dividing the change in TPH concentration over the 20-day time interval. When 20-day biodegradation rates are plotted as a function of initial TPH concentration, the R^2 value of 0.92 indicates a direct proportionality (Figure 1). This indicates TPH biodegradation in groundwater at the site follows first-order biodegradation kinetics. From the slope, a first order rate constant of 0.023 day^{-1} is observed. In this study particular focus was given to groundwater samples with low TPH concentrations, as they pose the greatest concern with biodegradability after weathering. When plotted against 20-day biodegradation rate, it was observed that three of the samples (204-A, A8-8 and A8-6) fell below the regression line. Hydrocarbons in groundwater in the vicinity of these monitoring wells appear to biodegrade, but with a lower first order rate constant. For these samples, nitrate, nitrite and ortho-phosphate levels were below the practical quantification limits. Thus the lack of nutrients in these samples may have led to the

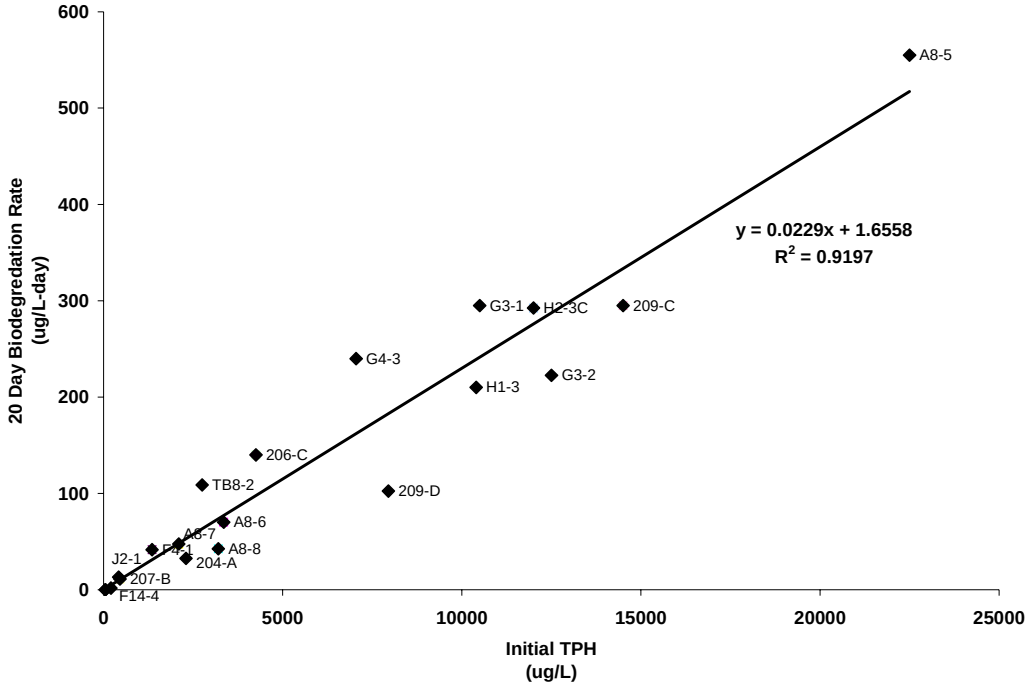

FIGURE 1. 20-Day TPH biodegradation vs. initial TPH concentrations. Trendline and R^2 = 0.9197 indicates a direct proportionality in data.

lower rates of biodegradation observed. Overall, samples with low TPH concentrations followed similar first order kinetics as high TPH samples which indicates that biodegradability of hydrocarbons is not hindered by chemical changes between groundwater with high vs. low TPH concentrations.

Simulated Distillation (SIMDIS) TPH Analysis. SIMDIS shows the equivalent carbon chain length distribution for the hydrocarbons in each groundwater sample. After 20 days, significant biodegradation was observed for all equivalent carbon ranges. For many samples the shorter chain hydrocarbons were completely biodegraded in 20 days. These results suggest there is not a recalcitrant component identifiable through SIMDIS analysis. These observations were similar for all groundwater samples studied.

Effect of Distance from Source on Biodegradation. Some of the groundwater samples used in this study were part of four recognizable plumes. Twenty-day biodegradation rates for these samples decreased with increasing distance from source for all four plumes (Figure 2). However, this trend may be misleading because wells at farther distances may exhibit lower biodegradation rates due to lower TPH concentrations as expected for first order kinetics. To clarify this trend, specific TPH utilization rate was calculated for each well by dividing degradation rate by TPH concentration (Figure 3). Plotting these values versus distance from source zone indicates only a slight decrease in specific TPH utilization rates as groundwater travels downgradient. Observed decreases vary from 5 to 46% for the four plumes studied. As discussed above, lower nutrient concentrations for downgradient samples may also have contributed to lower specific TPH utilization rates. Many of the groundwater samples in the plumes were below the practical quantification limit for the nutrients nitrate, nitrite and ortho-phosphate. More detailed chemical analyses of the hydrocarbons could help explain the decline of specific TPH utilization rates over

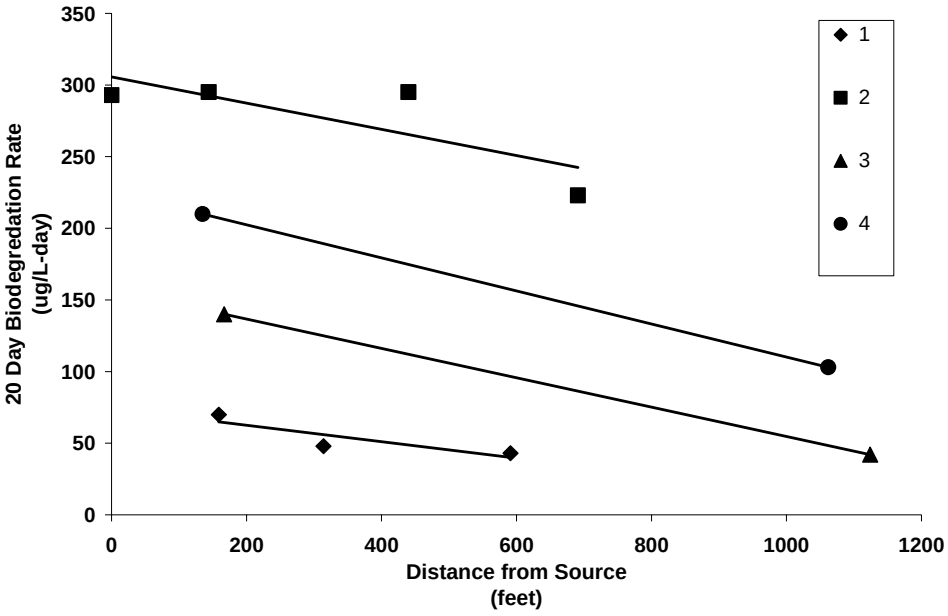

FIGURE 2. 20-day biodegradation rate vs. distance from source zone for selected plumes.

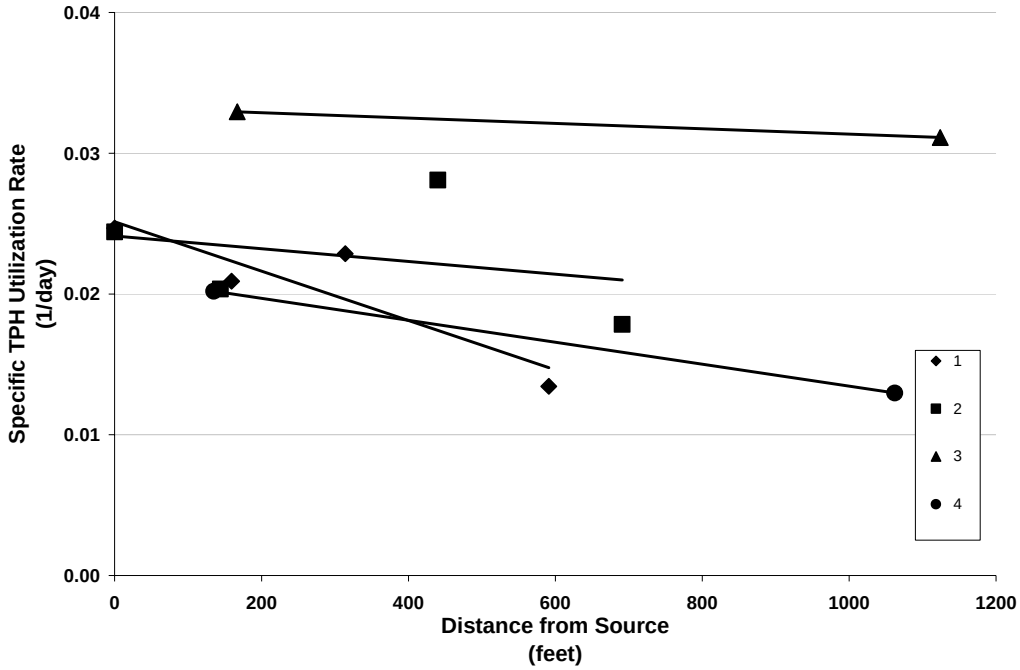

FIGURE 3. Specific TPH utilization rate vs. distance from source zone for selected plumes. Rate was calculated by dividing degradation rate by TPH concentration.

distance from source. Regardless, decreased biodegradability did not prevent biodegradation to still be greater than 50% of the rate for fresh material.

Toxicity Results. The Microtox® analyzer interpolates the measured toxicity effect of a series of sample dilutions to find the effective concentration where 50% of the bioluminescent test bacteria are killed (EC_{50}). In this study the percent effect at full concentration value was calculated to compare the toxicity of all samples. A linear regression was used to extrapolate to percent effect at full concentration. Unlike EC_{50}, the higher this value, the more toxic the sample. Plotting percent effect at full concentration vs. TPH concentration shows toxicity increases with increasing TPH concentration (Figure 4), as expected. However, it is also seen that for many samples within similar TPH ranges, a large range of toxicity is observed. More detailed chemical analyses of these samples would be useful to determine if there are differences in chemical composition. Microtox® toxicity was also determined after 20 days of biodegradation for 21 of the samples. After 20 days a large portion of these samples, which were initially very toxic, resulted in toxicity too low to allow calculation of EC_{50}, suggesting a significant reduction in toxicity in this short time frame.

To further examine the effect of weathering on toxicity, Microtox® toxicity as percent effect at full concentration was plotted versus distance from source zone (Figure 5). Only one plume exhibited a slight decrease in toxicity over distance. For the other plumes higher or equal toxicity levels were observed for weathered samples. This finding is significant because it suggests that toxicity is not consistently decreasing with distance from source zone. However, many assumptions were used in this plume analysis, including the exclusion of additional sources and contaminants into the plumes.

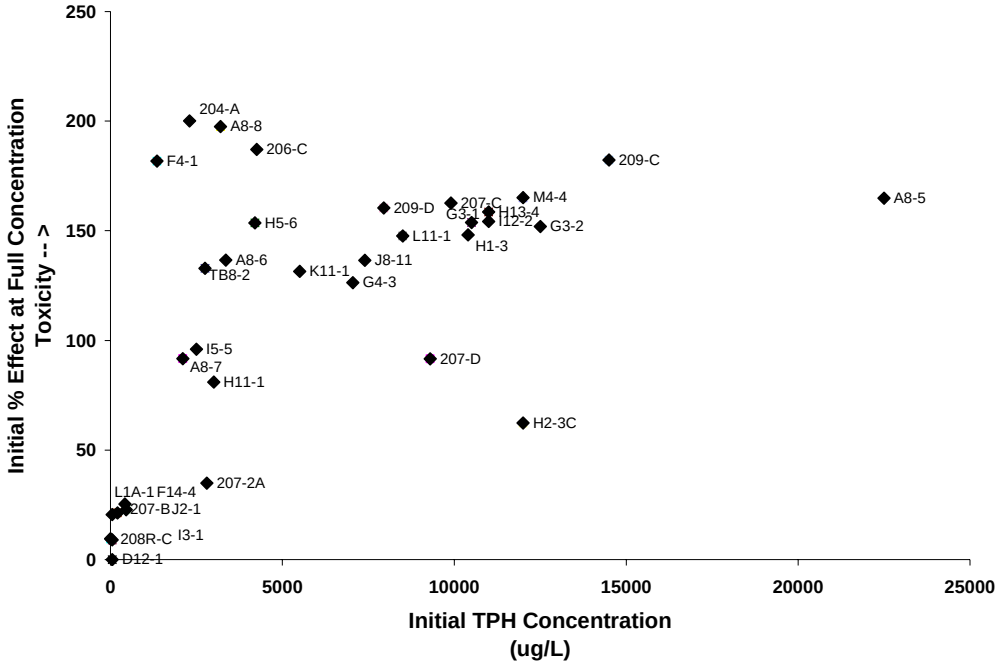

FIGURE 4. Toxicity as measured by initial % effect at full concentration vs. initial TPH concentration for all groundwater samples.

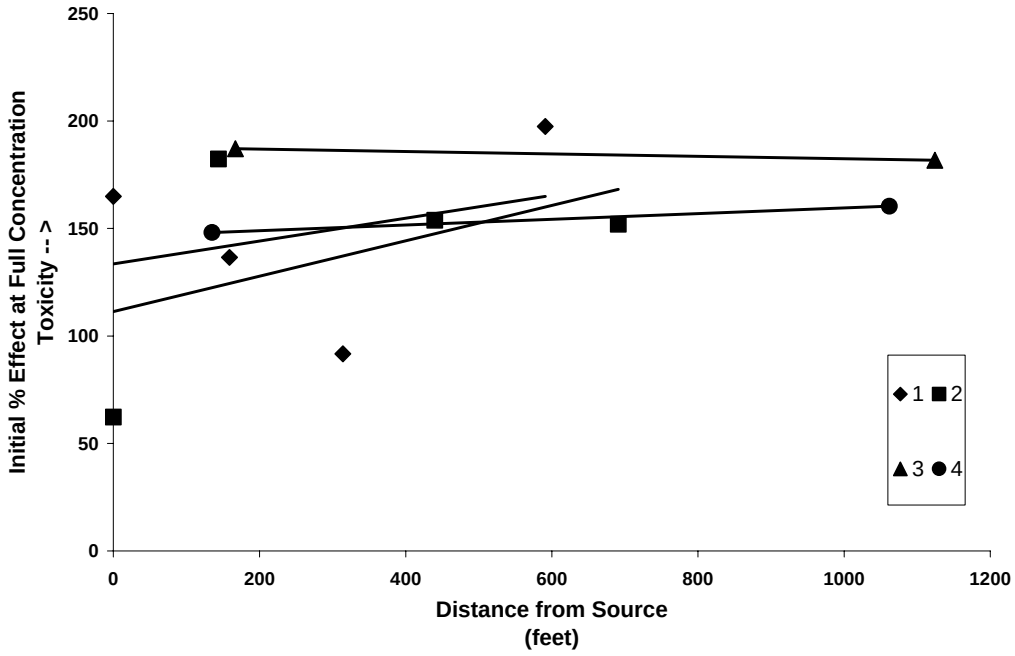

FIGURE 5. Toxicity (% effect full concentration) vs. distance from source.

CONCLUSIONS

Hydrocarbons in all groundwater samples were able to biodegrade to a level of low TPH concentration. Weathering decreased the observed first-order rate constants, but biodegradation was sustainable. However, after the period of most active biodegradation (20 days) slight amounts of both TPH and toxicity remained, suggesting a possible asymptote in degradation. Microtox toxicity was rapidly reduced (20 days) for most samples, but groundwater with low initial TPH concentrations did not significantly change in toxicity over the 20-day period. It may be that the toxicity was already so low that statistically significant reductions in toxicity could not be observed for these samples with low TPH concentration. Thus the ultimate feasibility of natural attenuation at this site will depend partly on the choice of appropriate endpoints for natural attenuation.

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