Natural Attenuation: Hydrocarbon Biodegradability along Vertical Profiles

Unocal-Sponsored EBI Research Project

Guadalupe Restoration Project

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Executive Summary

The sustainability of natural hydrocarbon biodegradation at the former Guadalupe Oil Field (GOF) was investigated by measuring the biodegradability of hydrocarbons in groundwater collected along vertical profiles using nested wells. In a companion EBI project, the microbial communities along these vertical transects were studied by principal investigator Chris Kitts using terminal restriction fragment (TRF) analysis of DNA extracted from the samples. A similar study was conducted by EBI one year prior to this project using groundwater collected from 34 different monitoring wells horizontally distributed throughout the GOF. This prior study showed that hydrocarbon biodegradation followed first-order kinetics regardless of well location, suggesting that hydrocarbon biodegradation by natural attenuation is consistently sustainable throughout the site (see Natural Attenuation Final Report December 2004). However, the wells used in that prior study were screened over a range of depths, so the groundwater analyzed was a mixture of groundwater from different depths, and therefore from different redox conditions. This study was extended this year to examine hydrocarbon biodegradability along vertical transects to ensure that groundwater collected from different redox zones is consistently biodegradable. Groundwater samples were collected from three nested wells, with five sampling depths, along an isolated plume transect at the former GOF. These groundwater samples contained diluent used at Guadalupe (mid-cut petroleum distillates) at concentrations ranging from non-detect (ND) to 3.2 mg/L total petroleum hydrocarbon (TPH). The biodegradability of the hydrocarbons in these groundwater samples was measured in the laboratory under aerobic conditions at 19°C (similar to *in-situ* temperature) for 20 days. Initial and final TPH concentrations were determined using gas chromatography, and CO_2 evolution over the 20-day period was monitored using a MicroOxymax respirometer. Toxicity was evaluated using the Microtox® method to observe changes in hydrocarbon toxicity during biodegradation. Inorganic nutrient concentrations, and total organic carbon [TOC] concentrations were also measured to fully characterize the samples. Initial TPH concentrations for the first well along the plume transect (Well # K5-7) ranged from 400 to 3,200 ug/L, and varied greatly with depth. Significant biodegradation was observed in 20 days for samples from depths with initial TPH concentrations greater than 500 ug/L. For these samples the observed first-order degradation rate constant was 0.014 day^{-1}. For depths with initial TPH concentrations less than 500 ug/L, little or no hydrocarbon biodegradation was observed. It is possible that the remaining hydrocarbons under the redox conditions prevalent at these depths are recalcitrant, but the lack of observed biodegradation may also have been the result of limited analytical ability for detecting changes in TPH concentration with such low initial TPH concentrations. The second and third wells down-gradient along the plume transect exhibited very low initial TPH concentrations at all depths, and as a result it was not possible to observe statistically significant biodegradation for groundwater samples from these wells. Initial TPH concentrations were at or below 700 ug/L for the second well (Well # I6-2) and were non-detect for most depths of the third well (Well # H6-2). Initial and final TPH concentrations after 20 days biodegradation were difficult to distinguish statistically at these low concentrations. Significant reductions in Microtox® toxicity were observed over the 20-day incubation period for all groundwater samples with detectable initial TPH concentrations. A follow-up study is underway to analyze one additional nested well with higher TPH concentrations.

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Table of Contents

1. Introduction

Natural attenuation is important at the former Guadalupe Oil Field GOF) because it is the most likely means of cleaning up residual hydrocarbon contamination following more active remediation efforts such as excavation. During the 2003-2004 academic year, the EBI team conducted an extensive research project to characterize natural attenuation at the site and to ascertain the sustainability of aerobic biodegradation of residual weathered hydrocarbons. Groundwater samples were collected from 34 different monitoring wells and fully characterized in terms of total petroleum hydrocarbon (TPH) concentration, aerobic biodegradation kinetics, respiration rates and toxicity, as well as complete assays of inorganic nutrients and microbial populations. Results of this project were reported in December 2004, and showed that hydrocarbon biodegradation followed first-order kinetics regardless of well location, suggesting that hydrocarbon biodegradation by natural attenuation is consistently sustainable throughout the site. However, the wells used in that prior study were screened over a range of depths, so the groundwater analyzed was a mixture of groundwater from different depths, and therefore from different redox conditions. This study was therefore extended this year to examine hydrocarbon biodegradability along vertical transects to ensure that groundwater collected from different redox zones is consistently biodegradable.

Another drawback of the previous study was that many of the 34 wells sampled were impacted by plumes from more than one source zone. By selecting nested wells from an isolated plume, we hoped to examine hydrocarbon biodegradability along a plume from a single source. The vertical profiles were also expected to provide valuable information on different electron acceptor processes because the redox potential varies with depth.

For this study, Groundwater samples were collected from three nested wells, with five sampling depths, along an isolated plume transect at the former GOF (K5 Area). Analyses the same as those conducted in the previous natural attenuation study were run on each sample, including measurement of 20-day aerobic biodegradation rate, respirometry, and Microtox® toxicity. Inorganic nutrients, sulfate and dissolved Fe(II) should also be measured for each depth. In a companion study (Chris Kitts, PI) the microbial communities at each sampling depth were characterized using terminal restriction fragment (TRF) analyses.

Additional details of this project can be found in the MS thesis of Drew Lassen.

2. Background on Natural Attenuation

Natural Attenuation at the former Guadalupe Oil Field (GOF):

Natural attenuation is an important mechanism for reducing total petroleum hydrocarbon (TPH) concentrations in source zones and groundwater plumes at the former Guadalupe Oil Field (GOF). Used in conjunction with other more active remediation approaches, natural attenuation is expected to provide the final polishing step for removing residual hydrocarbon contaminants at the site. There are several fundamentally important questions remaining regarding natural attenuation of diluent-affected groundwater at the Guadalupe site. One is whether or not observed hydrocarbon biodegradation is sustainable over long time periods. Significant effort was made during the 2003-2004 academic year to address this question, and the results were encouraging. Measurement of aerobic 20-day biodegradation rates in the laboratory for groundwater samples from 21 different GOF monitoring wells showed that TPH degradation rates are directly proportional to TPH concentration, as expected for first order kinetics (Dreyer, 2004). First order rate constants decreased only slightly with distance from source zones, indicating sustained biodegradation kinetics after weathering (Dreyer, 2004). Laboratory column studies also showed sustained TPH biodegradation over a 150-day period (Cunningham, 2004). These results are especially encouraging because published research has shown diminishing biodegradation for aged contaminants for a variety of reasons (Efroymson and Alexander, 1995).

It is also important to establish that hydrocarbon biodegradation at the Guadalupe site is consistent along vertical profiles as described here. Data from such vertical profiles could allow us to more completely characterize natural attenuation processes. Vertical profiles could provide insight into natural attenuation sustainability by measuring biodegradability as a function of weathering associated with depth at a single location where presumably all hydrocarbons are from the same source. The vertical profiles should also provide valuable information on different electron acceptor processes because the redox potential varies with depth. By comparing electron acceptor concentrations at different depths and examining other indicators, we expect to determine the dominant natural attenuation mechanism for each depth.

Natural Attenuation EPA Guidelines:

The US EPA defines monitored natural attenuation as:
"…The reliance on natural attenuation processes (within the context of a carefully controlled and monitored site cleanup approach) to achieve site-specific remediation objectives within a time frame that is reasonable compared to that offered by other more active methods." (EPA, 1999)

The 'natural attenuation processes' mentioned in the above description includes various physical, chemical, and biological mechanisms that serve to reduce the environmental impact of a contaminant over time. With respect to TPH plume dynamics, the accumulation of TPH within a plume volume is driven by the dissolution of non-aqueous phase contaminants, and processes such as volatilization, dispersion, and biodegradation. The relative rates of such

processes determine the efficiency of natural attenuation processes at Guadalupe. The work presented in this report pertains specifically to biodegradation as the primary means of hydrocarbon biodegradation, which is reviewed by Cookson (1995). For more information on the mechanisms of natural attenuation see Vance (2002).

As a result of the increased attention on monitored natural attenuation (MNA), the EPA has provided thorough guidelines for its use at contaminated sites. The guidelines emphasize and justify the EPA's support of MNA only after a sufficient amount of sound technical analyses have been performed to demonstrate a sufficient understanding of contaminant location, identity, movement, and degradation. These data must also indicate that natural attenuation processes are efficient and sustainable within the timeframe required to achieve remediation objectives (EPA, 1999).

The EPA supports a three-tiered approach to justifying the use of MNA. The first level of research is suggested to focus on providing "…historical groundwater and/or soil chemistry data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points" (EPA, 1999). Furthermore, this data should also show that decreasing concentrations are not "solely a result of plume migration" (EPA, 1999). If the first level of research is not sufficient in proving the efficacy and sustainability of natural attenuation mechanisms, a second level of research should be initiated to more closely examine historical groundwater and soil chemistry data. This second tier of research is intended to provide a deeper understanding of the rates and mechanisms of natural attenuation processes (EPA, 1999). When historical groundwater and soil chemistry data does not yield a sufficient understanding of natural attenuation processes, the EPA suggests the use of laboratory microcosm studies to "directly demonstrate the occurrence of a particular natural attenuation process at the site and its ability to degrade the contaminants of concern" (EPA, 1999). The nested well project of this thesis is part of a third-tier effort to show the rate and sustainability of biological degradation processes at Guadalupe.

3. Methodology

Groundwater Sampling:

An isolated plume was preferred for the current research in order to minimize spatial variations in plume characteristics caused by dispersion from other source zones. By selecting wells along an isolated plume, any changes in contaminant composition could be attributable to the process of natural attenuation. The K5 area plume was selected to provide groundwater samples for this project, as it appears to have developed from a single source zone. This plume extends westerly from its source as a result of groundwater flow towards the Pacific Ocean.

Sampling within this plume was performed at the nested wells designated as K5-7, I6-2, and H6-2 (Figure 1). Groundwater collection began at the K5-7 nested well on November 11, 2004, and ended with the completion of H6-2 analyses on May 23, 2005. These nested wells differ from standard wells in their lowermost 10-20 feet of length, which consists of a very fine cylindrical screen made of either PVC or stainless steel tubing. This design allows groundwater to enter at different depths while keeping the well free of silt and soil. These wells are intended to minimize the vertical mixing of groundwater and retain the *in-situ* concentration profiles of groundwater constituents.

Groundwater samples were taken from five depths at K5-7 and H6-2, but limitations imposed by the water table height allowed only four depths to be sampled at I6-2. The map of the former Guadalupe Oil Field shown in Figure 1 indicates the location of these wells within the K5 plume area.

Groundwater extracted from all three wells was placed into amber glass carboys and refrigerated immediately to retain sample integrity. Samples were promptly delivered to Zymax Envirotechnology (San Luis Obispo, CA) or B.C. Labs (Bakersfield, CA) where initial TPH analyses were performed within one week. 20-day aerobic biodegradation experiments and respirometry were measured in the laboratory at Cal Poly less than twelve hours from the time of sampling. TOC and toxicity analyses were also completed at Cal Poly within one week of sampling. Inorganic nutrients were analyzed by BC Laboratories. Fe(II) was analyzed in the field by Bob Pease (LFR).

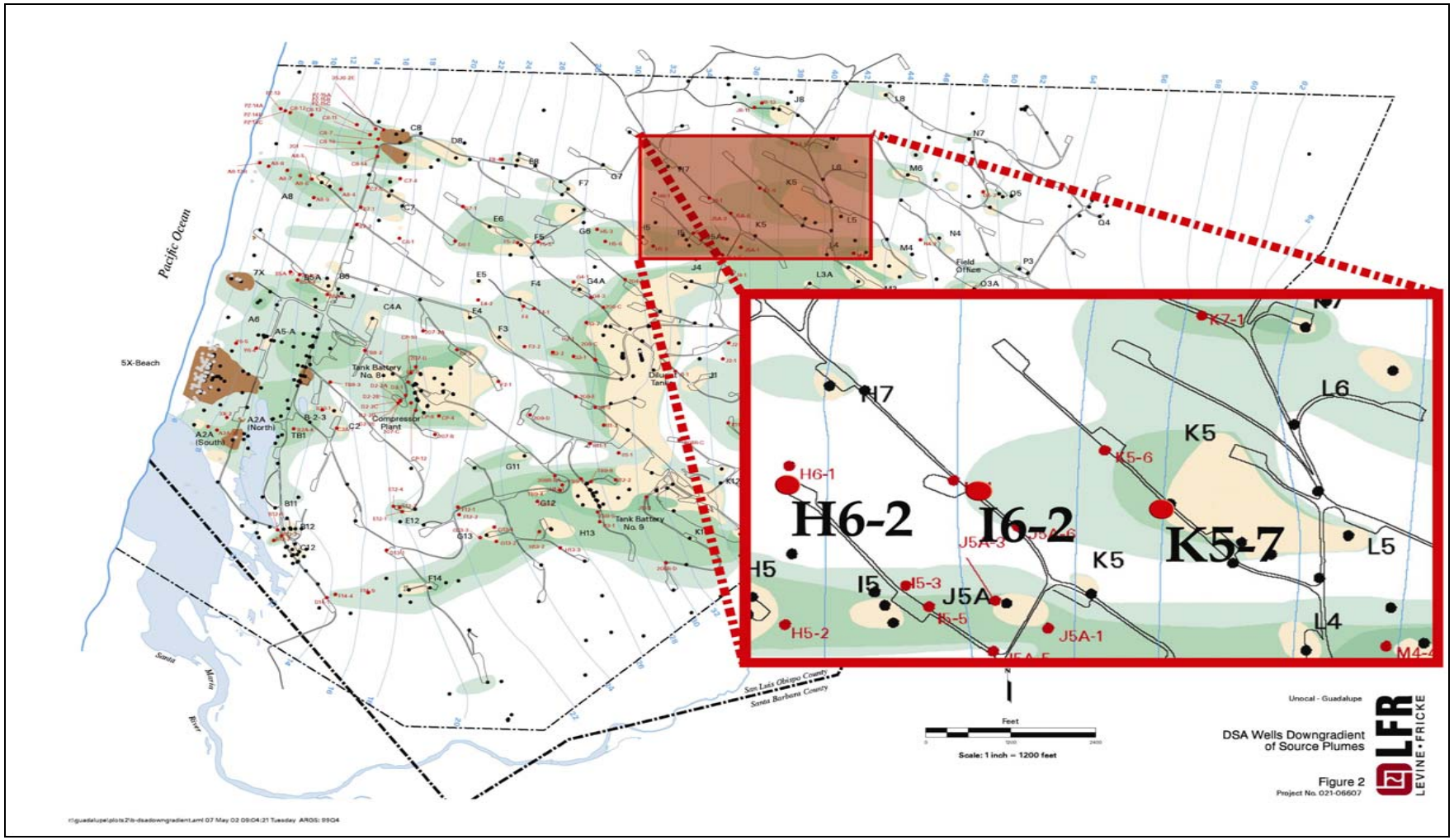


Figure 1. Schematic of well locations at Guadalupe K-5 area. Sampling wells are indicated by red dots; former oil wells are indicated by black dots. Inset shows current project location. Courtesy of LFR.

Measurement of 20-day Biodegradation Rates:

Groundwater samples from each depth of each nested well were incubated in the laboratory under aerobic conditions for 20 days, and initial and final TPH concentrations were measured as described below. The fresh groundwater samples were placed in 2-liter glass bottles within 6 hours of receipt from the field site. Samples were maintained at 19°C using a temperature-controlled, recirculating water bath. A magnetic stirrer was used in each bottle to minimize the settling of colloidal particles and biomass, and to enhance the exchange of CO_2 and O_2 between the headspace and the water. Air was supplied by the respirometer, as described below. Initial and final TPH concentrations were measured in duplicate.

Total Petroleum Hydrocarbon (TPH) Analyses:

One of two analytical laboratories in central California was employed to perform the TPH analyses. Zymax in San Luis Obispo, CA conducted these analyses until they went out of business on April 18, 2005. Testing performed after that date was done at BC Labs in Bakersfield, CA. Quantifying the low amounts of TPH present in Guadalupe groundwater required that 1-liter samples first be extracted into 180 ml of methylene chloride and concentrated down to 1 ml. Gas chromatography was then performed on the concentrate using a capillary column and mass spectrometer detector. A technique called simulated distillation (SIMDIST) was employed to show the distribution of hydrocarbons by equivalent carbon chain length. The ability to monitor degradation rates within these smaller ranges was useful for determining the biodegradability of hydrocarbons as a function of equivalent chain length. Compositional data was reported for compounds having elution times indicative of petroleum hydrocarbons in the 10-to-40 equivalent-carbon range.

Respirometry:

During the entire 20-day incubation, the groundwater sample bottles were connected to a Columbus Instruments Micro-Oxymax respirometer to monitor CO_2 evolution. The respirometer was programmed to purge the headspaces with ambient air every two hours in order to sufficiently replace the oxygen used in cellular respiration. With a headspace volume of 200-300 ml, this refresh rate supplied roughly 100 times the amount of oxygen necessary to meet the demand for hydrocarbon biodegradation in a typical two-liter groundwater sample. An infrared absorbance detector on the Mirco-Oxymax unit analyzed the CO_2 concentration of purged headspace gas. Cumulative CO_2 evolution for the full 20 days was calculated from these data.

Microtox® Toxicity Analyses:

Quantitative toxicity analyses were performed on groundwater samples both before and after the 20-day degradation period using an SDI Microtox® unit. The Microtox® method provides toxicity data by measuring the light emission level of bioluminescent bacteria before and after exposure to a test sample. The intensity of light emission from these bacteria is proportional

to their metabolic rate, which is diminished by the toxicity of their environment. A change in luminescence is reported in terms of percent effect, which is mathematically defined as:

$$\% \, Effect = \frac{I_{ot} - I_t}{I_{ot}} \tag{1}$$

where I_{ot} is the baseline light intensity and I_t is the measured light intensity at t minutes after reagent exposure. I_{ot} is determined for each test sample by assuming that any change in control sample luminescence will reflect a change in the baseline test sample luminescence:

$$I_{ot} = I_o \left[\frac{I_{Ct}}{I_{Co}} \right] \tag{2}$$

where I_{Ct} is the luminescence of the control sample at time t, I_{Co} is the luminescence of the control sample at time t=0, and I_o is the luminescence of the test sample at time t=0. The use of I_{ot} compensates for any change in luminescence that is not attributed to the addition of a test sample.

The Microtox® Omni software can also be used to calculate the EC50 of the samples. The EC50 represents the concentration at which a sample inhibits the luminescence of the bacteria by 50%, and is comparable to the more commonly used 'lethal dosage'. EC50's are calculated by introducing the parameter 'gamma' (Γ_t), whose mathematical definition is:

$$\Gamma_t = \frac{I_{ot}}{I_t} - 1 \tag{3}$$

Γ_t is plotted versus concentration on a double-log plot for each sample time. A straight line through data points on this graph can be extrapolated or interpolated to determine the EC50. This occurs where $\Gamma = 1$, or when $I_{ot} = 2\, I_t$.

Alternatively, toxicity can be reported as 'percent effect' by calculating the percent reduction in bioluminescence that would be expected for a full-strength sample. This is determined by extrapolating the percent effect for each dilution of the sample out to 100% sample strength. This method provides the more intuitive parameter that increases with increasing toxicity and is well suited for comparing samples with very low toxicity (Dreyer, 2004).

Total Organic Carbon (TOC) Analyses:

A series of total organic carbon [TOC] measurements were performed on samples both before and after the 20-day aerobic degradation period. Each groundwater sample was prepared for TOC testing in two ways. The first preparation involved homogenizing approximately 30 ml of a well-mixed sample using an ultrasonic probe. The probe was set at maximum power and immersed in the sample for approximately one minute. This preparation yielded a homogeneous liquid that resisted settling and allowed for more consistent TOC measurements. The second preparation involved filtering approximately 30 mL of a well-mixed groundwater sample through a Millipore 0.22 um filter membrane. This preparation facilitated the quantification of dissolved-phase TOC. Comparison to the TOC values of sonicated vs. filtered samples allowed quantification of particulate vs. dissolved TOC. All samples were analyzed using a Shimadzu TOC-5000A. Five test repetitions were performed in order to establish the precision of measurement.

4. Results

Physical/Chemical Well Data:

Sampling depths for each nested well are shown along with physical/chemical data in Table 1. As expected, initial TPH concentrations were highest for the first well along the plume transect (Well # K5-7). For this first well the TPH concentrations ranged from 400 to 3,200 ug/L and varied greatly with depth (Table 1). The second and third wells down-gradient along the plume transect (I6-2 and H6-2) exhibited very low initial TPH concentrations at all depths (Table 1).

Ferrous iron concentrations increased with increasing depth (Table 1), suggesting lower redox potential with increasing depth. However, little or no dissolved methane was observed in any of the wells at any depth (Table 1) so methanogenic conditions were not indicated. For the first well (K5-7) dissolved oxygen concentrations varied randomly with depth, ranging from less than 0.5 mg/L for the middle depths to about 2.5 to 3.0 mg/L for the deepest and shallowest samples (Table 1). For the second well (I6-2), dissolved oxygen concentrations were consistently low at 0.5 to 1.1 mg/L. The dissolved oxygen gradient was very strong for the last well along the transect (H6-2), ranging from 7.9 mg/L near the water table down to less than 0.7 mg/L at the bottom of the well (Table 1). This result was surprising because there was little TPH detected in this well and therefore low expected oxygen consumption due to hydrocarbon biodegradation. The oxygen gradient in this well may have been caused by oxygen consumption by biodegradation of natural organic material.

20-Day Biodegradation Rates:

Table 2 and Figure 2 show the concentrations of TPH in all groundwater samples before and after the 20 days of biodegradation. For the first well (K5-7), which exhibited the highest initial TPH concentrations, significant TPH degradation was observed over the 20-day period only for groundwater collected from depths where the initial TPH concentration was greater than 500 ug/L (K5-7AA, K5-7A, and K5-7D) (Figure 2). For these samples the average observed first-order degradation rate constant was 0.014 day^{-1}. For depths with initial TPH concentrations less than 500 ug/L, little or no hydrocarbon biodegradation was observed. It is possible that the remaining hydrocarbons under the redox conditions prevalent at these depths were recalcitrant, but the lack of observed biodegradation may have been the result of limited analytical ability for detecting changes in TPH concentration with such low initial TPH concentrations. Simulated distillation analysis of the hydrocarbon distributions (see Appendix) do not indicate any major differences in hydrocarbon composition that would explain the observed differences in biodegradation rates.

The second and third wells down-gradient along the plume transect exhibited very low initial TPH concentrations at all depths, and as a result it was not possible to observe statistically significant biodegradation for groundwater samples from these wells. Initial TPH concentrations were at or below 700 ug/L for the second well (Well # I6-2) and were all non-detect for the third well (Well # H6-2) (Table 2 and Figure 2).

Table 1. Physical-chemical data for each nested well.

Well Name	Screen Interval (feet bgs)	Date Sampled	Dissolved Oxygen	Ferrous Iron	Oxygen	Methane	Carbon Dioxide	Dissolved Iron	Nitrate as Nitrogen (NO3-N)	Sulfate	Ammonia as Nitrogen	Nitrite as Nitrogen (NO2-N	Ortho-phosphate	TPH Diluent (C12-C32)
			Field	Field	RSK 175	RSK 175	RSK 175	EPA 6020	EPA 300.0	EPA 300.0	EPA 350.1	EPA 353.2	EPA 365.1	GC/MS DILU
			mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
K5-7D	24-25	11/15/04	2.60	<0.01	**2.72**	**0.014**	6.03	na	na	na	na	na	na	**1.8**
K5-7D	24-25	11/15/04	2.60	<0.01	na	na	na	na	na	na	na	na	na	**1.8**
K5-7D	24-25	03/23/05	0.46	<0.01	**5.85**	**0.005**	3.25	**0.066**	**0.05** J	**34**	na	na	na	--
K5-7C	26.75-27.75	11/15/04	1.02	<0.01	**2.24**	**0.080**	8.11	na	na	na	na	na	na	**0.37**
K5-7C	26.75-27.75	11/15/04	1.02	<0.01	na	na	na	na	na	na	na	na	na	**0.41**
K5-7C	26.75-27.75	03/23/05	0.51	<0.01	**5.33**	**0.017**	4.12	**0.12**	**0.03** J	**50**	na	na	na	--
K5-7B	29.5-30.5	11/15/04	0.70	**0.08**	**2.59**	<0.001	8.03	na	na	na	na	na	na	**0.45**
K5-7B	29.5-30.5	11/15/04	0.70	**0.08**	na	na	na	na	na	na	na	na	na	**0.55**
K5-7B	29.5-30.5	03/23/05	0.40	**0.20**	**5.19**	**0.130**	5.41	**0.31**	<0.1	**49**	na	na	na	--
K5-7A	32.25-33.25	11/15/04	1.19	**0.14**	**2.70**	**0.002**	10.09	na	na	na	na	na	na	**2.9**
K5-7A	32.25-33.25	11/15/04	1.19	**0.14**	na	na	na	na	na	na	na	na	na	**3.4**
K5-7A	32.25-33.25	03/23/05	0.45	**0.10**	**6.22**	**0.003**	6.20	**0.19**	<0.1	**73**	na	na	na	--
K5-7AA	35.25-36.25	11/15/04	3.09	**0.23**	**4.52**	**0.003**	5.50	na	na	na	na	na	na	**2.5**
K5-7AA	35.25-36.25	11/15/04	3.09	**0.23**	na	na	na	na	na	na	na	na	na	**2.3**
K5-7AA	35.25-36.25	03/23/05	2.20	**0.20**	**6.70**	**0.002**	4.00	**0.42**	<0.1	**140**	na	na	na	--
I6-2D	66.25-67.25	12/13/04	--	na	na	na	na	na	na	na	na	na	na	**0.37**
I6-2D	66.25-67.25	12/13/04	--	na	na	na	na	na	na	na	na	na	na	**0.56**
I6-2D	66.25-67.25	03/23/05	1.13	**0.39**	**6.96**	**0.029**	2.72	**0.6**	<0.1	**34**	na	na	na	--
I6-2C	69.75-70.75	12/13/04	--	na	na	na	na	na	na	na	na	na	na	**0.23**
I6-2C	69.75-70.75	12/13/04	--	na	na	na	na	na	na	na	na	na	na	**0.22**
I6-2C	69.75-70.75	03/23/05	0.64	**0.47**	**5.20**	**0.007**	5.33	**0.61**	<0.1	**64**	na	na	na	--
I6-2B	73.25-74.25	12/13/04	--	na	na	na	na	na	na	na	na	na	na	**0.66**
I6-2B	73.25-74.25	12/13/04	--	na	na	na	na	na	na	na	na	na	na	**0.6**
I6-2B	73.25-74.25	03/23/05	0.54	**1.89**	**5.00**	**0.003**	5.16	**2.1**	<0.1	**130**	na	na	na	--
I6-2A	76.75-77.75	12/13/04	--	na	na	na	na	na	na	na	na	na	na	**0.35**
I6-2A	76.75-77.75	12/13/04	--	na	na	na	na	na	na	na	na	na	na	**0.33**
I6-2A	76.75-77.75	03/23/05	0.52	**1.90**	**5.16**	**0.003**	4.43	**3.1**	<0.1	**140**	na	na	na	--
H6-2D	63.7-64.7	04/04/05	7.87	<0.01	**9.71**	<0.001	1.07	na	**4.6**	**17**	**0.01** J	<0.02	**0.96**	<0.05
H6-2D	63.7-64.7	04/04/05	7.87	na	na	na	na	na	na	na	na	0.96	na	<0.05
H6-2C	66.6-67.6	04/04/05	6.67	<0.01	**8.52**	<0.001	2.04	na	**6.3**	**19**	**0.082**	<0.02	**0.58**	<0.05
H6-2C	66.6-67.6	04/04/05	6.67	na	na	na	na	na	na	na	na	na	na	<0.05
H6-2B	69.4-70.5	04/04/05	4.42	<0.01	**6.44**	<0.001	3.00	na	**8.1**	**36**	**0.059**	<0.02	**0.41**	<0.05
H6-2B	69.4-70.5	04/04/05	4.42	na	na	na	na	na	na	na	na	na	na	<0.05
H6-2A	72.4-73.4	04/04/05	0.69	**0.39**	**3.41**	<0.001	3.85	na	**0.79**	**52**	**0.051**	**0.048**	**0.14**	**0.051**
H6-2A	72.4-73.4	04/04/05	0.69	na	na	na	na	na	na	na	na	na	na	**0.066**
H6-2AA	76.3-77.3	04/04/05	0.68	**2.11**	**3.21**	<0.001	3.77	na	<0.10	**100**	**0.47**	<0.02	**0.14**	**0.075**
H6-2AA	76.3-77.3	04/04/05	0.68	na	na	na	na	na	na	na	na	na	na	**0.060**

Table 2. Complete analytical results for TPH concentrations, 20-day biodegradation rates, respiration rates, TOC and toxicity for all nested-well groundwater samples from the three monitoring wells.

Well Screen (depth, ft)	*Initial TPH (ug/l)*	*Final TPH (ug/l)*	*first-order rate constant (1/day)*	*20-day CO2 Evolution (ul)*	*Initial Filtered TOC (mg/l)*	*Initial Sonicated TOC (mg/l)*	*Final Filtered TOC (mg/l)*	*Final Sonicated TOC (mg/l)*	*EC50 Initial (%)*	*EC50 Final (%)*
K5-7AA (35.8)	2400	2150	0.0055	42744	42.49	37.62	31.80	31.83	72.50	178.60
K5-7A (32.8)	3150	2300	0.0157	53596	35.04	39.51	35.85	40.31	200.00	NT***
K5-7B (30)	500	640	-0.0123	47079	24.41	24.7	NA**	25.17	357.10	NT***
K5-7C (27.3)	390	365	0.0033	45180	21.14	26.16	NA**	22.49	500.00	1666.70
K5-7D (24.5)	1800	1150	0.0224	46160	29.65	64.18	NA**	37.19	833.30	NT***
I6-2A (73.8)	340	550	-0.0240	45768	46.66	79.05	28.64	84.21	6.69E+08	44148.51
I6-2B (70.3)	630	875	-0.0164	45668	34.54	75.17	22.10	82.10	231.75	11539.70
I6-2C (66.8)	225	475	-0.0374	39902	23.77	90.5	18.85	95.67	1.23E+13	3588.43
I6-2D (63.3)	465	335	0.0164	30368	32.74	76.51	14.82	91.47	404.23	21513.29
H6-2AA (76.8)	67.5	ND*	NA**	70972	21.43	24.59	21.23	26.40	2210.86	413.80
H6-2A (72.9)	58.5	ND*	NA**	60583	17.89	19.77	20.00	24.75	4184.57	3008155.72
H6-2B (70.0)	ND*	ND*	NA**	51211	20.41	29.07	21.04	31.63	7867.35	3074.75
H6-2C (67.1)	ND*	ND*	NA**	43923	16.23	26.09	18.23	22.73	3206.82	1387.01
H6-2D (64.2)	ND*	ND*	NA**	25868	14.69	16.68	22.23	18.69	13875.27	42488.71

** ND = Nondetectable concentration; value below 50 ug/l*
*** NA = Not available*
**** NT = Nondetectable toxicity*

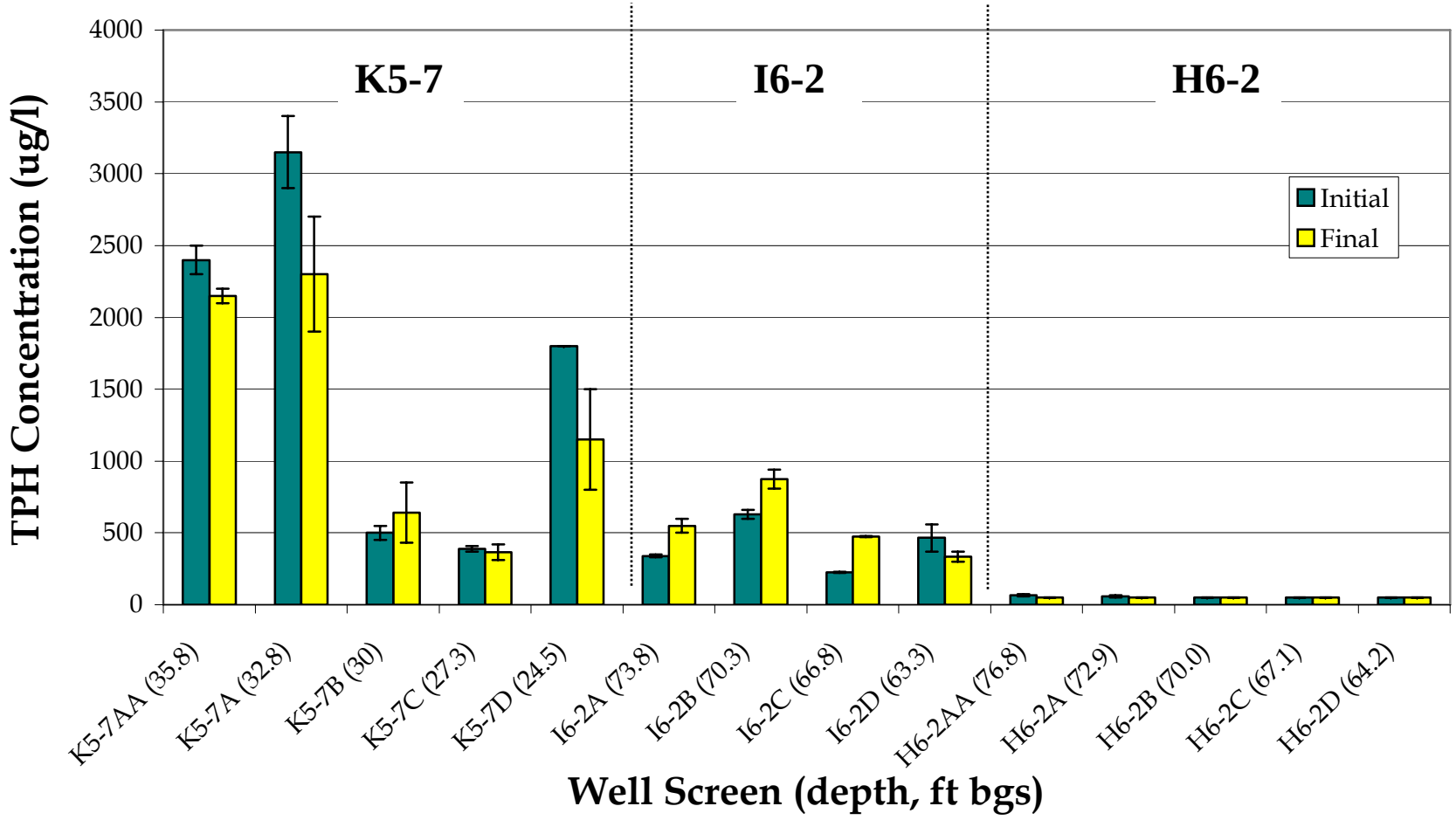


Figure 2: Initial and final TPH concentrations for all groundwater samples. Duplicate analyses were performed on each sample. The high and low error bars indicate the two values obtained. Actual data points represent the average of these values.

Toxicity results:

Overall, the Microtox® toxicities of the groundwater samples were low because of the low TPH concentrations (Table 2). Initial toxicities were highest at the K5-7 location (Figure 3), a result expected considering the higher TPH concentration of K5-7 groundwater. The Microtox® toxicity was dramatically reduced after 20 days of biodegradation for all groundwater samples with initial TPH concentration above the detection limit (Figure 3). Reductions in toxicity were observed even when TPH concentrations were low.

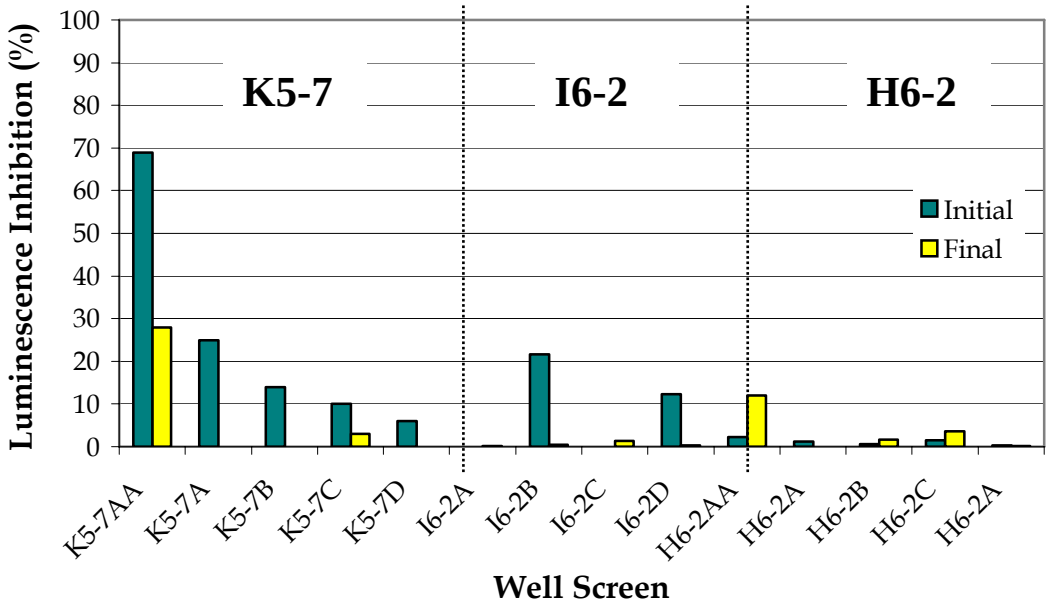


Figure 3. Microtox toxicity of each groundwater sample before and after 20 days of biodegradation in the laboratory.

For the initial samples before biodegradation, Microtox® toxicity generally increased with increasing initial TPH concentration (Figure 4). After 20 days of biodegradation in the lab, the final Microtox® toxicity was very low for almost all of the groundwater samples (Figure 4). This suggests that the hydrocarbon compounds biodegrading most rapidly are likely to be the hydrocarbons that contribute most to Microtox® toxicity.

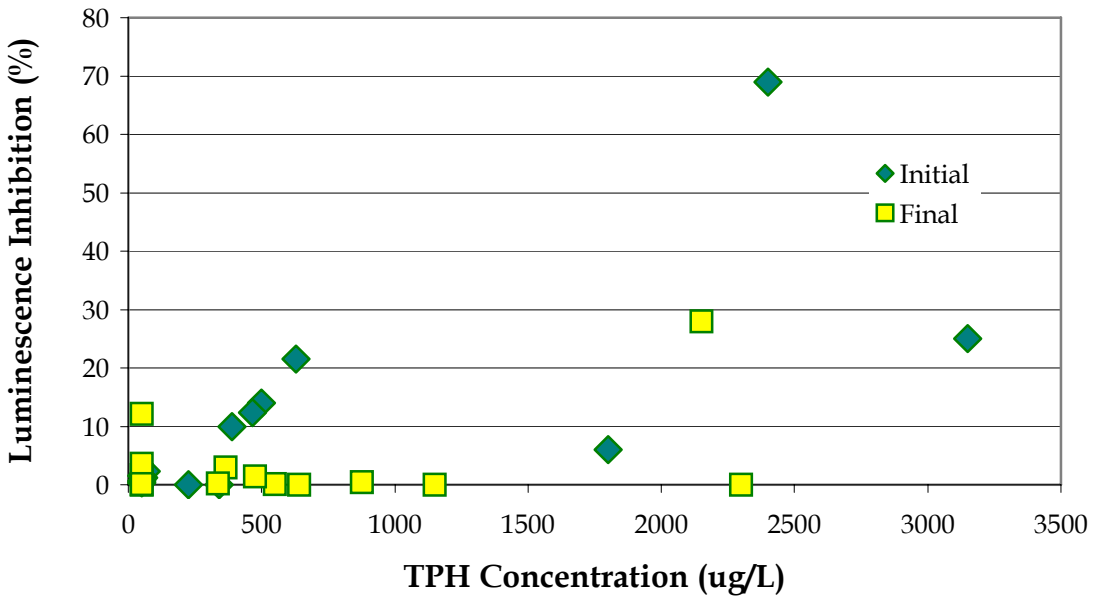


Figure 4. Correlation of Microtox toxicity with TPH concentrations before and after biodegradation.

Respirometry Results:

Figure 5 shows the total volume of CO_2 produced over 20 days by each two-liter groundwater sample. Significant CO_2 production was observed in all samples regardless of initial TPH concentration, suggesting that there may be significant biodegradation of natural organic material in these samples.

Figure 6 shows the volume of CO_2 produced as a function of time during the 20-Day Biodegradation experiment. Note that CO_2 production in control samples (DI water) was minimal.

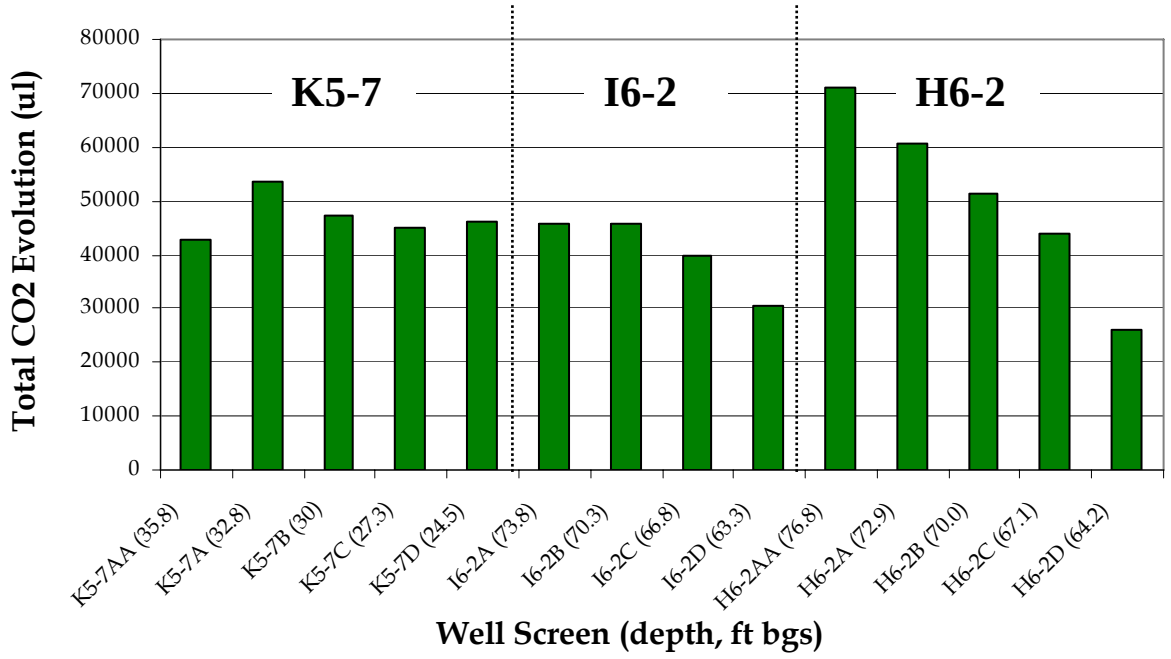
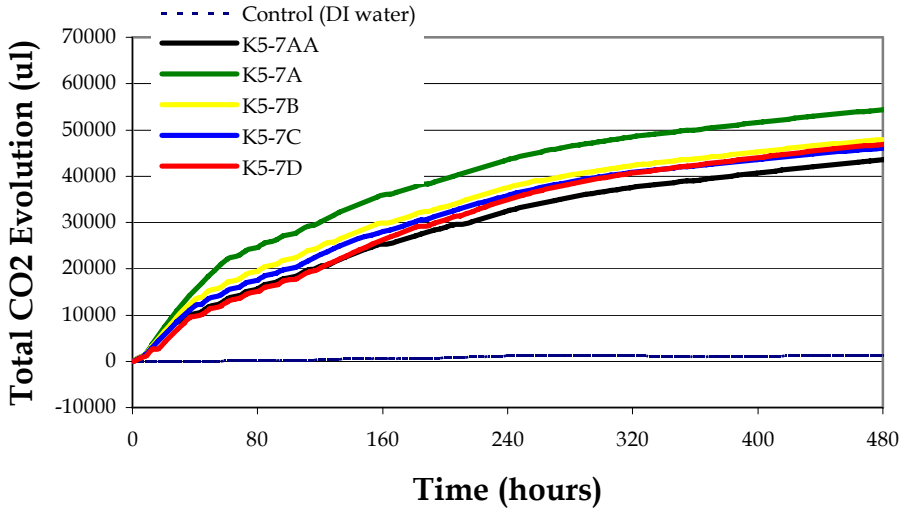
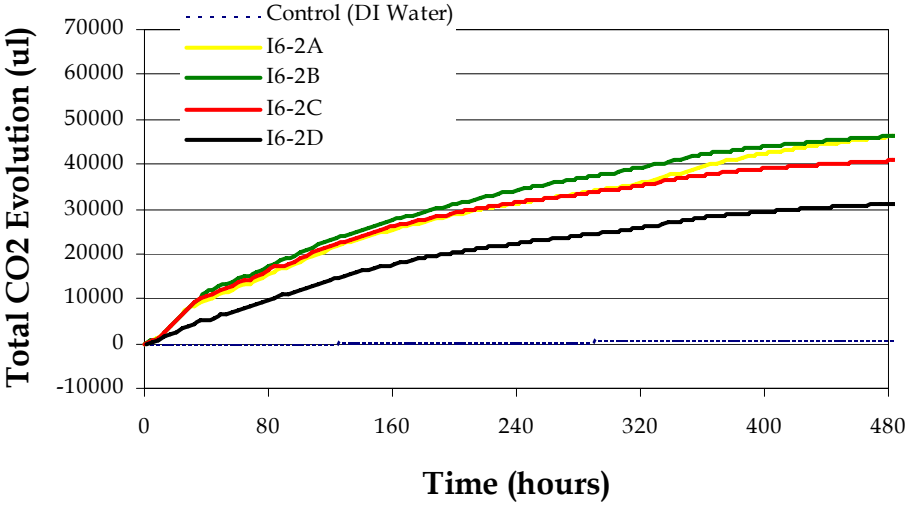


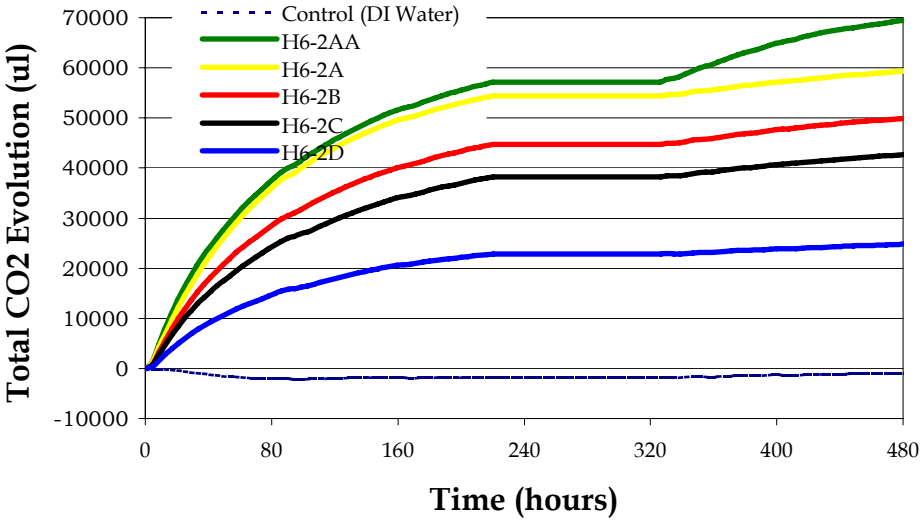
Figure 5. 20-day cumulative CO_2 evolution from groundwater samples under aerobic conditions.



K5-7



I6-2



H6-2

Figure 6. Cumulative CO_2 production for groundwater from each of the nested wells.

Total Organic Carbon Concentrations:

TOC was measured for each of the groundwater samples both after sonicating to homogenize suspended particulate material and after filtering. In this way dissolved TOC could be observed separately from total TOC. For Wells K5-7 and H6-2 the TOC concentration was similar for unfiltered/homogenized and filtered samples, indicating that nearly all of the TOC was in the dissolved phase (Figure 7). In contrast, unfiltered/homogenized TOC was significantly higher than filtered TOC for all samples from Well I6-2 (Figure 7), suggesting that a large portion of the TOC was in the particulate phase.

The measured TOC concentrations are more than an order of magnitude greater than the TPH concentrations (Table 2). This suggests that much of the organic carbon is associated with natural organic material, which is consistent with the conclusions made from the respirometry results. Very little change in TOC concentration was observed after 20 days of biodegradation (Table 2).

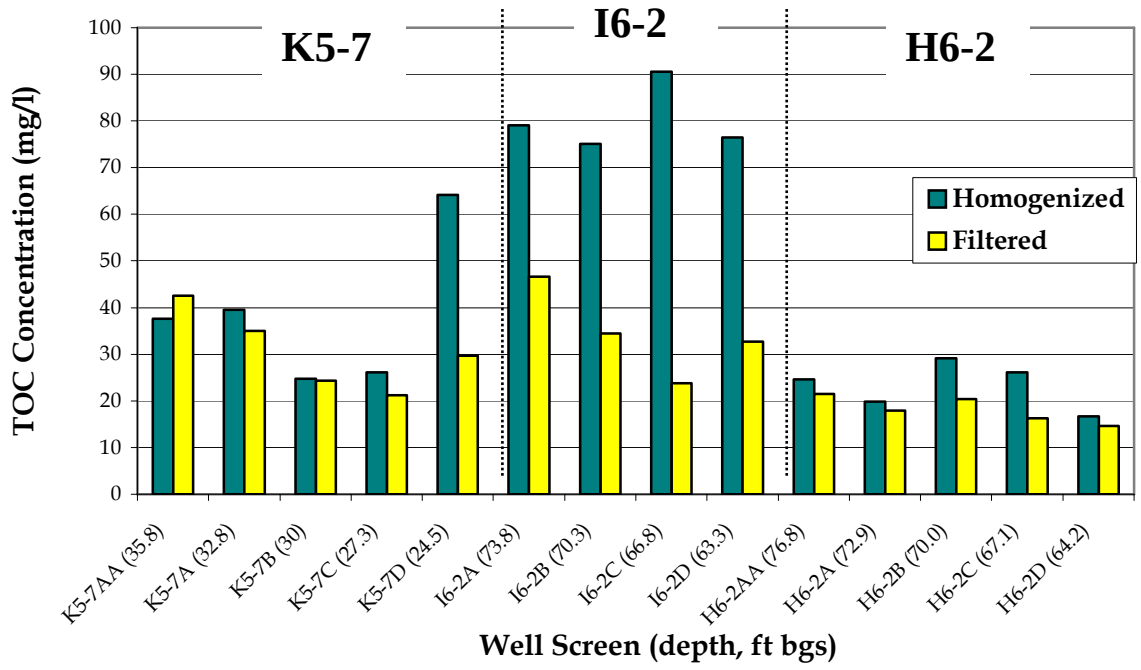


Figure 7: Measured TOC concentrations for both filtered and homogenized initial groundwater samples.

Inorganic Nutrient Concentrations:

It is important to evaluate nutrient concentrations for each of the samples to determine if biodegradation may have been limited by nutrient availability for any of the groundwater samples. Table 3 shows the concentrations of sulfate, nitrate, nitrite, ammonium, and ortho-phosphate in all tested samples. Adequate nitrogen and phosphate nutrients were available in all groundwater samples, except for H6-2AA and H6-2A, which contained low phosphate concentrations (Table 3). However, for these H6-2 samples, nutrient availability was not an issue because TPH concentrations were at or below the detection limit. Nutrient data were not available for Well I6-2. Clearly the limited biodegradation observed for two of the K5-7 samples (B and C) cannot be attributed to lack of N and P nutrients.

Table 3: Concentrations of key inorganic nutrients in groundwater samples from Wells K5-7, I6-2, and H6-2.

	Sulfate (ppm)	Nitrate (ppm)	Nitrite (ppm)	Ammonium (ppm)	ortho-Phosphate (ppm)
K5-7AA	120	<PQL	<PQL	0.2	0.65
K5-7A	81	<PQL	<PQL	0.06	0.9
K5-7B	57	<PQL	<PQL	0.1	3.9
K5-7C	54	<PQL	<PQL	0.34	6.5
K5-7D	48	<PQL	<PQL	0.54	7.9
I6-2A					
I6-2B					
I6-2C					
I6-2D					
H6-2AA	100	<PQL	<PQL	0.47	0.14
H6-2A	52	0.79	0.048	0.051	0.14
H6-2B	36	8.1	<PQL	0.059	0.41
H6-2C	19	6.3	<PQL	0.082	0.58
H6-2D	17	4.6	<PQL	0.01	0.96

PQL=Practical Quantitation Limit

5. Conclusions

These laboratory biodegradation experiments showed that diluent hydrocarbons were biodegraded under aerobic conditions, but the amount of biodegradation observed varied with initial TPH concentration. For groundwater samples with initial TPH concentrations greater than 500 ug/L, significant biodegradation was observed in 20 days. For these samples the observed first-order degradation rate constant was approximately 0.014 day^{-1}. This first-order rate constant is about half of that observed in a previous Guadalupe study with horizontally-distributed wells (Dreyer, 2004) and in laboratory soil columns (Cunningham, 2004). More detailed kinetic analyses were attempted, but because of the limited number of samples with sufficiently high TPH concentrations, such analyses were not deemed warranted (Lassen, 2005).

For groundwater samples collected from depths for which the initial TPH concentrations were less than 500 ug/L, little or no hydrocarbon biodegradation was observed. It is possible that under the redox conditions prevalent at these depths the readily biodegraded hydrocarbons had already been biodegraded *in-situ*, leaving a recalcitrant hydrocarbon fraction behind. However, since these TPH concentrations were so low, the lack of observed biodegradation may have been an analytical anomaly caused by our limited analytical ability for detecting changes in TPH concentration with such low initial TPH concentrations. A follow-up study is underway to analyze an additional vertical profile with higher TPH concentrations. Ideally the nested well chosen for this follow-up study will provide a series of groundwater samples along the vertical profile that all have initial TPH concentrations much greater than 500 ug/L. This will allow us to confirm that diluent hydrocarbons are equally biodegradable in groundwater collected over a range of depths and redox conditions.

Microtox® toxicity analyses indicated that groundwater toxicity increases with inceasing TPH concentration, as expected and observed previously (Dreyer, 2004). More importantly, significant reductions in Microtox® toxicity were observed over the 20-day incubation period for all groundwater samples with detectable initial TPH concentrations. These results are very encouraging because significant reductions in toxicity were consistently obtained after a very short incubation period (20 days). Perhaps the most important toxicity observation is that Microtox® toxicity decreased significantly for samples K5-7B and K5-7C for which no reduction in TPH had been observed. This suggests that the hydrocarbon compounds that were causing the initial toxicity in these samples were readily biodegraded in 20 days, even though this biodegradation did not cause a measurable decrease in TPH at this detection range.

No correlations were observed between TPH concentration and respiration rate or between TPH concentration and TOC concentration. The TOC analyses indicated that TPH compounds accounted for only a small portion of the total organic carbon concentrations within the groundwater samples. Further, significant respiration rates were observed in samples with non-detectable levels of TPH. This suggests that there is a significant fraction of organic material besides TPH in the samples that is contributing to CO_2 production.

In addition to sampling an additional nested well, it is also recommended that more detailed chemical analyses be performed to elucidate any compositional differences among TPH compounds as a function of location or distance from source zones. In the present study the only chemical analysis performed was GC analysis with simulated distillation (SIMDIS). The SIMDIS analysis did not reveal any obvious differences between the equivalent carbon chain length distribution of hydrocarbons in groundwater samples exhibiting rapid biodegradation *vs.* those that did not biodegrade significantly in 20 days (see Appendix). In case the differences in biodegradability are due to chemical composition differences, then it would be useful to conduct more detailed chemical analyses of the hydrocarbons. This type of data may be useful in explaining variations in biodegradation rates for samples collected under different redox conditions.

Another important area for future study is biodegradation rates under anaerobic conditions. All of the biodegradation rates reported in the current study were under aerobic conditions with excess aeration provided in the laboratory. So in this study we collected groundwater from a variety of redox conditions, but then changed the conditions to aerobic to determine a simple 20-day biodegradation rate. Another valuable area for study would be to collect groundwater under anaerobic conditions and observe biodegradation rates in the lab under a variety of controlled redox conditions with different electron acceptors. Such a study is currently underway using anaerobic soil columns, and an anaerobic microcosm study is also being initiated.

An additional value of this study was the changes in microbial populations observed as a function of depth and distance from source zone in the plume. In the previous study with horizontally-distributed wells, there were no correlations observed between physical-chemical well data and microbial populations. This lack of correlation was likely the result of vertical mixing in the wells that were screened over a range of depths. This mixing would mask any effects of redox indicators. However, in the present study with nested wells, the microbiology team was able to use TRF analyses to find strong correlations between redox indicators and microbial community populations. These results are described in a separate final report submitted by Christopher Kitts.

6. References

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APPENDIX

SIMULATED DISTILLATION PLOTS FOR NESTED WELL K5-7

INITIAL SAMPLES ONLY

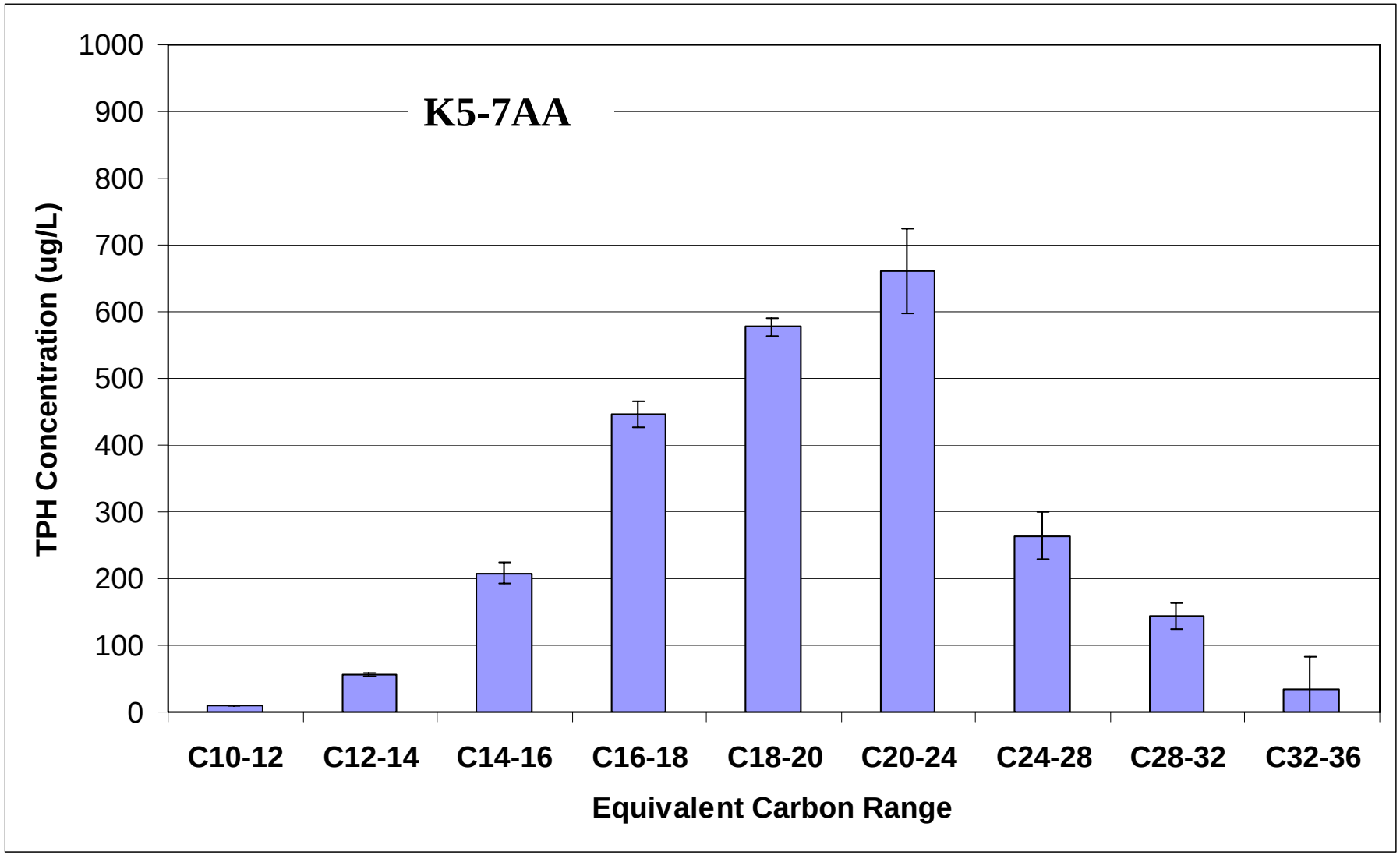

Figure A-1. SIMDIS plot for Well K5-7AA

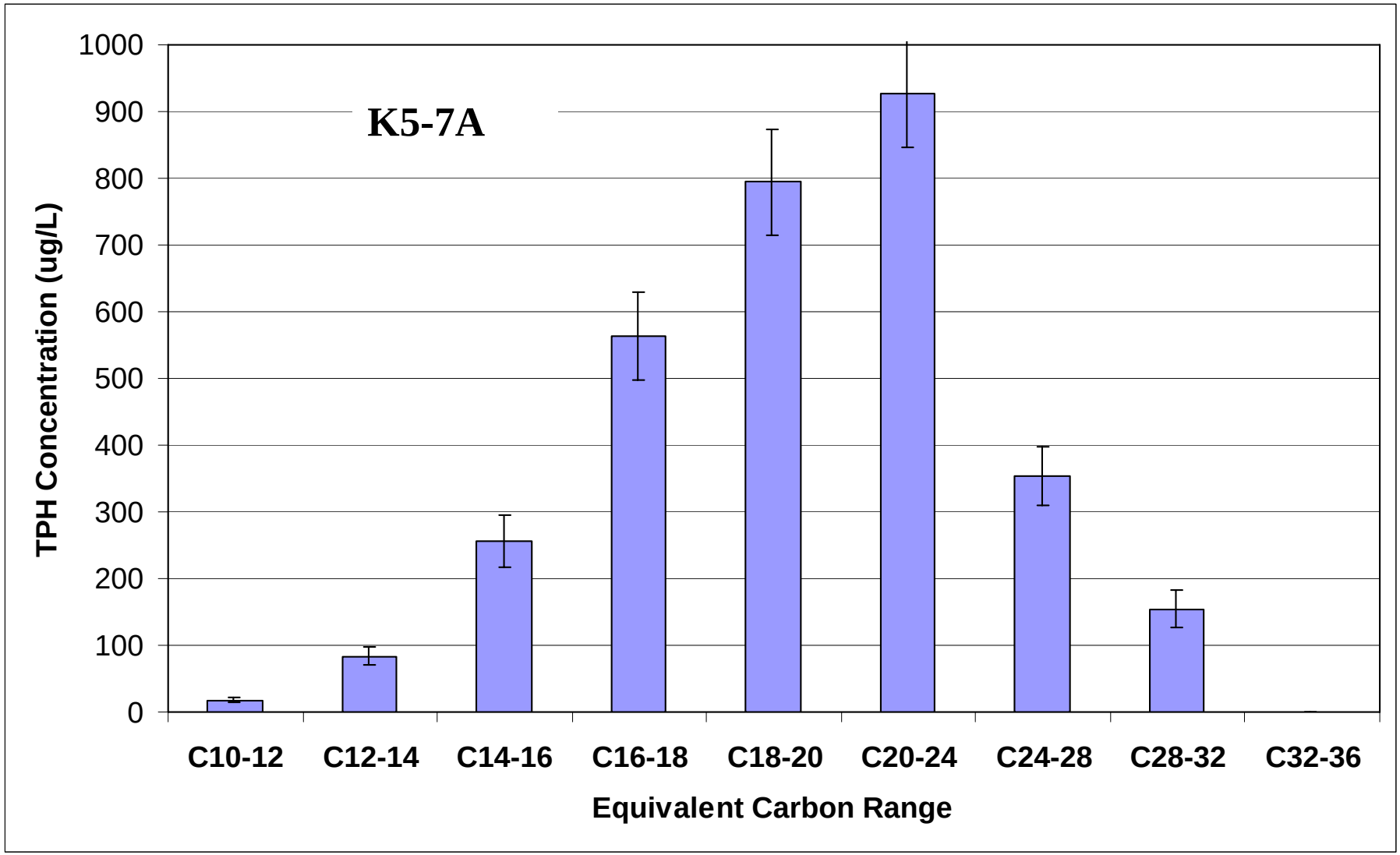

Figure A-2. SIMDIS plot for Well K5-7A

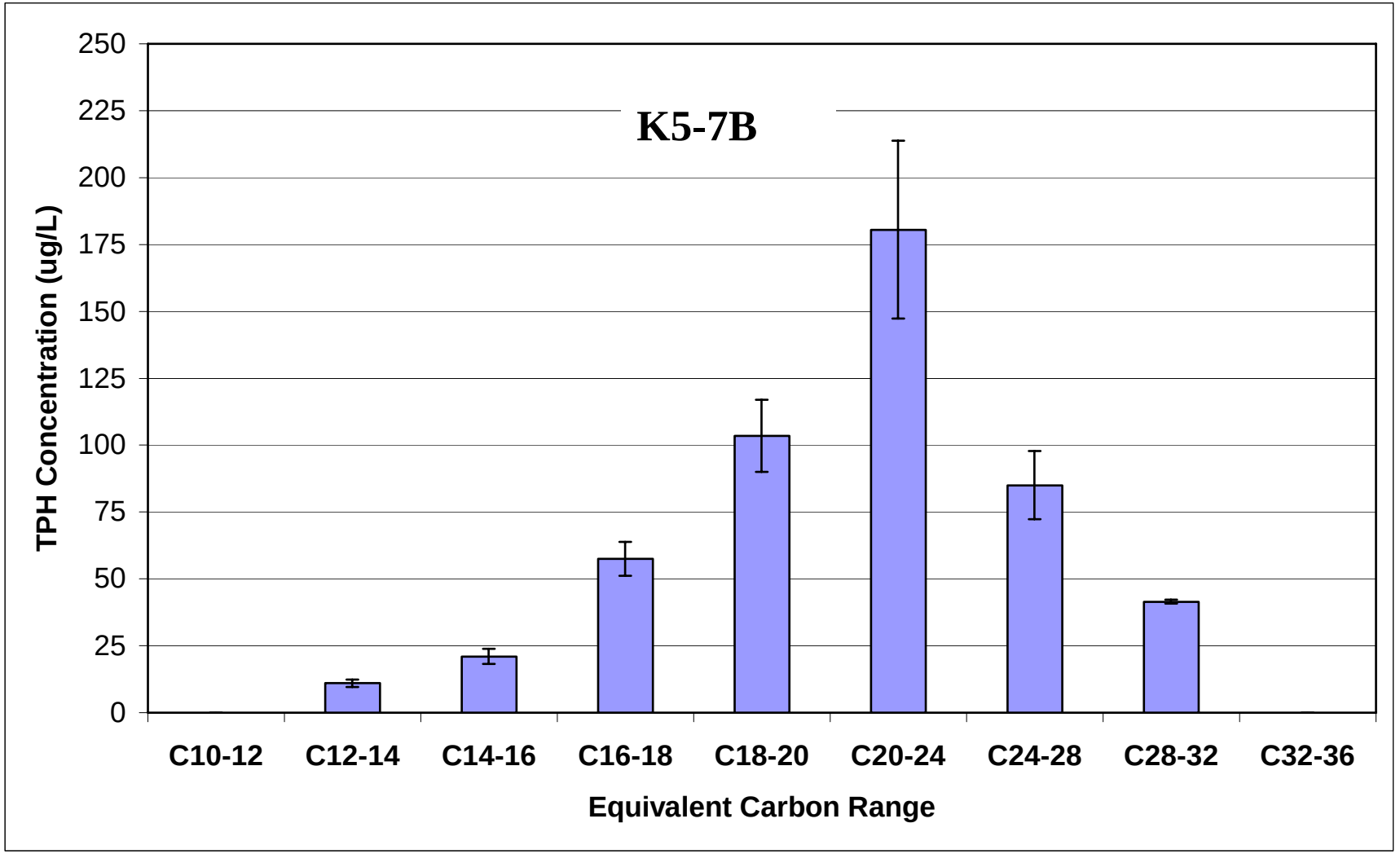

Figure A-3. SIMDIS plot for Well K5-7B

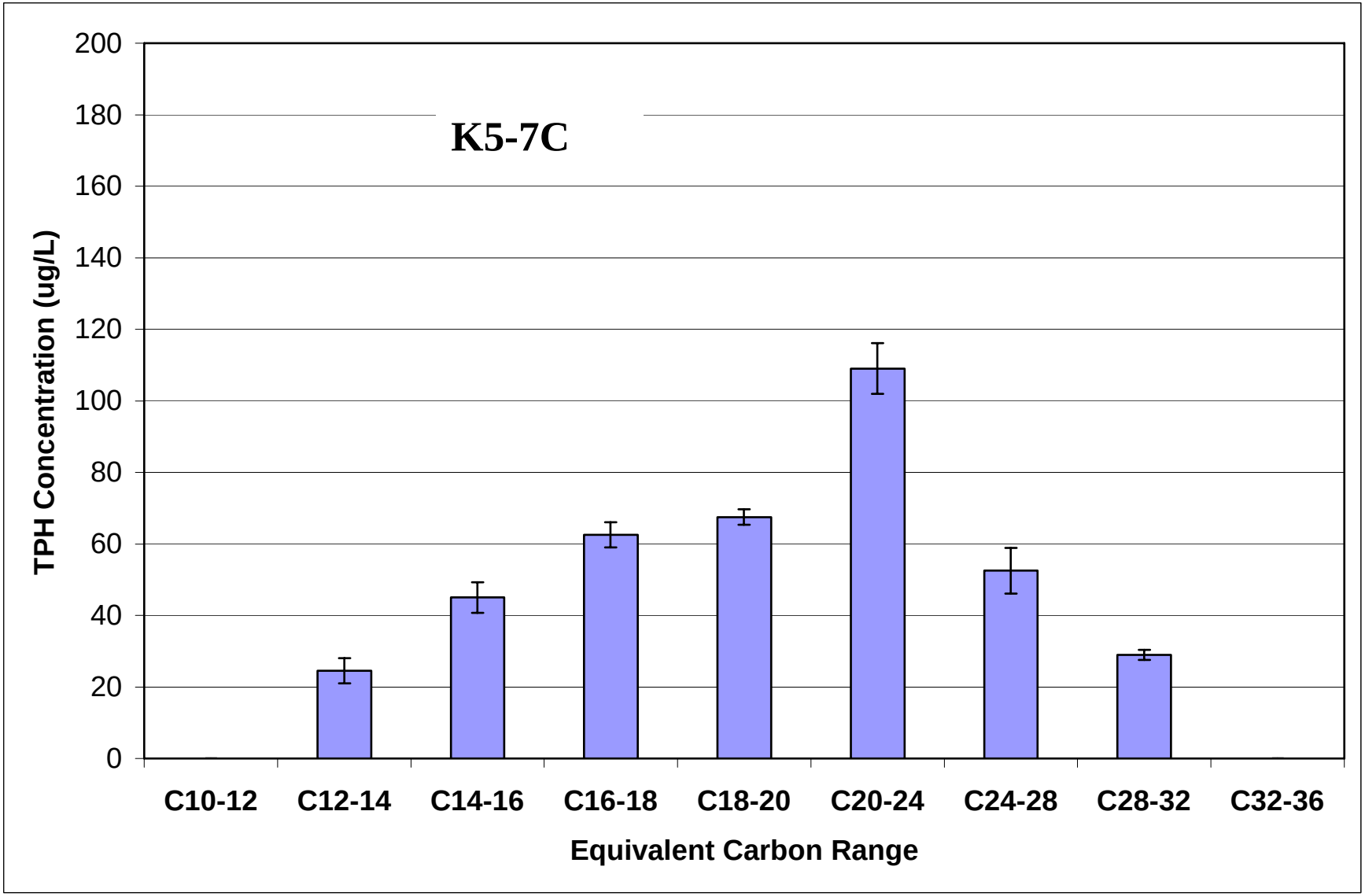

Figure A-4. SIMDIS plot for Well K5-7C

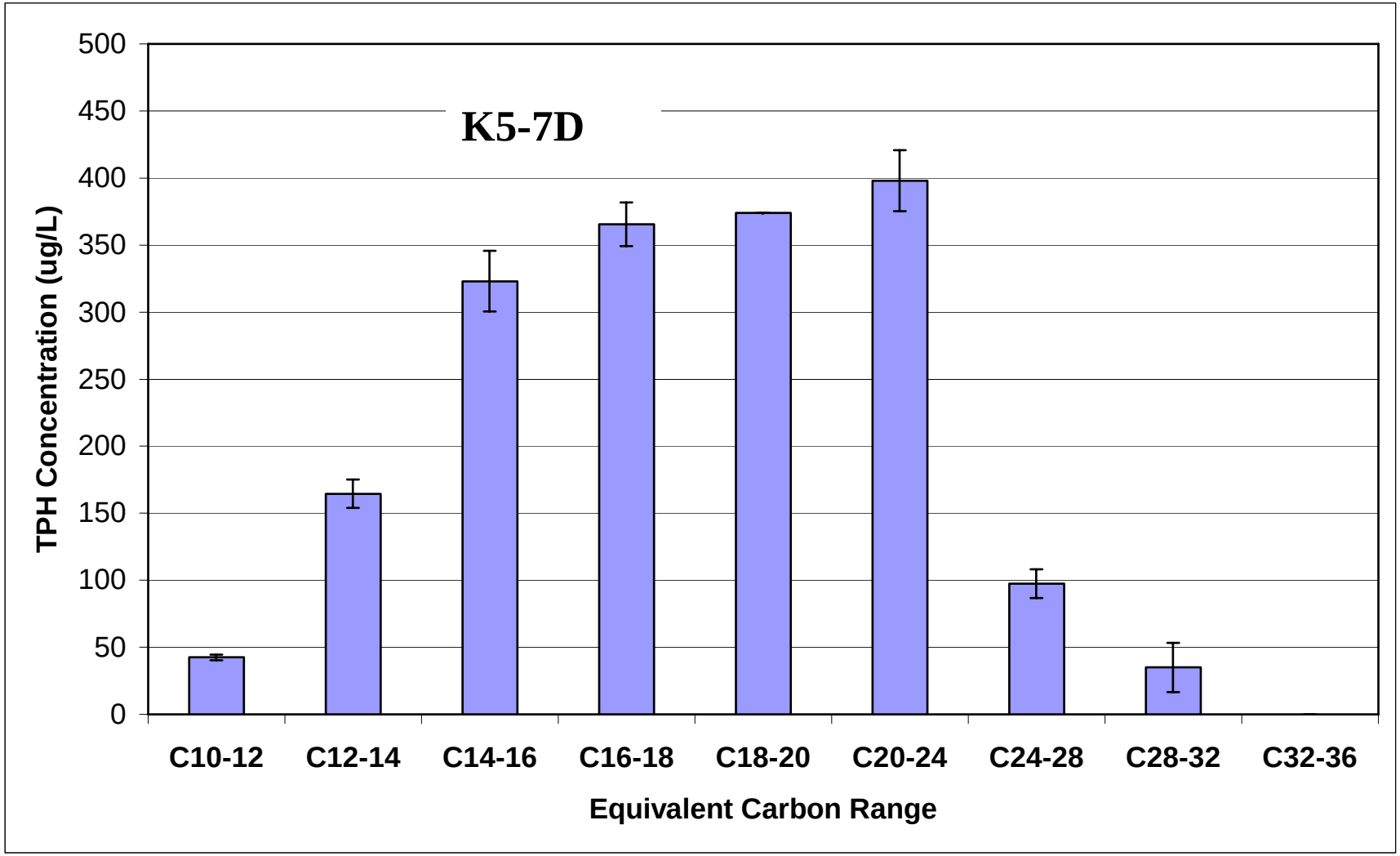

Figure A-5. SIMDIS plot for Well K5-7D