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Prepared in cooperation with the College of Engineering, Technology, and Computer Science, Tennessee State University

Partnership of Environmental Education and Research: A Compilation of Student Research, 1999–2008

"...the state is our laboratory." Dean Decatur Rogers (ret.) Tennessee State University

Open-File Report 2010–1291

U.S. Department of the Interior
U.S. Geological Survey

Cover photographs. *Collage:* students involved in research at Tennessee State University; *background photo:* Tennessee State University campus (from Google Earth images).

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Edited by Michael W. Bradley, Patrice Armstrong, and Thomas D. Byl

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Foreword

Tennessee State University College of Engineering and the U.S. Geological Survey developed a collaborative partnership to coordinate and develop a synergy between the university and USGS research in bioremediation, to provide cross-discipline research opportunities to the students, and to develop research programs in hydrology, water quality, and bioremediation. The collaboration was eventually titled PEER—Partnership for Environmental Education and Research.

PEER made significant contributions to the Tennessee State University Environmental Program in three major areas: Learning, Inquiry, and Engagement.

1. PEER-directed *Learning* took place in STEM-based pre-college programs, Bachelor of Science Civil and Environmental Engineering Program, and Master of Environmental Engineering Program. PEER directed *Learning:*

    - stimulated student interest in the field of environmental engineering and science,
    - helped mentor undergraduate and graduate students through classes, internships, and directed research, and
    - promoted student development through participation in regional and national professional conferences.

2. PEER established a focused, sustained, and quality-directed program of *Inquiry*, which resulted in science fair projects for pre-college youth, award-winning capstone projects for Bachelor of Science Engineering Seniors and regional and national award winning Master's Theses. PEER focused *Inquiry:*

    - helped students make a connection between laboratory research and field trials,
    - promoted scientific advances in quantifying biodegradation of contaminants in karst aquifers,
    - added to our understanding of how to stimulate biodegradation of recalcitrant compounds in groundwater, and
    - demonstrated wetland remediation of urban non-point source contamination.

3. PEER-directed *Learning*, focused *Inquiry*, and broad based engagement resulted in an enhanced, enriched, multi-disciplined, team-engaged quality Environmental Engineering Program. PEER broad based *Engagement:*

    - improved the TSU Environmental Program by bringing in significant grants and contracts,
    - promoted cross-discipline research, uniting teams of engineers, biologists, chemists, mathematicians, and other earth scientists, and
    - helped advance USGS research in natural remediation.

TSU and USGS have both benefited from the partnership. Significant advances have been made in fields of karst hydrology and contaminant remediation. More importantly, environmental engineering students have benefited the most. The students learn what it takes to get out of the class room, to get on the ground—in the mud and muck of wetlands and marshes—to use their education and the experiences from PEER to solve urgent real-world environmental problems.

As you read this document and marvel at the depth, breadth and quality of the students' work, take particular note of the PEER research faculty, research scientists, research institutions and research organizations that contributed to the exciting achievements of the TSU students!

Dr. Decatur Rogers, Former Dean
College of Engineering, Technology, and Computer Science
Tennessee State University, Nashville, TN 37209

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Conversion Factors

Inch/Pound to SI

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Area	
square foot (ft^2)	929.0	square centimeter (cm^2)
square mile (mi^2)	2.590	square kilometer (km^2)
	Volume	
gallon (gal)	3.785	liter (L)
cubic foot (ft^3)	0.02832	cubic meter (m^3)
	Flow rate	
acre-foot per day (acre-ft/d)	0.01427	cubic meter per second (m^3/s)
foot per second (ft/s)	0.3048	meter per second (m/s)
gallon per minute (gal/min)	0.06309	liter per second (L/s)
gallon per day (gal/d)	0.003785	cubic meter per day (m^3/d)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8×°C)+32$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

$$°C=(°F-32)/1.8$$

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C).

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

Note: Any use of trade, product, or firm names in this document is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Photographs used in this report were by U.S. Geological Survey employees unless otherwise noted.

Partnership of Environmental Education and Research: A Compilation of Student Research, 1999–2008

Edited by Michael W. Bradley[1], Patrice Armstrong[2], and Thomas D. Byl[1]

Abstract

The U.S. Geological Survey (USGS) Tennessee Water Science Center and the College of Engineering and Technology at Tennessee State University developed a Partnership in Environmental Education and Research (PEER) to support environmental research at TSU and to expand the environmental research capabilities of the USGS in Tennessee. The PEER program is driven by the research needs to better define the occurrence, fate, and transport of contaminants in groundwater and surface water. Research in the PEER program has primarily focused on the transport and remediation of organic contamination in karst settings. Research conducted through the program has also expanded to a variety of media and settings. Research areas include contaminant occurrence and transport, natural and enhanced bioremediation, geochemical conditions in karst aquifers, mathematical modeling for contaminant transport and degradation, new methods to evaluate groundwater contamination, the resuspension of bacteria from sediment in streams, the use of bioluminescence and chemiluminescence to identify the presence of contaminants, and contaminant remediation in wetlands.

The PEER program has increased research and education opportunities for students in the College of Engineering, Technology, and Computer Science and has provided students with experience in presenting the results of their research. Students in the program have participated in state, regional, national and international conferences with more than 140 presentations since 1998 and more than 40 student awards. The PEER program also supports TSU outreach activities and efforts to increase minority participation in environmental and earth science programs at the undergraduate and graduate levels. TSU students and USGS staff participate in the TSU summer programs for elementary and high school students to promote earth sciences. The 2007 summer camps included more than 130 students from 20 different States and Washington DC.

Introduction

The U.S. Geological Survey (USGS) Tennessee Water Science Center and the College of Engineering, Technology, and Computer Science at Tennessee State University (TSU) developed a partnership in environmental education and research (PEER). The partnership was initially developed in 1998 between TSU and the USGS to support environmental research at TSU and to expand the environmental research capabilities of the USGS in Tennessee. The objectives of the partnership are to conduct environmental research and to promote environmental education through research and hands-on activities. Dean Decatur Rogers (TSU, retired) in describing one benefit, said of the PEER program, “The entire State is now our laboratory.” Research conducted by USGS scientists, TSU professors, and students in the PEER program has been presented in a wide range of local, State, national and international conferences.

The PEER program is driven by research needs to better define the occurrence, fate, and transport of contaminants in groundwater and surface water. Contamination of surface water and groundwater affects the adequacy of water resources to support healthy ecosystems and to meet the ever-increasing demands for municipal, agricultural, and industrial use. Research conducted through the PEER program in the USGS Tennessee Water Science Center currently is focused on the occurrence, fate, and transport of contaminants in karst terrain, in unconsolidated aquifers, and in wetlands.

The PEER program includes students in the engineering, environmental engineering, chemistry, and biology programs. USGS scientists assist and mentor students in defining research topics and mentor students in proper data collection, documentation, and quality assurance and quality control. Students in the PEER program have worked in USGS Water Science Centers in Nashville, Tennessee, Louisville, Kentucky, Indianapolis, Indiana, and Columbia, South Carolina and in the USGS Biological Science Center in Rolla, Missouri.

The PEER program also supports TSU outreach activities and efforts to increase minority participation in environmental and earth science programs at the undergraduate and graduate levels. The PEER program has increased research and education opportunities for the College of Engineering and Technology. Students in the program have participated in state, regional and nation al conferences with more than 140 presentations since 1998 and more than 40 student awards. USGS scientists have developed new curriculum for the environmental engineering programs. PEER students and USGS staff participate in the TSU summer programs for elementary and high school students to promote earth sciences. The 2007

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summer camps included more than 130 students from 20 States and from Washington DC.

The PEER program and the involvement of USGS were instrumental in assisting TSU in receiving a grant from the National Science Foundation (NSF) to increase the number of minority students in earth sciences (geology, hydrology, and environmental science and engineering). The NSF grant has allowed TSU, with assistance from the PEER program and the USGS, to develop programs at TSU with the National Park Service (Mammoth Cave) to encourage, develop, and mentor minority students in the earth sciences. TSU, through the PEER program, has been recognized by the American Society for Engineering Education (ASEE) for educating, on average, more than 10 percent of the Nation's African-American students entering graduate programs in environmental engineering; this percentage increases to 30 percent during some years (Dr. Decatur Rogers, former Dean of Engineering ret, TSU, oral comm., 2006). Research conducted on natural attenuation by students in the PEER program was featured in an article on environmental research at historically black colleges and universities (Witherspoon, 2008).

The PEER program has included more than 70 students in studies in seven general research topics at 10 locations across Tennessee (fig. 1). The research has resulted in more than 50 abstract, articles, and presentations at professional conferences at the local, regional, national, and international level (table 1). The purpose of this report is to provide a description of the PEER program including descriptions of the research areas, a summary of the accomplishments in awards and outreach activities, and a compilation of abstracts and papers developed through the USGS and TSU research.

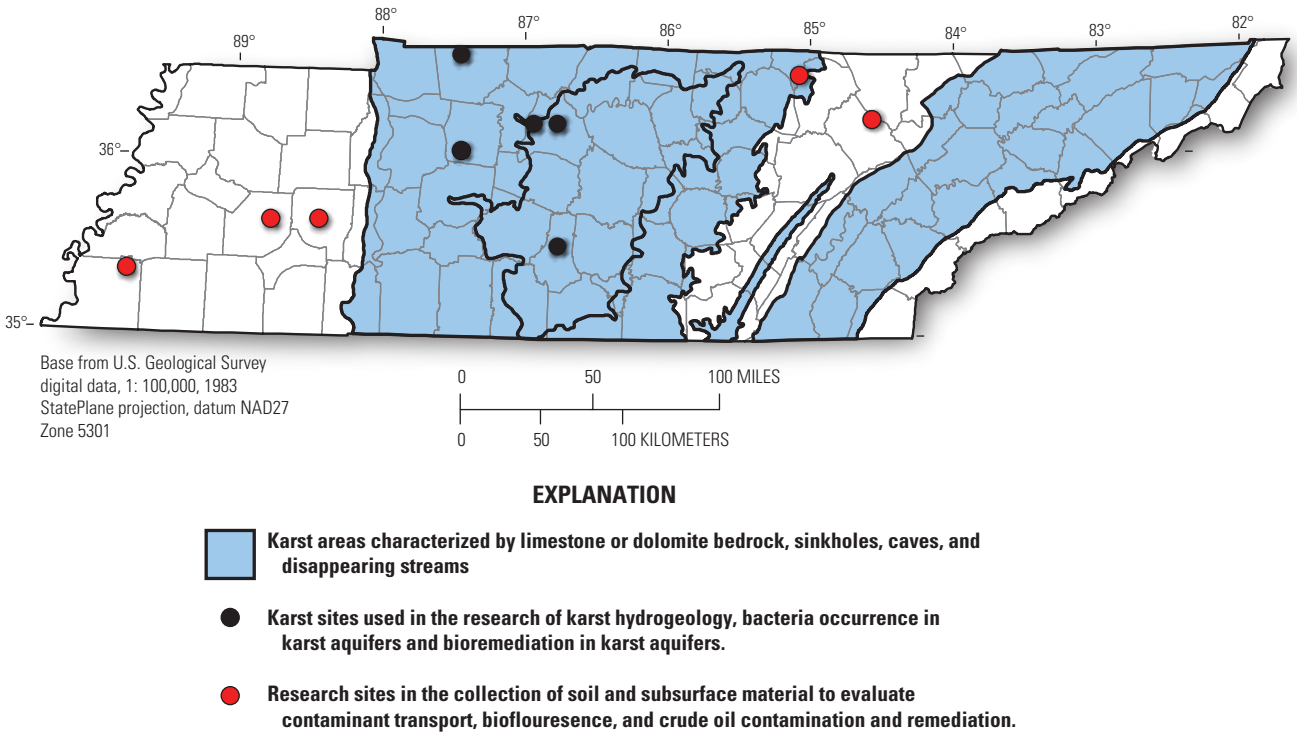

Figure 1. Karst areas in Tennessee and location of research sites for the U.S. Geological Survey/Tennessee State University Partnership in Environmental Education and Research Program, 1998–2008.

PEER Research Areas

More than two-thirds of the State of Tennessee is underlain by limestone and dolomite with karst features such as caves, sinkholes, and disappearing streams (figure 1). Contamination in karst areas can be difficult to fully characterize and remediate. Groundwater and surface water in karst settings are linked in an interactive system that can be complex and difficult to predict or model. Temporal variations in natural water quality and contaminant concentrations and highly variable geochemical, biological, and hydrogeological processes affect the fate and transport of contaminants in karst. Research conducted through the PEER program can provide additional information to better understand these processes and to help improve strategies for pollution prevention, mitigation, and remediation in karst.

Research in the PEER program has focused primarily on the transport and remediation of organic contamination in karst settings. Accordingly, most of the abstracts and student presentations have described contaminant occurrence and transport, natural and enhanced bioremediation, and geochemical conditions in karst aquifers. As the activities in karst areas expanded, PEER research included mathematical modeling for contaminant transport and degradation, the adaptation of residence-time-distribution (RTD) and Lattice-Boltzmann models to karst aquifers, the occurrence and role of bacteria in karst aquifers, and the occurrence and fate of different contaminants in karst aquifers.

Although the PEER activities have focused on karst settings, research has expanded to a variety of media and settings. Research has been conducted on new methods to evaluate groundwater contamination, the resuspension of bacteria from sediment in streams, the use of bioluminescence and chemiluminescence to identify the presence of contaminants, and contaminant remediation in wetlands.

The research compiled in this report is grouped by subject. The first group contains abstracts and papers that describe the research conducted in karst areas, including bioremediation, research on modeling contaminant degradation in karst aquifers, and other research on karst hydrology. Additional research conducted through PEER includes methods in contaminant evaluation, contaminant transport and remediation in wetland and streams, the use of bioluminescence to monitor water quality, and the degradation and transport of other contaminants in the environment.

Karst—Bioremediation

Initial research conducted through the USGS and TSU partnership supported USGS investigations of contamination in karst areas of Tennessee in general (Wolfe and others, 1997). Research examining bioremediation in karst aquifers was generally lacking and many felt that karst aquifers were not suitable for bioremediation due to rapid flow conditions, a perceived relatively low surface to volume ratio and perceived absence of bacteria. Conceptual models developed by Wolfe and others (1997) indicated that contaminants in karst systems occurred not only in the active, large conduit, but also along small fractures, bedding planes and other isolated features. Groundwater and contaminants in the isolated areas could provide an environment for biodegradation if the bacterial and geochemical conditions were suitable.

PEER students conducting research in this area documented the presence of bacteria in karst systems that were suitable for biodegradation, documented the occurrence of natural and enhanced bioremediation in karst systems, utilized laboratory microcosm studies to simulate degradation in karst aquifers, and defined geochemical conditions suitable for biodegradation. Research was conducted in Dickson, Marshall, and Montgomery Counties, Tennessee.

Table 1. Summary of student participation, research areas, and awards for students in the PEER program, 1998–2008.

Category	Number of abstracts	Number of students	Number of Awards*
Karst bioremediation	22	25	8
Karst modeling	10	8	5
Karst hydrology	5	7	1
Methods	5	6	—
Wetlands and streams	6	11	4
Luminescence	4	8	3
Other contaminants	5	11	1

* Does not include University awards

Microcosm Study to Assess the Potential for Intrinsic Bioremediation at a Karst Site

P.R. Franklin[1], S.D. Williams[2], J.J. Farmer[2], T.D. Byl[2]

The potential for bioremediation of trichloroethylene (TCE) is being examined at a karst site in Middle Tennessee where "pump and treat" wells are used to slow the movement of ground water away from the site and to remove some of the TCE. Ground-water samples were collected from nearby wells and analyzed to identify geochemical indicators of bioremediation (electron donors and acceptors), and to monitor trends in the concen¬trations of TCE and intermediate degradation products. Bacteria associated with the degradation of TCE were identified in water samples using the RNA oligonucleotide hybridization technique. Water-quality data indicate that both aerobic and anaerobic conditions occur in the contaminated karst aquifer. Sulfate-reducing and methanogenic bacteria, both associated with the reductive dechlorination of TCE (an anaerobic process), were present in samples from anaerobic wells. Methanotrophs and ammonia-oxidizing bacteria, both associated with co-metabolic degradation of TCE (an aerobic process), were present in samples from aerobic wells. Aerobic and anaerobic bacteria were identified in samples from wells that fluctuated in dissolved-oxygen concentration. Microcosm studies of ground water collected from the karst aquifer suggest that both biodegrada¬tion pathways are active. The rate of biodegradation was rapid in some samples, with a TCE half- life of 35 days. Degradation products indicative of the reductive dechlorination process were detected in other samples at 10 months. Additional water-quality and hydrologic monitoring of the karst aquifer are being conducted to determine temporal and spatial changes in water quality and to evaluate how these changes may affect the biodegradation processes at the site.

Published as Franklin, P.R., Williams, S.D., Farmer, J.J., Byl, T.D., Microcosm study to assess the potential for intrinsic bioremediation at a karst site: *in* Crabtree, L.R., Bradley, M.W., Blunt, Tiffany, and Pierre, Salnave, eds., 1999, Proceedings from the Ninth Annual Tennessee Water Resources Symposium, April 1999, Nashville, Tennessee: Tennessee AWRA, p. 1B-51.

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Biodegradation of Fuel in a Karst Aquifer

Kamalah Minor[1], Raushanah Muhammad[1], Tavy Wade[1], Allyn Allison[2], and Tom D. Byl[3]

Complex hydrogeologic conditions coupled with poorly understood biodegradation processes in karst aquifers have led many to believe that the potential for natural attenuation of petroleum fuel hydrocarbons is limited. This research addressed the capacity for biodegradation processes in karst. Groundwater samples were collected for bacteria and geochemical analysis from two monitoring wells (MCI-1 and MCI-4) in a karst bedrock aquifer. Water from the MCI-1 well has consistently tested positive for fuel contamination during the past 3 years of semi-annual monitoring. Water from MCI-4 has been relatively uncontaminated during the same time period. Bacteria concentrations were 50% greater in ground-water samples from the fuel-contaminated well. Additionally, bacteria isolated from fuel-contaminated ground-water samples readily grew on Petri dishes with dissolved toluene and benzene as the only source of food. Water from the less contaminated MCI-4 well had a greater dissolved oxygen concentration (6.4 milligrams per liter) than the fuel-contaminated water (dissolved oxygen less than 0.1 milligram per liter). Also, where the oxygen concentrations were diminished, geochemical evidence indicated that anaerobic processes were active. This evidence includes elevated levels of ammonia, sulfide, and ferrous iron in the fuel-contaminated ground-water samples. Based on these results, biodegradation of fuel constituents in the karst aquifer is indicated, and therefore, natural attenuation should not be disregarded because of preconceptions about low microbial activity in karst aquifers.

Published as Minor, K., Muhammad, R., Wade T., Allison, A., and Byl, T., Biodegradation of fuel in a karst aquifer *in* Tennessee Section, American Water Resources Association, 2001, Proceedings from the Eleventh Annual Tennessee Water Resources Symposium, April 2001, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-6.

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Comparison of Different Lactic Acid Formulations Used to Enhance Biodegradation of Perchloroethylene

Ahlam Ary[1], LyTreese Hampton[1], and Tom D. Byl[2]

A sanitary landfill situated on a karst terrain in northern Tennessee has leaked chlorinated solvents, such as perchloroethylene (PCE) and trichloroethylene (TCE), into a karst aquifer. Some biodegradation apparently has occurred based on the presence of breakdown products such as cis-dichloroethylene (cDCE) found in water from wells screened in the karstic bedrock. Sulfur-reducing bacteria, which are known to biodegrade chlorinated solvents, have been identified in water collected from these same bedrock wells. Previous studies have found that sulfur-reducing bacteria are stimulated by sodium lactate. A study was conducted to evaluate different formulations of lactic acid to determine which was the best for PCE biodegradation by indigenous karst bacteria. Water from the karst bedrock wells was collected and used to make 250-milliliter water microcosms. The microcosms were stocked with 2.5 milligrams per liter PCE and different formulations of lactic acid, including sodium lactate, lactic acid, magnesium lactate, iron lactate, crude lactate, methyl-lactate, potassium lactate, calcium lactate, ethyl-lactate, propyl-lactate, and ammonium lactate. The concentrations of the lactic acids were normalized so that each microcosm had the equivalent of 50 lactic-acid (reducing) electrons for each PCE molecule. Preliminary results indicate that all of the lactic acids stimulated the reductive dechlorination of PCE to TCE and cDCE. Trace amounts of trichloroethane (TCA) and 1,1-dichloroethylene also were detected in some of the microcosms. Within 4 weeks, a 40- to 60-percent decrease in PCE occurred in the treated microcosms compared to the sterile control microcosms. A concurrent rise in TCE also occurred during the same time period. The microcosms treated with ammonium lactate had PCE concentrations initially drop twice as fast compared to the other treatments during the first 10 days. But after 21 days, no significant difference between the ammonium lactate treatment and the other treatments was observed. After 45 days incubation, the microcosms treated with iron-lactate and magnesium lactate resulted in a 90-percent reduction of the initial PCE. The other lactic acid treatments contributed to a 50-to 70-percent reduction of the initial PCE after a 45-day incubation period. The sterile controls had a 30-percent reduction in PCE caused by abiotic processes such as volatilization and sorption in the same time period. In conclusion, ammonium lactate initially appears to stimulate PCE biodegradation, but the effect diminishes within 21 days. Iron and magnesium lactate appear to maintain enhanced PCE biodegradation over a longer period of time than the other lactic acid formulations tested.

Published as Ary, A., Hampton, L., and Byl, T.D., Comparison of different lactic acid formulations used to enhance biodegradation of perchloroethylene *in* Tennessee Section, American Water Resources Association, 2003, Proceedings from the Thirteenth Tennessee Water Resources Symposium, April 9–11, 2003, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-2.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

[2] U.S. Geological Survey

Microbial Strategies for Degradation of Organic Contaminants in Karst

Tom D. Byl[1], Gregg E. Hileman[1], Shannon D. Williams[1], David W. Metge[2], and Ron W. Harvey[2]

Approximately 40 percent of the United States east of the Mississippi River is underlain by various types of karst aquifers (Quinlan, 1989) and more than two-thirds of the State of Tennessee is underlain by carbonate rocks and can be classified as karst (Wolfe and others, 1997). Potential industrial sources of ground-water contamination are common in karst regions; however, the fate and transport of contaminants such as fuels in karst areas are poorly understood because of the distinctive hydraulic characteristics of karst aquifers (Field, 1993). Ground-water models that predict the fate and transport of contaminants in sandy aquifers have limited application to karst aquifers. Most natural attenuation and bioremediation guidelines specify that they are not applicable in fractured rock or karst aquifers (U.S. Environmental Protection Agency, 1997).

The lack of studies examining biodegradation in karst aquifers may be due to the widespread perception that contaminants are rapidly flushed out of karst aquifers. In highly developed and well-connected conduit systems, the rate of contaminant migration is expected to be much faster than the rate of biodegradation. Field (1993) states that remediation techniques such as ground-water extraction or bioremediation are impractical in karst aquifers dominated by conduit flow; however, he also states that the belief that contaminants are rapidly flushed out of karst aquifers is a popular misconception. Large volumes of water may be trapped in fractures along bedding planes and other features isolated from active ground-water flowpaths in karst aquifers (Wolfe and others, 1997). In areas isolated from the major ground-water flowpaths, contaminant migration may possibly be slow enough that biodegradation could reduce contaminant mass if favorable microorganisms, food sources, and geochemical conditions are present (Byl and Williams, 2000; Byl and others, 2001). The capacity for biodegradation processes in a karst setting was evaluated at sites in Tennessee and Kentucky.

The potential for biodegradation of trichloroethylene (TCE) was studied in a karst aquifer at Lewisburg, Tennessee. This site was selected because of the presence of TCE degradation by-products in the karst aquifer, available site hydrologic and chlorinated-ethene information. Additional chemical, biological and hydrological data were gathered to evaluate if the occurrence of TCE degradation by-products in the karst aquifer was the result of biodegradation in the aquifer or simply transport into the aquifer. Geochemical analysis established that sulfatereducing conditions, essential for reductive dechlorination of chlorinated solvents, existed in parts of the contaminated karst aquifer. Geochemical conditions in other areas of the aquifer fluctuated between anaerobic and aerobic conditions and contained compounds associated with cometabolism, such as ethane, methane, ammonia and dissolved oxygen. A large, diverse bacteria population inhabits the contaminated aquifer. Bacteria known to biodegrade TCE and other chlorinated solvents, such as sulfate-reducers, methanotrophs, and ammonia-oxidizers, were identified from karst-aquifer water using the RNA-hybridization technique. Results from microcosms using raw karst-aquifer water found that aerobic cometabolism and anaerobic reductive dechlorination degradation processes were possible when appropriate conditions were established in the microcosms. The chemical and biological results provide circumstantial evidence that several biodegradation processes are potentially active in the karst aquifer. Additional site hydrologic information was developed to determine if appropriate conditions persisted long enough in the karst aquifer for these biodegradation processes to be significant. Continuous monitoring devices placed in four wells during the spring of 1998 documented a dual phase ground-water flow system within the karst aquifer. Dynamic areas were present within the karst aquifer where active flow occurred, as well as, stable areas in the karst aquifer that were isolated from active flow. The pH, specific conductance, low

Published as Byl, T.D., Hileman, G.E, Williams, S.D., Metge, D.W. and Harvey, R.W. Microbial Strategies for Degradation of Organic Contaminants in Karst *in* Aiken, G.R., and Kuniansky, E.L., eds., 2002, U.S. Geological Survey Artificial Recharge Workshop proceedings, April 2–4, 2002, Sacramento, California: U.S. Geological Survey Open-File Report 02–89, p. 61–62.

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Microbial Strategies for Degradation of Organic Contaminants in Karst—Continued

dissolved oxygen levels and low oxidation-reduction potentials changed very little in the stable areas isolated from active flow. The stable areas in the karst aquifer had geochemical conditions and bacteria conducive to reductive dechlorination of chlorinated ethenes. The dynamic areas of the karst aquifer associated with active flow fluctuated between anaerobic and aerobic conditions in response to rain events. Associated with this dynamic environment were bacteria and geochemical conditions conducive to cometabolism. In summary, multiple lines of evidence developed from biological, chemical and hydrological data demonstrate that a variety of biodegradation processes were active in this karst aquifer.

A second karst-aquifer site contaminated with jet fuel was also investigated. The site is located at an airfield in southern Kentucky. Ground-water samples were collected for bacteria and geochemical analysis from several contaminated monitoring wells in an unconsolidated regolith and karst aquifer that had varying concentrations of dissolved fuel. Bacteria counts ranged from approximately 700,000 bacteria per milliliter to 1.2 million depending on the well and sample collection time. These bacteria counts were derived using two methods, direct counts and BART growth tests, and the results of the two tests were within 20 percent of each other. These numbers are much greater than previously reported when tryptic soy agar was used to quantify heterotrophic bacteria in the same wells (Byl and others, 2001). Bacteria from the fuel-contaminated part of the karst aquifer had a 5% lighter buoyant density and a wider range of sizes than the bacteria from the non-contaminated well. Additionally, bacteria isolated from fuel-contaminated ground-water samples readily grew with dissolved gasoline as the only source of food. Static microcosms (n=3) set up using aerated raw karst water spiked with benzene at 1 mg/L established a biodegradation rate of 50% loss (T1/2) in 3 days. Sterile control microcosms had less than 10% benzene loss over the same time period. Additional field evidence that biodegradation was taking place in the aquifer was established by measuring geochemical indicators. The wells with screens intersecting non-contaminated sections of the aquifer had greater dissolved oxygen concentrations (generally above 2 milligrams per liter) than those intersecting more contaminated sections (dissolved oxygen less than 0.1 milligrams per liter). Also, where the oxygen concentrations were diminished, geochemical evidence indicated that anaerobic processes were active. This evidence includes elevated levels of ammonia, sulfide and ferrous iron in the fuel-contaminated ground-water samples. Based on these results, biodegradation of fuel constituents in the karst aquifer is indicated, and therefore, natural attenuation should not be disregarded because of preconceptions about low microbial activity in karst aquifers.

Optimum pH for Biodegradation of Benzene and Toluene in a Karst Aquifer

Natascha Morris[1], Gregg Hileman[2], and Tom D. Byl[2]

Jet fuel leaking from underground pipes contaminated the regolith and karst aquifer underlying a south-central Kentucky airfield. Benzene and toluene, components of jet fuel, were detected in seven fuel-contaminated wells at the airfield. Ground-water pH measured in these seven wells ranged from 5 to 11. Some microorganisms are capable of degrading aromatic hydrocarbons such as benzene and toluene, but the rate of metabolism varies with pH. A study was conducted to determine the optimum pH for biodegradation of benzene and toluene by bacteria indigenous to the karst aquifer. Batch microcosms (three replicates per treatment and sampling time) were set up using karst bacteria enriched with benzene and toluene. The pH of the water in the microcosms was adjusted to pH 2, 5, 7, 9, and 12. Little to no biodegradation occurred outside the pH range of 5 to 9; pH values higher than 9 or lower than 5 caused the rate of biodegradation to decrease rapidly. Tests of biochemical oxygen demand verified that dissolved-oxygen consumption stopped when pH values were outside the range of 5 to 9. Microcosms with a pH of 5 showed the greatest decrease in benzene and toluene concentrations (approximately an 80-percent reduction in 6 days), followed by pH values of 7 (70-percent reduction), 9 (65-percent reduction), 2 (25-percent reduction), and 12 (10-percent reduction).

During a 3-month pump-and-treat remediation process, ground-water pH was monitored in the seven fuel-contaminated wells. The pH of the water in six of the seven wells ranged from 5.2 to 6.8. One well, which initially had a pH of 11 attributed to grout water from a newly installed well 9 feet away, retained a pH above 10 during the 3-month remediation. Increased biological activity was observed in most of the wells because of the remediation. However, bacterial growth and oxygen consumption of fuel biodegradation were not indicated in the well where the pH was greater than 10. These results demonstrate that fuel biodegradation can be slowed by very high or low pH values.

Published as Morris, N., Hileman, G., and Byl, T.D., Optimum pH for biodegradation of benzene and toluene in a karst aquifer *in* Tennessee Section, American Water Resources Association, 2003, Proceedings from the Thirteenth Tennessee Water Resources Symposium, April 9–11, 2003, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-20.

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Enhanced Biodegradation of TCE in a Karst Aquifer Using Lactic Acid, Molasses, and Soy Milk

Koushik Chakraborti[1], Gregg Hileman[2], LyTreese Hampton[1], Martin Greene[1], and Tom D. Byl[2]

A sanitary landfill situated on a karst terrain in northern Tennessee has leaked chlorinated solvents, primarily trichloroethylene (TCE), into a karst aquifer. TCE has been found in water samples collected from eight wells screened in the karst bedrock aquifer. Some anaerobic biodegradation apparently has occurred in four of the eight wells tested based on the appearance of trace amounts of breakdown products. Sulfur-reducing bacteria have been found in all eight wells. In July 2002, a mixture of dye, lactic acid, molasses, and soymilk was injected into six of the eight wells to determine if the mixture would enhance reductive dechlorination of TCE. Water samples were collected and electronic monitoring devices were placed in selected wells over a period of 4 months following injection to monitor changes for geochemistry and concentrations of TCE and breakdown products, and dye. Prior to injection, TCE concentrations ranged from 1 part per billion (ppb) to 74 ppb. After approximately 4 weeks, there was a noticeable decrease in TCE and cis-dichloroethylene (cDCE) concentrations in all but one of the wells. After 4 months, there was an 85- to 100-percent decrease in TCE concentrations in the eight wells, and a 65- to 100-percent decrease in cDCE concentrations in six of the eight wells. Two wells showed an increase in cDCE as TCE degraded to cDCE. Concurrent with decreases in TCE and cDCE concentrations were increases in sulfide concentrations. No dissolved oxygen was found in any of the wells 1 week after the mixture was injected. Dye concentrations decreased at varying rates during the 4-month sampling period, indicating that the injection mixture was being degraded or transported down-gradient. Trace amounts of 1,1-dichloroethylene (1,1-DCE) and trichloroethane (TCA) were found in some well-water samples, possibly due to secondary abiotic chemical reactions. These data indicate that a mixture of lactic acid, molasses, and soy milk enhanced biodegradation of TCE in a karst aquifer. However, further work is needed to determine how long the enhancement will last.

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Evaluating Oxygen-Releasing Compounds to Enhance Fuel Biodegradation by Free-Living Bacteria

Lashun King[1], LyTreese Hampton[1], and Tom D. Byl[2]

Anaerobic biodegradation of fuels is generally slower than aerobic biodegradation. During an anaerobic experiment, almost 100 days were required to biodegrade 2.5 milligrams per liter (mg/L) benzene and toluene in liquid-karst microcosms to less than 1 microgram per liter. However, it took less than 1 week to biodegrade the same amount of benzene and toluene under aerobic conditions. Considering that water in many karst aquifers moves at a relatively rapid pace, it follows that aerobic conditions are better whether efficient biodegradation processes are desired. The objective of this study was to evaluate if oxygen-release compounds (ORCs) enhance fuel biodegradation by free-living bacteria found in karst aquifers. One ORC that was evaluated is hydrogen peroxide (H_2O_2). Hydrogen peroxide can be toxic to many bacteria in high concentrations (3 percent volume:volume), but is relatively nontoxic in low concentrations (less than 0.1 percent). Two H_2O_2 will break down into oxygen (O_2) and water (2 H_2O). In a preliminary study, 250-milliliter liquid-karst microcosms were spiked with benzene and toluene. Half of the microcosms also were enriched with H_2O_2 to a final concentration of 30 milligrams per liter (mg/L), which would break down into 15 mg/L of dissolved O_2. The microcosms enriched with H_2O_2 continued to biodegrade benzene and toluene at a fast rate, whereas biodegradation in the non-enriched microcosms slowed down after 2 days. After 7 days, the H_2O_2-enriched microcosms had biodegraded three times as much benzene and toluene as the non-enriched microcosms. These preliminary results indicate that biodegradation by free-living bacteria found in karst aquifers can be enhanced by H_2O_2. Additional ORCs to be evaluated include magnesium peroxide and calcium peroxide. These ORCs differ from H_2O_2 by generating hydroxide ions, in addition to oxygen, which raise the pH significantly; they also tend to become solid and diffuse slowly into the water column.

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Biorestoration of a TCE-Contaminated Karst Aquifer by Stimulating Sulfate-Reducing Bacteria

Gregg Hileman[2], Koushik Chakraborti[1], and Tom D. Byl[2]

A sanitary landfill situated on a karst terrain in northern Tennessee has leaked chlorinated solvents, primarily trichloroethylene (TCE), into a karst aquifer. TCE has been found in water samples collected from eight wells screened in the karst bedrock aquifer. In July 2002, a mixture of dye, methyllactate, sodium lactate, molasses, and soymilk was initially injected into six of the eight wells to determine if the mixture would enhance biological reductive dechlorination of TCE. The mixture was later injected into a 7th well. Water samples were collected and electronic monitoring devices were placed in selected wells following injection to monitor changes in bacteria, geochemistry, TCE and breakdown products, and dye. After 8 months, there was an 85- to 100-percent decrease in TCE concentrations in the injection wells, and a 65- to 100-percent decrease in cDCE concentrations in six of the eight wells. Two wells showed an increase in cDCE as TCE degraded to cDCE. Concurrent with decreases in TCE and cDCE, there were 10-fold increases in sulfur-reducing bacteria & sulfide. No dissolved oxygen was found in any of the wells 1 week after the mixture was injected. Also, the lactic acid was metabolized into acetic acid after 4 months.

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Supplements to Enhance Groundwater-Microbial Growth and Biodegradation Processes

LeMiracle Hendking[1], Patricia Burton[1], and Tom D. Byl[2]

Recent research found that there is a large diverse bacteria population in karst aquifers. These bacteria have been shown to biodegrade fuels and chlorinated solvents in liquid microcosms. In one chlorinated solvent experiment, bacteria ceased biodegrading PCE. Biodegradation resumed only after vitamin B12 was added. Thus it appears that the karst conduit water is oligotrophic and requires additional vitamins to sustain a vigorous bioremediation. To test this hypothesis, microcosms were established using karst bacteria and toluene. The growth of bacteria was monitored over time. Then different vitamin supplements were provided and continued bacteria growth was evaluated. Bacteria supplied with a mixture of complete vitamin B (B1, B2, Niacin, B6, Folate, B12, Biotin, Pantothenic acid) grew 50% faster and 5-times more than control with no supplement. Vitamin B12 alone did not have a significant influence on growth or number. Bacteria supplied a complete vitamin supplement (Vitamins A, all the B's, C, D, E, and various minerals) also grew faster than controls, but did not remain viable as long as the complete–B supplement. The optimum pH for biodegradation of toluene by karst bacteria was also found to be 6–8. Based on these results, future studies will evaluate different buffers to maintain optimum pH in conjunction with different concentrations of the B-vitamins.

Published as Hendking, L., Burton, P. and Byl, T.D., Supplements to Enhance Groundwater-Microbial Growth and Biodegradation Processes *in* Tennessee Section, American Water Resources Association, 2004, Proceedings from the Fourteenth Tennessee Water Resources Symposium, March 31–April 2, 2004, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-25.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

[2] U.S. Geological Survey

The Role of Free-Living and Attached Bacteria in Processing Contamination in Karst Aquifers

By Tom Byl[2], Kelly Ray[1], Chad Walden[1], Valetta Watson[1], and Roger Painter[1]

In karst aquifers, biodegradation can be accomplished both by surface-attached and free-living bacteria. Biodegradation by attached bacteria is dependent upon and limited by the relatively low surface area to volume ratio (SA/V) of karst aquifers. Biodegradation due to free-living bacteria, however, is not limited by SA/V, but by residence time. The objective of this research was to determine if free-living karst bacteria contributed as much to the removal of ammonia (NH3) as attached, indigenous karst bacteria. These results were compared with the results of a toluene biodegradation study conducted using the same set up (Painter and others, 2005 KIG). The experimental setup included flow-through karst microcosms with high and low SA/V ratios. The low SA/V ratio system consisted of three 1-L cylinders connected together with non-stick tubing. The high SA/V ratio karst system was similar except the cylinders were packed with glass beads to increase the SA/V ratio by approximately 500%. Microscopic examination confirmed that bacteria colonized the interior surfaces of the lab karst systems. Fresh spring water containing between 10,000 and 20,000 indigenous karst bacteria was continuously pumped through each system. A known quantity of NH3 was added as a food source and measured at the exit port. Flow rates were similar and residence time differences were compensated for with the residence-time distribution (RTD) formula described by King and others (2005). First-order NH3-biotransformation rate constants were 0.17 day-1 for the low SA/V system and 0.27 day-1 for the high SA/V system. In the previous toluene study, the first-order rate constants were 0.014 hour-1 for the low SA/V system and 0.016 hour-1 for the high SA/V ratio system leading Painter (2005) to conclude that free-living bacteria contribute as much to toluene biodegradation processes as attached bacteria in karst aquifers. This study suggests that this is not the case with respect to NH3 biotransformation in karst systems.

Published as Byl, T., Ray, K., Walden, C., Watson, V., and Painter, R., The role of free-living and attached bacteria in processing contamination in karst aquifers, *in* Kuniansky, E.L., ed., 2008, U.S. Geological Survey Karst Interest Group Proceedings, Bowling Green, Kentucky, May 2008: U.S. Geological Survey Scientific Investigations Report 2008–5023, p. 68.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

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Comparison of Redox Measurements and Geochemical Conditions in a Contaminated Karst Aquifer and Their Influence on Biodegradation

R. Darlington[1], Gregg Hileman[2], and Tom Byl[2]

Redox measurements measure the capacity of a system for oxidation or reduction. The capacity is determined by which inorganic ions are present and which redox processes are taking place. These redox processes identified by the redox measurements characterize the biodegradation process in a karst aquifer. Biodegradation is important because by this process bacteria break down organic contaminants in a contaminated karst aquifer. Many scientist and engineers hold the view that redox probes are incapable of producing an accurate determination of the redox processes taking place in an aquifer system and consequently whether biodegradation is taking place. Expensive geochemical analyses are considered more accurate. However by comparing redox measurements, using redox probes, with redox processes indicated by geochemical analyses, this study proves that redox probes can be used to identify the different biological redox processes. The inorganic ions considered in this study are O_2, H_2S, NO_3^-, Fe_3^+, SO_4^{2-} and CO_2 and these are called terminal electron acceptors (TEAs). TEA characterization is traditionally done using geochemical tests. Ground water samples were collected from clean and contaminated wells screened in the karst bedrock formation every three weeks using bailers. Field tests of temperature, pH, alkalinity, specific conductance and dissolved oxygen were conducted. In addition to the field tests, laboratory tests using a spectrophotometer were done to determine the concentration of sulfate, nitrate, soluble iron (2+), ammonia and sulfide in the water. Additional chemical information was collected using YSI datasonde units that recorded redox potential, DO, pH, specific conductance and temperature every 15 minutes. The units were deployed for months in clean and contaminated wells. These data were used to observe trends in aquifer geochemistry and depth overtime. Comparison of the two data sets showed correlation's between the concentration of TEAs and the datasonde redox measurements. However there was a lag time between when the units were deployed and when accurate readings were achieved. Once the probes reached a true equilibrium with the aquifer they provided an accurate assessment of aquifer redox conditions and thus an indication of biodegradation efficiency.

Presented as an unpublished poster session at the Tennessee Section, American Water Resources Association, 2002 conference. Tennessee Section, American Water Resources Association, 2002, Proceedings from the Twelfth Annual Tennessee Water Resources Symposium, April 2002, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association [variously paged].

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Comparison of Water Geochemistry and Redox Electrodes to Identify Terminal Electron Acceptor Processes in an Aquifer

Ramona Darlington[1] and Tom D. Byl[2]

Several metabolic pathways of fuel biodegradation exist that have different efficiencies based on the terminal electron acceptor process (TEAP) present in the aquifer. The TEAP is determined by the oxidation-reduction (redox) potential of the aquifer, which is a measure of the system's capacity to give (oxidation) or receive (reduction) electrons. Because biodegradation can be an effective way to remove organic contaminants from ground water, depending on the TEAP, accurate redox measurements are essential. A study was initiated to determine whether a meter equipped with a platinum redox electrode could identify the TEAPs as effectively as geochemical analysis of the ground water. YSI* datasonde units equipped with a redox electrode, dissolved oxygen (DO), pH, specific conductance, temperature, and depth probes were deployed for 4 to 18 months in uncontaminated and fuel-contaminated wells screened in a karst bedrock formation. The datasonde units were serviced (probes re-calibrated, data downloaded, and batteries changed) every 2 to 3 weeks. Groundwater samples also were collected from the same wells during the datasonde servicing. The water samples were tested for temperature, pH, alkalinity, specific conductance, and DO within minutes of sample collection. Additional water samples were placed in clean glass amber bottles, packed on ice, and brought back to the laboratory for sufate, nitrate, soluble iron (Fe^{2+}), ammonia, and sulfide analyses. These data were used evaluate trends in aquifer geochemistry, redox potential, and depth over time. An equilibrium time of 2 to 4 hours was needed for the YSI datasonde redox electrodes to equilibrate with their surroundings. Comparison of the geochemical and datasonde data showed a good correlation between the concentration of geochemical constituents and the datasonde redox measurements, indicating the potential to use a platinum redox electrode to identify TEAPs in a contaminated aquifer., ammonia and sulfide in the water. Additional chemical information was collected using YSI datasonde units that recorded redox potential, DO, pH, specific conductance and temperature every 15 minutes. The units were deployed for months in clean and contaminated wells. These data were used to observe trends in aquifer geochemistry and depth overtime. Comparison of the two data sets showed correlation's between the concentration of TEAs and the datasonde redox measurements. However there was a lag time between when the units were deployed and when accurate readings were achieved. Once the probes reached a true equilibrium with the aquifer they provided an accurate assessment of aquifer redox conditions and thus an indication of biodegradation efficiency.

Published as Darlington, R., and Byl, T.D., Comparison of water geochemistry and redox electrodes to identify terminal electron acceptor processes in an aquifer, *in* Tennessee Section American Water Resources Association, 2003, Proceedings from the Thirteenth Tennessee Water Resources Symposium, April 9–11, 2003, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-7.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

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Free-Living Bacteria Versus Attached Bacteria: Which Contributes More to Bioremediation?

Fuzail Faridi[1], Tom Byl[2], and Roger Painter[1]

Researchers have implied that natural bioremediation in karst or fractured rock is unlikely to occur because of the lack of bacteria biofilm in karst aquifers. It has been stated that hydrologic and geologic characteristics of fractured rock aquifers are not suited for natural bioremediation because small microbial populations exist. If bioremediation in bedrock aquifers is dependent upon contact between surface-attached bacteria and contaminants, then bioremediation would be limited by the low surface area to volume ratio (SA/V) of karst aquifers. However, a quantitative basis for accepting or rejecting the assumption that attached bacteria dominate bacteria the biodegradation process in karst conduits has not been shown. The objective of this research was to determine if free-living karst bacteria contributed as much to toluene biodegradation as attached bacteria. To accomplish this objective, two flow-through reactor systems were established. One reactor system consisted of three 1-liter cylinders connected together with Teflon® tubing for a total open volume of 3.3 liters. The second reactor system was similar to the open system except the cylinders were filled with flat, circular glass beads that increased surface area to volume ratio approximately 500 percent compared to the open system. Raw karst water containing live indigenous bacteria was pumped through each system. The flow rate was slightly less in the high SA/V system than in the open system to compensate for the reduced volume space. Additionally, the residence-time distribution of each system was established using rhodamine dye in order to calculate biodegradation rates as a function of residence-time distribution. Results from the conservative tracer studies and toluene degradation studies were used to mathematically determine first-order degradation-rate constants for both systems. The resulting first-order rate constants were 0.014 hour-1 for the open system and 0.016 hour-1 for the high SA/V ratio system, respectively. If surface-attached bacteria were the main contributors to the biodegradation process and the SA/V ratio was increased 500 percent, a significantly higher biodegradation rate should have occurred in the high SA/V reactor. The biodegradation process was predominantly a result of free-living bacteria in the open volume. The 15 percent increase in the degradation rate for a 500 percent increase in SA/V indicates that attached bacteria may have contributed to the observed biodegradation rate.

Published as Faridi, F., Byl, T.D., and Painter, R., Free-living bacteria versus attached bacteria: which contributes more to bioremediation?, *in* Tennessee Section, American Water Resources Association, 2005, Proceedings from the Fifteenth Annual Tennessee Water Resources Symposium, April 2005, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-6.

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Quantifying Peroxide-Enhanced Toluene Biodegradation in a Single-Well Injection

Lashun King[1], Kendra Smith[1], and Tom D. Byl[2]

Microcosm studies indicate that anaerobic biodegradation of toluene is generally 50 times slower than aerobic biodegradation. Because of the potential for the rapid transport of dissolved contaminants in karst conduits, aerobic conditions are needed to enhance bioremediation. This study was divided into two phases. The objective of the first phase was to evaluate oxygenrelease compounds (ORCs) to enhance fuel biodegradation by free-living bacteria found in karst aquifers. The objective of the second phase was to develop a numerical method to quantify the rate of enhanced biodegradation using a single well for injection and monitoring. In the first phase of this study, the ORCs evaluated were hydrogen peroxide (H_2O_2), calcium peroxide (CaO_2), and magnesium peroxide (MgO_2). The H_2O_2 molecules break down into oxygen (O_2) and water (H_2O). The CaO_2 and MgO_2 break down in the presence of water into O_2 and either CaOH or MgOH, respectively. In this study, 2.25-liter liquid-karst microcosms (for example, flasks containing water and free-living karst bacteria) were spiked to 100 micrograms per liter (µg/L) toluene, and different ORC concentrations were added. Sterile controls also were established with toluene and ORCs to verify that toluene removal resulted from biological processes. Additional controls with live bacteria, but no ORC supplements, also were established for comparison. Microcosms enriched with 3 milligrams per liter (mg/L) H_2O_2, CaO_2, or MgO_2 all showed greater than 95 percent toluene removal in 7 days, as compared to 45 percent removal in live microcosms with no ORCs. When the microcosms were enriched with 300 mg/L H_2O_2, CaO_2, or MgO_2, only the H_2O_2 treatment elicited a reduction in toluene of greater than 99 percent in 7 days. The other peroxide treatments had slightly enhanced toluene removal compared to the live control, but generally were not effective at this higher concentration. The decline in MgO_2 and CaO_2 performance possibly was caused by the simultaneous release of hydroxide, which was found to inhibit biodegradation processes.

In the second phase of the project, a numerical method capable of quantifying biodegradation was developed by coupling the equation for residence-time distribution to a first-order rate of biodegradation. This numerical method was evaluated in a laboratory simulation. The simulation included a single-well injection of H_2O_2 and sodium chloride (conservative tracer) into a 5-gallon carboy containing karst water with 100 micrograms per liter toluene. The carboy was connected to a pump that delivered a constant flow of fresh bacteria-containing karst water (3 milliliters per minute) through the 5-gallon carboy, thereby diluting and transporting the conservative tracer from the carboy. The toluene was also diluted and transported from the carboy, but was also subject to biodegradation processes since it is a non-conservative chemical. The rate of toluene removal predicted by the numerical model and the observed rate of removal in the experiment were within close agreement (18 percent), confirming the numerical approach.

Published as King, L., Smith, K., and Byl, T.D., Quantifying peroxide-enhanced toluene biodegradation in a single-well injection, *in* Tennessee Section, American Water Resources Association, 2005, Proceedings from the Fifteenth Annual Tennessee Water Resources Symposium, April 2005, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-11.

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Evaluating Oxygen-Releasing Compounds to Enhance Toluene Biodegradation by Free-Living Bacteria

Lashun King[1], Khalid Woods[1], LyTreese Hampton[1], and Tom D. Byl[1,2]

Microcosm studies done in our lab found that anaerobic biodegradation of toluene was generally 25 to 50 times slower than aerobic biodegradation. Considering the potential for rapid transport of dissolved contaminants in karst conduits, it follows that aerobic conditions are desired for enhancing bioremediation. The objective of this study was to evaluate the ability of three oxygen release compounds (ORCs) to enhance fuel biodegradation by free-living bacteria found in karst aquifers. The ORCs that were evaluated were hydrogen peroxide (H_2O_2), calcium peroxide (CaO_2) and magnesium peroxide (MgO_2). The H_2O_2 molecules will break down into oxygen (O_2) and water (H_2O). The CaO_2 and MgO_2 will break down in the presence of water into O_2 and either CaOH or MgOH, respectively. In this study, 2.25-liter liquid-karst microcosms (i.e., flasks containing water and free-living karst bacteria) were spiked to 100 µg/L toluene and different ORC concentrations were added. Sterile controls were also established with toluene and ORCs to verify toluene removal was due to biological processes. Additional controls with live bacteria, but no ORC supplements were also established for comparison. Microcosms enriched with 3 mg/L H_2O_2, CaO_2, or MgO_2 all showed >95% toluene removal in 7 days, as compared to, 45% removal in live microcosms with no ORCs. When the microcosms were enriched with 300 mg/L H_2O_2, CaO_2, or MgO_2, only the H_2O_2 treatment elicited a >99% reduction in toluene in 7 days. The other peroxide treatments had slightly enhanced toluene removal compared to the live control, but were generally not effective at this higher concentration. The decline in MgO_2 and CaO_2 performance was possibly due to the simultaneous release of hydroxide, which was found to inhibit biodegradation processes. Other parameters that will be discussed in the poster are dissolved oxygen concentration, bacteria concentration, and pH. Based on these results H_2O_2 appears to be the best ORC candidate for enhancing biodegradation in karst aquifers.

Published as King, L., Woods, K., Hampton, L., and Byl, T.D., Evaluating Oxygen-Releasing Compounds to Enhance Toluene Biodegradation by Free-Living Bacteria, *in* Tennessee Section, American Water Resources Association, 2004, Proceedings from the Fourteenth Tennessee Water Resources Symposium, March 31–April 2, 2004, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-18.

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Lactate Induction of the Ammonia Mono-Oxygenase Enzyme and PCE Cometabolism

LyTreese Hampton[1], Roneisha Graham[1], and Tom D. Byl[2]

Water containing bacteria was collected from a PCE-contaminated karst aquifer in northern-central Tennessee to establish liquid, 1-liter microcosms. The microcosms were spiked with known concentrations of perchloroethylene (PCE) and 11 different formulations of lactic acid. The ammonia-lactate formulation caused a rapid removal of PCE and oxygen (O_2). Similar results achieved using a second set of microcosms established with ammonia-lactate to re-test the removal rate of PCE and O_2 indicated a possible co-metabolic PCE-removal process. Although only one report of PCE-cometabolism was found in the literature, we hypothesized that ammonia-oxidizing bacteria indigenous to the karst aquifer were capable of cometabolizing PCE with the ammonia mono-oxygenase (AMO) pathway. To test this hypothesis, microcosms were established using different forms of ammonia (ammonia-lactate, ammonia-chloride, ammonium plus sodium lactate), reference controls (sterile, live-no food, sodium lactate, sterile + ammonia lactate), and ammonia mono-oxygenase inhibitor (2-chloro-6-(trichloromethyl) pyridine). Microcosms treated with ammonia-lactate had the most rapid reduction of PCE and O_2, followed by the ammonium + Na-lactate treatment. The other live microcosms treated with ammonia also experienced significant drops in PCE and O_2 after 24 hours. The control (sterile and live-no food) microcosms did not experience a significant drop in PCE in the same time period. After 24 hours, the rapid PCE removal in all the ammonia-treated microcosms slacked off, due to the consumption of the oxygen. Tests with the AMO inhibitor did not prevent the PCE removal or O_2 consumption, indicating the inhibitor did not work on this particular AMO enzyme or bacteria. It is possible that the lactate stimulates AMO or protects the enzyme from inhibition. Additional tests need to be conducted to characterize the optimum pH, stoichiometric balance, and different AMO inhibitors. Nonetheless, these preliminary results provide strong evidence that karst bacteria indigenous to this aquifer can cometabolize PCE.

Published as Hampton, L., Graham, R., and Byl, T.D., Lactate induction of the ammonia mono-oxygenase enzyme and PCE cometabolism, *in* Tennessee Section, American Water Resources Association, 2005, Proceedings from the Fifteenth Annual Tennessee Water Resources Symposium, April 2005, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-9.

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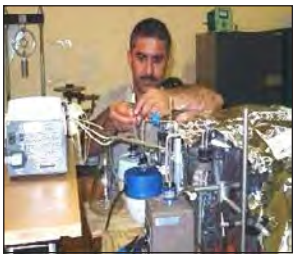
Biodegradation of Toluene as it Continuously Enters a 5-Liter Karst System

The collaborative research on this project was presented at different conferences by the two student researchers.

a) Shawkat Kochary[1], Roger Painter[1], and Tom Byl[2]

b) Fuzail Faridi[1], Roger Painter[1], and Tom Byl[2]

Contamination releases can occur as slow, long-term spills rather than as instantaneous spills. These continuous releases can result in a steady state of contaminants that can last months to years. Predicting the fate and transport of these contaminants in a karst aquifer is especially challenging because of the complex hydrogeology and uncertainties in residence time. The objective of this research was to adapt the residence-time distribution (RTD) biodegradation model, which was developed to predict the biotransformation of a single spill in a karst aquifer, for a continuous input of contaminants. Theoretically, the RTD for a karst system calculated from either a pulse- or a continuous-input tracer study would be identical, but mathematical manipulation of the data for the two approaches is quite different. Determination of the RTD from a continuous input requires numerical differentiation of tracer response data as opposed to numerical integration for the pulse approach. Three experimental runs were conducted involving the application of a continuous input: (1) rhodamine dye alone to establish RTDs for the systems, (2) sterile toluene (25 micrograms per liter) to quantify abiotic sorption, and (3) toluene with karst bacteria to quantify biodegradation. The three replicate karst systems were each 5 liters and had a continuous flow rate of 3.3 milliliters per minute. The difference between the RTD-based model prediction and the experimental toluene conversions was 17 percent. The continuous-input approach (numerical differentiation) had the tendency to magnify experimental and random errors in the tracer response data as compared to the pulse-input method (numerical integration).



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a) Published as Kochary, S., Painter, R. and Byl, T.D., Biodegradation of Toluene as it continuously enters a 5-liter karst system, *in* Tennessee Section, American Water Resources Association, 2005, Proceedings from the Fifteenth Annual Tennessee Water Resources Symposium, April 2005, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-12

b) Unpublished poster presented at Society of Environmental Toxicology and Chemistry (SETAC), 2005 conference

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Ammonia Oxidation by Bacteria Collected from a Karst-Bedrock Well

Kelly Ray[1] and Tom Byl[2]

Elevated ammonia concentrations can pose an environmental and health problem in groundwater. Animal and human wastes, fertilizers and decaying plant material all contribute to ammonia-nitrogen into the subsurface. Ammonia issues can be exacerbated in karst systems where it can enter directly through sinkholes or disappearing streams without any filtration. The rate of ammonia oxidation and optimum conditions for autotrophic nitrifying bacteria in a karst system are not known. The objective of this study was to characterize the rate of ammonia oxidation using bacteria indigenous to a karst aquifer in middle Tennessee. Liquid microcosms (300 mL, n=3) were established with an initial ammonia concentration of 20 mg/L. The pH in one-third of the microcosms was adjusted to either pH 3 with HCl, pH 10 with NaOH, or 7 (no addition). The growth in bacteria was monitored using a spectrophotometer. Ammonia concentrations were also measured through time. The microcosms with the greatest rate of ammonia-oxidation were pH 7, followed by pH 10 and pH 3, respectively. The growth pattern also confirmed that bacteria incubated at pH 7 had the greatest growth. Bacteria incubated at pH 3 or 10 did not demonstrate an appreciable increase in optical density, indicating they did not replicate to any significant degree. It is hypothesized that bacteria incubated at pH 3 were not able to take up ammonia as fast because the ammonia is ionized (NH4+) at this pH. It is possible that ionized ammonia less able to transfer across the cell membrane than the un-ionized form. Further research is needed to answer this hypothesis.

Published as Ray, K., and Byl, T.D., Ammonia oxidation by bacteria collected from a karst-bedrock well, *in* Tennessee Section, American Water Resources Association, 2006, Proceedings from the Sixteenth Tennessee Water Resources Symposium, April 19–21, 2006, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-12.

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Ammonia Oxidation by Bacteria Collected from a Karst-Bedrock Well

By Kelly Ray[1], Roger Painter[1], and Tom Byl[2]

(NOTE – Although the abstract has the same title as previous abstract, this abstract represents a continuation of the research)

Elevated ammonia concentrations in groundwater pose health and environmental problems. In karst systems where water can enter directly through sinkholes or disappearing streams without any filtration, ammonia contamination can be exacerbated. The rate of ammonia oxidation by nitrifying bacteria in karst systems is not known. The objective of this study was to characterize the aerobic and anaerobic rate of NH_3 oxidation using bacteria indigenous to a karst aquifer in Middle Tennessee. Static batch reactors using indigenous karst bacteria collected from a spring established a first-order rate of NH_3-oxidation (k) = 0.0209 per day. Because this rate appeared to be slow, in a follow-up experiment, the effect of supplements and surface area were investigated. It was found that, in a flow-through karst system, a 500% increase in surface area to volume (SA/V) ratio increased the k value 54%. Addition of 1 g of lactate/L further increased the k value almost 10-fold. Because NH_3-oxidizing bacteria are autotrophs (CO_2 fixing), it is hypothesized the lactate stimulated the growth of symbiotic bacteria that significantly enhanced the activity of NH_3-oxidizing bacteria. Anaerobic NH_3 oxidation was also investigated using data collected in 2002 from an anaerobic karst site in northern Tennessee with high levels of NH_3. The tracer and ammonia data were entered into the RTDB model and yielded a calculated ammonia oxidation k of 0.0168/day. The observation of ammonia biotransformation at an anaerobic site is circumstantial evidence that the anaerobic ammonia oxidation pathway called anammox was active. Additional research, however, is needed to confirm if the anammox pathway was responsible for the observed anaerobic removal of NH_3.

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Free-Living Bacteria or Attached Bacteria: Which Contributes More to Bioremediation?

Roger D. Painter[1], Shawkat Kochary[1], and Tom D. Byl[2]

Researchers have implied that natural bioremediation in karst or fractured rock is unlikely to occur because of the lack of bacteria biofilm in karst aquifers. Hydrologic and geologic characteristics of fractured rock aquifers have been described as not being suited for natural bioremediation because of small microbial populations. If bioremediation in bedrock aquifers is dependent upon contact between surface-attached bacteria and contaminants, then bioremediation would be limited by the low surface area to volume ratio (SA/V) of karst aquifers. A quantitative basis, however, for accepting or rejecting the assumption that attached bacteria dominate the biodegradation process in karst conduits has not been shown. The objective of this research was to determine if free-living karst bacteria contributed as much to toluene biodegradation as attached bacteria. Two flow-through reactor systems were established to test the different biodegradation rates. Each reactor system consisted of four 1.24-liter cylinders connected together with glass tubing for a total open volume of approximately 5 liters. The second reactor system was similar to the open system except the cylinders were filled with acid-washed, circular glass spheres that increased surface area to volume ratio approximately fivefold compared to the open system. Rhodamine dye was used to calculate the different residence-time distributions in each system. A sterile control study established that less than 3 percent of the toluene was lost to abiotic processes. Next, raw water from a karst aquifer containing live, indigenous bacteria was pumped through each system for 5 days to establish a biofilm on the glass surfaces. Colonization of the surface was confirmed by microscope visualization before toluene was added to the systems. The resulting first-order rate constants were computed to be 0.014 per hour for the open system and 0.0155 per hour for the packed reactor system. If surface-attached bacteria were the main contributors to the biodegradation process and the SA/V ratio was increased fivefold, a significantly higher biodegradation rate should have occurred in the packed reactor. The results of this study indicate that the free-living bacteria indigenous to a karst aquifer contribute as much to the toluene biodegradation process as attached bacteria.

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Bacteria Induced Dissolution of Limestone in Fuel-Contaminated Karst Wells

Serge Mondesir[1] and Tom D. Byl[2]

Karst landscapes are formed in water-soluble geologic formations, such as limestone, in which dissolution processes have enlarged water-transmitting openings. Approximately 20 percent of the United States is underlain by carbonate rocks and is classified as karst, and 40 percent of the United States east of the Mississippi River is underlain by karst aquifers. Karst ground-water systems are extremely vulnerable to contamination. Many organic contaminants such as fuels can stimulate bacteria biodegradation and the production of carbon dioxide (CO_2). The increased respiration by bacteria in contaminated karsts aquifers can lead to a significant increase in CO_2 production and formation of carbonic acid.

A quantitative study was conducted to determine the effect of elevated concentrations of carbonic acid due to bacteria action on limestone dissolution. Sealed flasks were set up that contained 250 milliliters of distilled water, limestone fragments of known size and weight, and varying concentrations of CO_2. The flasks with elevated CO_2 concentration had a 3-fold increase in the rate of calcium carbonate dissolution. Water with elevated CO_2 concentrations had a slightly lower pH than water with the lower CO_2 concentrations, but the difference in pH was not statistically significant at the 0.05 confidence level. Further tests were done to determine if these lab results applied to field conditions. Water samples were collected from wells completed in karst aquifers. The CO_2 concentrations in water samples collected from fuel-contaminated wells were higher than in samples collected from wells with no fuel contamination. Also, the dissolved calcium was usually two or three times greater in the fuel-contaminated wells. The results have implications for redesigning geochemical models that predict conduit enlargement when fuel contaminants are present in karst aquifers

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Geochemical and Microbial Evidence of Fuel Biodegradation in a Contaminated Karst Aquifer in Southern Kentucky, June 1999

Tom D. Byl[1], Gregg E. Hileman[1], Shannon D. Williams[1], and James J. Farmer[1,2]

Complex hydrogeologic conditions coupled with poorly understood biodegradation processes in karst aquifers have led many to believe that the potential for natural attenuation of petroleum fuel hydrocarbons is limited. This research addressed the capacity for biodegradation processes in karst. Ground-water samples were collected for bacteria and geochemical analysis from several contaminated monitoring wells in an unconsolidated regolith and karst aquifer that had varying concentrations of dissolved fuel. Bacteria concentrations were greatest in ground-water samples containing the greatest fuel contamination. Additionally, bacteria isolated from fuel-contaminated ground-water samples readily grew in Petri dishes with dissolved gasoline fuel as the only source of food. The wells with screens intersecting less contaminated sections of the aquifer had greater dissolved oxygen concentrations (6.3 milligrams per liter) than those intersecting more contaminated sections (dissolved oxygen less than 0.1 milligrams per liter). Also, where the oxygen concentrations were diminished, geochemical evidence indicated that anaerobic processes were active. This evidence includes elevated levels of ammonia and ferrous iron in the fuel-contaminated ground-water samples. Based on these results, biodegradation of fuel constituents in the karst aquifer is indicated, and therefore, natural attenuation should not be disregarded because of preconceptions about low microbial activity in karst aquifers.

Published as Byl, T.D., Hileman, G.E., Williams, S.D., and Farmer, J.J, Geochemical and microbial evidence of fuel biodegradation in a contaminated karst aquifer in southern Kentucky. June 1999, *in* Eve L. Kuniansky, editor, 2001, U.S. Geological Survey Karst Interest Group Proceedings, Water-Resources Investigations Report 01–4011, p. 151–156.

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Karst—Contaminant Degradation Models

Karst aquifers are vulnerable to contamination and can have complex aquifer properties and flow characteristics. Ground-water models based on Darcy's Law have limited application in karst aquifers, especially at a local scale typical of contamination sites. Research conducted by TSU professors, PEER students, and USGS scientists developed and applied non-standard modeling techniques to flow and contaminant degradation in karst aquifers. PEER students developed the non-standard models including a residience-time-distribution (RTD) model and applied the Lattice Boltzmann method (LBM) to simulate flow rates in karst aquifers. The LBM is a simulation technique for complex fluid systems and was applied to PEER research to simulate flow in a laboratory-scale karst microcosm. The RTD model was coupled with a pseudo-first-order rate of biodegradation and then successfully modeled contaminant degradation through a laboratory-scale karst microcosm. The research continued with the evaluation of the model and the application of the RTD-biodegradation model to field sites. The results from the application of the LBM and RTD models indicate that these models can be applied to karst systems and can provide additional information to aid in characterizing and remediating contaminants in karst systems.

Numerical Modeling of Flow Simulations and Rate of Biodegradation in a Karst Microcosm

Valetta Watson[1], Roger Painter[1], Tom Byl[2]

The contamination of aquifers with petroleum hydrocarbons has lead to the development of several bioremediation strategies. Bioremediation has received special attention because it results in the mineralization of the petroleum hydrocarbon in the sub-surface. This eliminates the need for expensive excavation of the contaminated zone. In spite of its utility, bioremediation has not been aggressively pursued as a remediation option in karst aquifers. Skeptics theorize that bacteria concentrations in karst are negligible, and that contaminants are rapidly flushed out of a karst aquifer with little biodegradation. This research addresses the notion that insignificant biodegradation occurs in karst conduits. In this study, a dye tracer was used in a karst microcosm to gain information about its characteristics. We have utilized the Lattice Boltzmann method to simulate flow rates in karst aquifer microcosms. The Lattice-Boltzmann method (LBM) uses a statistical approach to solve the Navier-Stokes equations for fluid flow. The LBM uses the nearest neighbor relations on a micro scale to evolve the macroscopic dynamics of the system. Preliminary results show that the LBM can be used to model the complex interactions associated with karst aquifer flows.

Presented as an unpublished poster session during the 1999 Tennessee AWRA conference.

Crabtree, L.R., Bradley, M.W., Blunt, Tiffany, and Pierre, Salnave, eds., 1999, Proceedings from the Ninth Annual Tennessee Water Resources Symposium, April 1999, Nashville, Tennessee: Tennessee AWRA, 271 p.

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Numerical Simulation of Flow and Contaminant Transport in a Karst Aquifer Conduit

Valetta Watson[1], Roger Painter[1], and Tom D. Byl[2]

Computer models that simulate water flow or contaminant transport in unconsolidated aquifers have little transferability to karst aquifers because they are based on equations derived under the assumption of laminar flow. Flow through conduits developed in karst aquifers may be turbulent, and the locations of the conduits may not be well understood, limiting the usefulness of these models.

To address this issue, the Lattice-Boltzmann method (LBM), a powerful technique that can be used for computational modeling of complex fluid flow, was used to develop a numerical model to simulate water flow and contaminant movement in a theoretical karst conduit. The LBM is based on statistical physics and uses a numerical approach to solve the Navier-Stokes equations for fluid flow. In this study, two-dimensional images were generated using object grids that delineated the structure and surface of the karst conduit within which the fluid flow was being modeled. Fluid movement was visualized by assembling simulation outputs as a series of time-step animations. For the volume simulation outputs, transparency was used to indicate fluid density and color intensity was used to indicate fluid velocity.

The numerical results obtained from the simulations were compared against those obtained from a laboratory dye-tracer experiment to validate the model. An artificial karst conduit of varying dimensions was constructed in the laboratory by connecting a series of glass tubes ($\leq$ 2 inch diameter). Once a constant discharge was observed at the outlet, rhodamine dye was injected at the inlet of the conduit and measured at the outlet as a function of time. An analysis of the quantitative experiment indicated that distinct re-circulation zones existed within the artificial karst conduit. These same features were captured in the LBM simulation. The agreement between the LBM simulation and the laboratory study demonstrates the potential for using the LBM technique to model the transport characteristics and residence time of contaminants within a karst system.

Published as Watson, V., Painter, R. and Byl, T.D., Numerical simulation of flow and contaminant transport in a karst aquifer conduit *in* Tennessee Section, American Water Resources Association, 2003, Proceedings from the Thirteenth Tennessee Water Resources Symposium, April 9–11, 2003, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-38.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

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Use of Residence-Time Distribution Coupled with a Biodegradation Rate to Predict Toluene Removal in an Artificial Karst System

R. Painter[1], V. Watson[1], and T. Byl[2]

Approximately 40 percent of the United States east of the Mississippi River is underlain by karst aquifers (Quinlan, 1989). Karst ground-water systems are extremely vulnerable to contamination; however, the fate and transport of contaminants in karst areas are poorly understood because of the complex hydraulic characteristics of karst aquifers. Ground-water models developed using Darcy's Law coupled to rates of biodegradation are useful for predicting the fate of fuels in unconsolidated aquifers, but have little utility in karst conduits. Conceptual models developed for karst aquifers have a consistent theme of non-ideal flow, storage and active flow components. This research used a residence-time distribution (RTD) model approach that integrated residence times of contaminants isolated in storage areas with the residence time of contaminants moving through conduits coupled to a pseudo-first order rate of biodegradation. The microcosms consisted of 4 1-liter chambers connected with small glass tubing. A peristaltic pump provided a consistent flow of karst water from a 10-gallon reservoir. First, a quantitative dye study was done to establish the residence time distribution of the three systems. This was followed by a sterile toluene run to measure sorption of toluene to the microcosm systems. The third microcosm run incorporated karst bacteria and toluene. There was good agreement between the predicted toluene concentration by the RTD model and the experimental data. Eighty-seven micrograms of toluene was injected into each system. Recovery in the sterile systems ranged from 62.6 µg to 84.6 µg, indicating that sorption was minimal. Recovery of toluene in the biotic systems ranged from 36.5 to 41.6 µg indicating biodegradation occurred. The mean residence-time distributions for the 3 systems were ranged from 40 to 43 hours. The RTD model predicted 44, 48 and 52 percent toluene biodegradation for the 3 systems, respectively. The biodegradation calculated from the experiments was 44, 48 and 50 percent respectively. Thus, the RTD coupled to a pre-determined biodegradation rate appears to accurately predict the amount of toluene that the bacteria will degrade.

Published as Painter, R., Watson, V., and Byl, T.D., Use of Residence-Time Distribution Coupled with a Biodegradation Rate to Predict Toluene Removal in an Artificial Karst System, *in* Tennessee Section, American Water Resources Association, 2004, Proceedings from the Fourteenth Tennessee Water Resources Symposium, March 31–April 2, 2004, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p 2A-14.

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Development of a Computer Program that Uses Residence-Time Distribution and First-Order Biodegradation to Predict BTEX Removal in Karst Aquifers

Ryan Fitzwater[1], Roger Painter[1], Valetta Watson[1], and Tom D. Byl[2]

Approximately 40 percent of the United States east of the Mississippi River is underlain by karst aquifers. Karst groundwater systems are extremely vulnerable to contamination; however, the fate and transport of contaminants in karst areas are poorly understood because of the complex hydraulic characteristics of karst aquifers. Ground-water models developed using Darcy's Law coupled to rates of biodegradation are useful for predicting the fate of fuels in unconsolidated aquifers, but have little utility in karst conduits. Conceptual models developed for karst aquifers have a consistent theme of non-ideal flow, storage and active flow components. This research used a residence-time distribution (RTD) model approach that integrated residence times of contaminants isolated in storage areas with the residence time of contaminants moving through conduits coupled to a pseudo-first order rate of biodegradation. The microcosms consisted of 4 1-liter chambers connected with small glass tubing. A peristaltic pump provided a consistent flow of karst water from a 10-gallon reservoir. First, a quantitative dye study was done to establish the residence-time distribution of the three systems. This was followed by a sterile toluene run to measure sorption of toluene to the microcosm systems. The third microcosm run incorporated karst bacteria and toluene. The removal of toluene predicted by the RTD-biodegradation model and the experiment were within 2 percent agreement (n=3). The RTD-biodegradation model was transformed into a user-friendly program that utilizes MS Excel® with Visual Basic interfaces. The input sheet of this prototype program requires site information, a biodegradation rate, and the results of a quantitative tracer study. The results, or output pages, provide residence-time distribution graphs and various statistical calculations. The output pages also report the calculated amount of BTEX removed during transport through the karst aquifer based on RTD and biodegradation. Additional work is needed to incorporate dilution into the model.

Note: Any use of trade, product or firm names in this document is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Development of a Protocol for Enhanced Bioremediation in Karst Using a Single Injection Well

Tarra M. Beach[1], Lashun K. King[1], Roger Painter[1],and Tom D. Byl[2]

Approximately two-thirds of Tennessee and Kentucky are underlain by karst terrain. The groundwater aquifers in karst terrains are particularly susceptible to contamination. Once a contaminant has entered a karst-bedrock aquifer it is difficult to determine its precise flow-path through the bedrock. The contaminant may reside for long periods in stagnant areas of the aquifer or it may be rapidly transported through tortuous conduits. All the while, the contaminants are susceptible to biodegradation processes in the aquifer. Recently, the RTDB model was developed to calculate the contaminant biotransformation as a function of residence time in the aquifer. There have been two field tests successfully applying RTDB to contaminated karst sites with enhanced biodegradation. However, there is currently no standard protocol for the application of RTDB on karst sites. The objective of this research was to develop a general protocol for enhanced bioremediation of organic contaminants in karst terrains and the application of the RTDB model. Furthermore, since the discharge point is often off-site, unknown, or diffused, a single-well method was developed for the protocol. This protocol is based on data and reports from field studies done at Ft. Campbell. The protocol is divided into six steps. The first step is to gather historical data about the spill and develop background information about the site (geochemical, biological, and hydrological information). Second, based on the information collected in step one, an enhancement mixture is decided upon and calculations are done to determine how much enhancement-mixture should be injected. Third, the enhancement mixture mixed with a conservative tracer is injected at a depth equal to the conduit opening(s). Fourth, the tracer concentration is measured through time along with the chemical, hydrological and biological measurements. Fifth, the tracer data is used to calibrate the residence-time distribution portion of the RTDB model. Sixth, once calibrated, the RTDB model is used to quantify the amount and rate of contaminant biodegradation in the aquifer around the well. The protocol is intended to be flexible so it can be applied to a variety of contaminated karst sites.

Published as Beach T.M., King, L.M., Painter, R., and Byl, T.D., Development of a protocol for enhanced bioremediation in karst using a single injection well, *in* Tennessee Section, American Water Resources Association, 2006, Proceedings from the Sixteenth Tennessee Water Resources Symposium, April 19–21, 2006, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-4.

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Evaluating Peclet Values and the Role of Advection, Dispersion and Diffusion in Tracer Studies

Jameka Johnson[1], Carlton Cobb[1], Lonnie Sharpe[1], and Tom Byl[2]

Karst aquifers have been recognized as one of the most challenging geological media in terms of groundwater modeling. Numerical models based on Darcy's Law often are unable to accurately characterize contaminant flow through the heterogeneous fractures and dissolution features. Lab and field data were used to study dispersion, matrix diffusion and advection. The residence-time distribution (RTD) formula was used to calculate Peclet values, which are indicative of advection and dispersion properties. Based on field tracer studies published in the literature, it appears that when a tracer study was conducted with an artificial head (i.e., the dye is pushed or flushed through the system), the system was dominated by advection processes. When a natural water gradient was used, the system was dominated by dispersion processes and a longer tail is produced. However, it was not clear if the dispersion was due to turbulence or matrix dispersion. Lab experiments were conducted to try and differentiate between the hydrodynamic dispersion, heterogenous advection and matrix diffusion. The lab system, consisting of a plexiglass box holding limestone rocks with permeable fractures, was used to measure tracer diffusion under different hydrologic conditions. Preliminary results from those studies back the field observations, that pushing the system tends to result in plug-like flow (advective flow) and less matrix diffusion. Additional tracer studies are scheduled for a wetland to evaluate the Peclet values, dispersion and advection values for the system under storm- and base-flow. These findings will have implications for the interpretation of tracer tests designed to measure advection, dispersion and diffusion of contaminants as they transport through complex hydrologic systems.

Published as Johnson, J., Cobb, C., Sharpe, L., and Byl, T.D., Evaluating peclet values and the role of advection, dispersion and diffusion in tracer studies, *in* Tennessee Section, American Water Resources Association, 2007, Proceedings from the Seventeenth Tennessee Water Resources Symposium, April 17–19, 2007, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-10.

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Application of the RTD Model to Analyze the Fate and Transport of Ammonia in Laboratory Karst System

Kelly Ray[1], Roger Painter[1], and Tom Byl[2]

Elevated ammonia concentrations in groundwater pose health and environmental problems. The impact of ground water contamination can be exacerbated in karst systems where water can enter directly through sinkholes or disappearing streams without any filtration. Karst aquifers are highly heterogeneous and cannot be adequately described by Darcian principles used to characterize flow in sandy aquifers. The objective of this research was to determine if a residence time distribution (RTD) based model in conjunction with the advection dispersion equation could adequately describe the fate and transport of ammonia in a karst aquifer. To accomplish this task, a laboratory karst system was constructed to simulate the non-ideal flow. Feeding ammonia solution to this simulated system in a controlled fashion allowed the model to be validated by comparing the actual concentration versus time data at the systems effluent to the model predicted values. Static batch reactors using indigenous karst bacteria established a first-order rate of NH_3-oxidation with a k value of 0.0209 per day. These results suggest that ammonia degradation does not occur at significant rate in karst under ambient conditions. In a follow-up experiment, supplements and surface area were investigated. A flow-through karst system with a 400% increase in surface area to volume (SA/V) ratio increased the k value 54%. The addition of 1 g of lactate/L increased the k value almost 10-fold.

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Development and Verification of a Computer Program That Predicts Fuel Biodegradation in Karst Aquifers

Ryan Fitzwater[1], Patricia Burton[1], Roger Painter[1], and Tom Byl[2]

Approximately 40 percent of the United States east of the Mississippi River is underlain by karst aquifers. Karst ground-water systems are extremely vulnerable to contamination; however, the fate and transport of contaminants in karst areas are poorly understood because of the complex hydraulic characteristics of karst aquifers. Ground-water models developed using Darcy's Law coupled to rates of biodegradation are useful for predicting the fate of fuels in unconsolidated aquifers, but have little utility in karst conduits. Conceptual models developed for karst aquifers have a consistent theme of non-ideal flow, storage, and active flow components. This research used a residence-time distribution (RTD) model approach that integrated residence times of contaminants isolated in storage areas with the residence time of contaminants moving through conduits coupled to a pseudo-first order rate of biodegradation.

This RTD-biodegradation model was adapted for large sites with a known source-to-discharge connection, and, for small, singlewell, injection-monitoring systems. The first method assumes second points are measured (a release point and discharge point) and RTD is calculated using numerical integration. The second method assumes a single measuring point (as it leaves the release area) and uses differential integration to solve for RTD. These two approaches were tested in laboratory karst systems and fuel-contaminated field sites. The results found that the 2-point strategy worked well in the lab, but failed to account for dilution in the large-scale field study. Dilution must be factored into large scale site evaluations. The single-point strategy worked well in the lab and field studies. Both methods show great promise. These two numerical equations were converted to computer algorithms. Next a Microsoft Excel® 2002 and the Visual Basic® programming language were utilized to create a user friendly interface for the computer program. The RTD method that utilizes 2-monitoring points requires additional work on the dilution term before it is ready for use. The single point injection-monitoring method appears to be ready for use at contaminated karst sites.

Published as Fitzwater, R., Burton, P., Painter, R., and Byl, T.D., Development and verification of a computer program that predicts fuel biodegradation in karst aquifers, *in* Tennessee Section, American Water Resources Association, 2006, Proceedings from the Sixteenth Tennessee Water Resources Symposium, April 19–21, 2006, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-7.

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Residence Time Distribution Derived from Independent Gamma Distributions of Tracer Travel Distance and Linear Velocity

By Roger Painter[1], Tom Byl[2], and Valetta Watson[1]

The advection dispersion equation (ADE) is widely used as a predictor of residence time distributions (RTDs) for tracer breakthrough curves for karst systems. Solutions of the ADE for tracer breakthrough studies with near plug flow behavior are characteristically Gaussian in appearance. However, very few, if any, quantitative tracer studies result in tracer concentrations that are normally distributed about the mean residence time. While the symmetry of Gaussian breakthrough curves often correctly predicts finite tracer concentrations at zero time, it generally does not accurately predict actual tracer breakthrough curves, which invariably are characterized by relatively long tails. This suggests that a different conceptual approach may be appropriate for describing these systems in easily visualized terms. The objective of this project was to develop a more descriptive approach of tracer break-through data based on the gamma probability density function. The tracer travel distance and tracer linear velocity were assumed to be randomly distributed variables with gamma distributions. The RTD for tracer breakthrough curves was derived from the individual distributions of tracer travel distance and linear velocity. This approach was compared and contrasted with the traditional approach based on the ADE for modeling tracer breakthrough data at a karst site, as well as, modeling the rate of biodegradation of toluene in laboratory karst aquifers.

Originally published as Painter, R., Byl, T., and Watson, V., Residence time distribution derived from independent gamma distributions of tracer travel distance, *in* Kuniansky, E.L., ed., 2008, U.S. Geological Survey Karst Interest Group Proceedings, Bowling Green, Kentucky, May 2008: U.S. Geological Survey Scientific Investigations Report 2008–5023, p. 75.

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Adaptation of the Residence Time Distribution (RTD)-Biodegradation Model to Quantify Peroxide-Enhanced Fuel Biodegradation in a Single Karst Well

Lashun K. King[1,3], Roger D. Painter[1], and Tom D. Byl[2]

This field study was conducted to determine if a numerical model incorporating residence time distribution (RTD) coupled to a first-order rate of biodegradation (k') could be used to quantify toluene and benzene removal in a single karst-well injection system. This study involved injecting sodium chloride (NaCl) as a conservative tracer, as well as hydrogen peroxide (H_2O_2), to enhance aerobic biodegradation of toluene and benzene. A 100-gallon volume of fuel-contaminated karst aquifer water was pumped into a container. NaCl (1.25 kilograms) and 33 percent H_2O_2 (4 liters) were mixed into the water and injected back into the bedrock aquifer. The NaCl, dissolved oxygen, benzene and toluene concentrations were monitored for several weeks. Results show that benzene and toluene concentrations declined approximately 10 times faster than the NaCl concentrations, indicating enhanced biodegradation. The RTD was calculated by using the declining NaCl-concentration curve through time. The biodegradation rate was derived from the benzene and toluene data. The RTD-biodegradation formula (described in this paper) was used to predict and quantify the enhanced biodegradation of benzene and toluene in the karst aquifer. The RTD-biodegradation formula predicted benzene and toluene concentrations in the well through time to within 1 microgram per liter (µg/L) of the actual concentration. This close agreement between the RTD-biodegradation model prediction and the measured concentration confirms that this method can be used to quantify enhanced biodegradation in a single karst injection well.

Published as King, L.K., Painter, R.D., and Byl, T.D., Adaptation of the Residence Time Distribution (RTD)-Biodegradation Model to Quantify Peroxide-Enhanced Fuel Biodegradation in a Single Karst Well, *in* Kuniansky, E.L., ed., 2005, U.S. Geological Survey Karst Interest Group Proceedings, Rapid City, South Dakota, September 2005: U.S. Geological Survey Scientific Investigations Report 2005–5160, p. 174–179.

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Karst–Hydrology

The occurrence and movement of contaminants in a karst aquifer are controlled by the hydraulic properties of the aquifer and the volume and rate of ground-water flow through the aquifer. As part of the process to better understand ground-water contamintation, the PEER program has included research on karst hydrology and aquifer characteristics. Students in the PEER program have conducted studies on ground-water recharge in a karst system, evaluated land-use effects on water quality in small watersheds in Middle Tennessee, applied methods to evaluate advective versus dispersive contaminant transport, and characterized the geochemistry of a shallow, local aquifer discharging at springs near the TSU campus. The PEER students have conducted the field studies and measured water quality and springflow. The research was conducted at multiple site in Middle Tennessee and included the Mississippian limestone aquifers in Dickson and Montgomery Counties, and the Ordovician limestone aquifers in Davidson and Bedford Counties, Tennessee.

Advection Versus Dispersion as Determined by Single-Well Tracer Studies

Tarra M. Beach[1], Michael Bradley[2], Roger Painter[1], and Tom D. Byl[2]

Approximately two-thirds of Tennessee and Kentucky are underlain by karst terrain. The groundwater aquifers in karst terrains are particularly susceptible to contamination; once a contaminant has entered a karst-bedrock aquifer it is difficult to determine its precise flow-path through the bedrock. The contaminant may reside for long periods in stagnant areas of the aquifer or it may be rapidly transported through tortuous conduits. All the while, the contaminants are susceptible to biodegradation processes in the aquifer. Traditional dye trace studies carried out in karst terrains required two or more points of assessment. A dye, such as rhodamine or sodium chloride, would be injected into a well, sinkhole, or stream; that dye's length of time to resurface at a spring would be measured–this could lead to extremely long-term projects which could provide no conclusive results. Therefore, the objective of this research was to develop a tracer injection procedure that would involve one well. A quantity of sodium chloride was injected and its dissipation over time was monitored. The data collected yielded a response curve for the specific conductance of the sodium chloride injections, and in turn, permitted an examination of the properties of advection and dispersion in the area around the well. These properties indicated whether a site was suitable for remediation. Monitoring and production wells in Dickson, Tennessee and Fort Campbell, Kentucky were utilized to determine these hydraulic conductivity properties.

Published as Beach, T.M., Bradley, M.W. Painter, R. and Byl , T.D., Advection versus dispersion as determined by single-well tracer studies, *in* Tennessee Section, American Water Resources Association, 2007, Proceedings from the Seventeenth Tennessee Water Resources Symposium, April 17–19, 2007, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-3.

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Enhanced Groundwater Recharge Can Alleviate Storm-Water Flow and Dilute Groundwater Pollution in Karst Terrane

Derek Lovett[1], Nkechi Chieke[1], and Tom Byl[2]

Storm-water runoff is becoming a major issue as flash-flooding becomes a common theme in many low-lying areas. As water is diverted to streams, groundwater recharge is diminished. This project focused on the development of a numerical model to quantify enhanced groundwater recharge in karst terrains. A karst-lab model system was set up to analyze the effects of enhanced recharge on spring discharge and contaminant dilution. Recharge was quantified using a modified water-budget method by measuring discharge and water levels during a rain simulation. Placement of the infiltration-basin (up-gradient, down-gradient, dispersed) affected transport and dilution of contaminants. Placing the enhanced infiltration up-gradient of a spill resulted in rapid transport of the contaminant to the spring. Enhancing infiltration down-gradient of the groundwater spill delayed migration of the contaminant. When infiltration was distributed over the entire aquifer, dilution effects varied depending on the rate of discharge. Analyzing various infiltration scenarios increased understanding of transport and dilution effects, as well as assisted development of a numerical approach for quantifying recharge in karst aquifers. Applying this approach to the field required extensive knowledge of the basin hydrology and soil hydraulic conductivity properties. Management of groundwater resources can be better optimized by understanding the influence that infiltration basins have on an aquifer system.

Presented as an unpublished poster session at the 2007 Tennessee Section, AWRA conference.

Tennessee Section, American Water Resources Association, 2007, Proceedings from the Seventeenth Tennessee Water Resources Symposium, April 17–19, 2007, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association [variously paged].

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

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Characterization of Bacteria and Geochemistry of Springs in Nashville, Tennessee

Patrice Armstrong[1], Carlton Cobb[3], Brandon Cobb[3], Jennifer Stewart-Wright[4], Tom Byl[2]

The objective of the project was to evaluate the water quality of four limestone bedrock springs in an urban environment during a severe drought in the summer of 2007. Three of the springs were discovered on the Tennessee State University (TSU) campus in Nashville, TN in May, 2007. Two are located near a poultry research facility and a third near the TSU athletic center. An additional spring flowing from a cave in the Charlotte Park neighborhood of west Nashville (Carlos Cave) was also included in the study. The two TSU springs behind the poultry barns were sampled approximately every week from June through September, 2007. The cave and TSU athletic center springs were sampled less frequently.

Water quality parameters included temperature, specific conductance, and dissolved oxygen, pH, sulfate, nitrogen, *E. coli*, and bacteria Biological Activity Reaction Tests (BART). Continuous water-quality monitoring devices were installed at two of the springs to measure changes associated with different weather patterns. Water temperatures were very stable, ranging from 16°C in June to 19°C in September. Sulfate concentrations were consistently higher in the spring water than the receiving surface waters. Conversely, nitrogen levels were lower in the spring water (< 10 mg/L) than the surface waters. Fecal bacteria levels fluctuated randomly with no discernable correlation to weather pattern. BART tests confirmed the presence of denitrifying, iron-reducing, sulfur-reducing, and slime-producing bacteria at each of the springs. Spring discharges decreased at all sites as the drought continued but never decreased below 10 gallons per minute. The data showed that each spring had unique water quality characteristics reflective of the different hydrologic recharge areas that replenish them.

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Land-Use Effects on Bacteria Loads and Water Quality in Small Karst Catchments of the Upper Duck River Watershed

James J. Farmer[1]

Several streams in the upper Duck River watershed are listed by the Tennessee Department of Environment and Conservation as impaired by elevated bacteria levels. Potential sources of bacteria in the watershed include grazing, confined-animal operations, and domestic septic systems. The relative contributions of these sources to elevated bacteria levels are not known. The effects of land-use on stream water-quality in karst topography will be determined by studying four rural catchments in the upper Duck River watershed. Two of the study catchments will be small and in close proximity to each other to control for natural variability in water chemistry, geology, and physiography. One catchment will encompass the two small catchments, and the fourth will be located on the main stem of the Duck River. This study will be conducted in two phases. The first phase will characterize the water quality and land use across the study to identify spatial patterns. The second phase will investigate temporal variability of bacteria counts and the use of surrogates, such as turbidity, for predicting bacteria loads.

Published as Farmer, J.J., Land-use effects on bacteria loads and water quality in small karst catchments of the Upper Duck River watershed, *in* Tennessee Section, American Water Resources Association, 2008, Proceedings from the Eighteenth Tennessee Water Resources Symposium, April 2008, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. 2C-23.

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Methods in Contaminant Evaluation

Researchers in the PEER program were often faced with inadequate or an absence of established methods to address research questions about contaminants in karst aquifers. Karst aquifers are complex, and resource managers are often faced with trying to identify the best way to determine the sources and extent of contamination and to characterize ground-water flow conditions. Student researchers in the PEER program have adapted and pioneered methods to better characterize karst sites and to identify specific sources of bacterial contamination in karst areas. The students in the PEER program have adapted new methods and technology to evaluate ground-water quality in karst aquifers. The students have constructed and deployed diffusion bag samplers to monitor water quality and evaluate vertical movement in karst aquifers. The student retrieved the samplers from field sites, analyzed the water samples and evaluated the changes in water quality due to water movement in the aquifer. Student researchers also applied mathematic models to evaluate water movement and contaminant transport using water-level and water-quality data collected from single-well injection and withdrawal tests. The research conducted in the PEER program has provided new tools that can be used to better understand karst hydrology and contaminant transport.

Use of Polymerase Chain Reaction and Oligonucleotide Hybridization Probes to Determine the Source of Fecal Contamination in Karst Terranes

Tom D. Byl[1] and James J. Farmer[2]

Fecal contamination of surface and ground water remains a serious health problem in the U.S. and the world. This problem cannot be adequately addressed until the source of the contamination is known and remediated. At present, no standard monitoring technique exists that can identify the source of the bacteria. We have been working to modify and apply the molecular technique known as "oligonucleotide hybridization probes" to identify bacteria sources in the karst terrane of Middle Tennessee. The oligonucleotide hybridization probes can be used to target ribosomal RNA and DNA. Fecal bacteria unique to a host species can be identified using this molecular technique. Tests have been conducted using RNA hybridization probes that target universal sequences (all bacteria), *E. faecalis* (warm blooded animals), *Lachnospira multiparus* (ruminants), *Fibrobacter succinogenes* (ruminants), *Fibrobacter succinogenes* (ruminants), *Fibrobacter intestinales* (ruminants), *Bacteroides distasonis* (humans), *Bacteroides vulgates* (primarily human), *Bacteroides fragilis* (human) and *Salmonella sp.* (poultry & human pathogen). We were able to differentiate between these organisms in blind studies, but the concentration of bacteria had to be equal to or greater than 1,000 bacteria per liter before the cells were visually detected. The sensitivity was increased by using polymerase chain reaction (PCR) to amplify nucleic acid sequences on DNA (instead of RNA). PCR increased the sensitivity so that only 5 cells per liter were required for positive detection. Using PCR, we identified *Bacteroides fragilis*, a bacterium unique to humans, and *E. coli* in a water and biofilm sample collected from a karst spring-fed stream in Middle Tennessee. These preliminary results indicate this technique has potential to identify sources of fecal bacteria in hydrologically complicated karst terranes.

Originally published as Byl, T.D., and Farmer, J.J., Use of polymerase chain reaction and oligonucleotide hybridization probes to determine the source of fecal contamination in karst terranes, *in* Tennessee Section, American Water Resources Association, 2001, Proceedings from the Eleventh Annual Tennessee Water Resources Symposium, April 2001, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. 2B-41.

[1] U.S. Geological Survey

[2] Tennessee State University and U.S. Geological Survey

Effectiveness of m-TEC Agar for Quantifying *Escherichia coli* in the Duck River near Shelbyville, Tennessee

Allyson M. Morgan[1], James J. Farmer[2], Anthony O. Ejiofor[1], and Terrance L. Johnson[1]

The filter membrane method using m-TEC agar* for quantifying Eshcerichia coli (*E. coli*) concentration is based on the assumption that most of the lactose-fermenting, urease negative (LFUN) bacteria that grow on the agar are *E. coli*. Standard Methods (1995) published by the American Public Health Association recommends specific identification of LFUNs to determine the percentage of the bacteria that are E. coli; however, this procedure rarely is performed. A study was conducted to test the hypothesis that LFUN species can be highly variable in a specific watershed. Four water grab samples were collected from two sites on the Duck River near Shelbyville, Tennessee. Site 1 is upstream, and site 2 is downstream of Shelbyville. The samples were collected once during base-flow conditions and once during storm-flow conditions at each site. Water samples were filtered through 0.45-micrometer filters and placed onto m-TEC agar. Plates were incubated at 44.5°C for 24 hours. Filters were transferred to a pad saturated with urea containing phenol red. Isolates that remained yellow or brown after 15 minutes were considered to be urease negative. LFUN colonies were streaked onto tryptic soy agar for isolation. A total of 124 isolates were identified using the Biolog®* system. At site 1, *E. coli* composed 75 percent and 88 percent of the LFUN isolates from the base-flow and storm-flow samples, respectively. At site 2, 60 percent and 40 percent of the LFUN isolates were identified as *E. coli* for base-flow and storm-flow samples, respectively. The LFUN isolates that were not E. coli were identified as other enteric bacteria. These results indicate that for the samples from site 2, colony counts of LFUN bacteria would result in a poor quantification of *E. coli*.

Published as Morgan, A.M., Farmer, J.J., Ejiofor, A.O., and Johnson, T.L., Effectiveness of m-TEC agar for quantifying *Escherichia coli* in the Duck River near Shelbyville, Tennessee, *in* Tennessee Section, American Water Resources Association, 2003, Proceedings from the Thirteenth Tennessee Water Resources Symposium, April 9–11, 2003, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-19.

*Any use of trade, product, or firm name in this abstract is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

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Evaluation of Passive-Diffusion Samplers to Measure Dissolved Oxygen, Geochemistry, and Volatile Organic Compounds in Ground Water

Sumedha Namuduri[1], Gregg Hileman[2], Koushik Chakraborti[1], and Tom Byl[2]

The efficiency of fuel or solvent biodegradation is dependent upon the amount of dissolved oxygen and other geochemical electron acceptors present in the water. Accurate measurements of ground-water geochemistry are essential when evaluating the potential for biodegradation at a contaminated site. Collecting ground-water samples with a bailer or pump can disrupt the concentration of constituents in the water and provide misleading information about the aquifer. The objective of this study was to determine if geochemical constituents and volatile organic compounds (VOCs) could be monitored more effectively using passive-diffusion (PD) samplers as compared to disposable bailers. Initially, laboratory experiments were conducted to determine whether polyethylene or cellulose-dialysis tubing should be used to make the PD samplers. PD samplers were constructed by filling polyethylene or cellulose-dialysis tubing with ultra-pure water, sealing the ends, and placing the samplers in 4-liter bottles of water with a known geochemistry and VOC concentration. The samplers were removed from the water at specific time intervals, and the contents of the samplers and bottled water were analyzed to determine the VOC concentration, DO, dissolved iron (Fe^{2+}), nitrate (NO_3^{2-}), sulfate (SO_4^{2-}), sulfide (S^{2-}), pH, alkalinity, and specific conductance. Results of laboratory studies indicate that the concentration of VOCs and DO in the polyethylene bags were at equilibrium with the bottled water by 48 hours. The polyethylene bags were not suitable for measuring pH, specific conductance, or any inorganic constituents. The dialysis tubing was better suited for measuring pH, specific conductance, and dissolved inorganic compounds such as Fe^{2+}, NO_3^{2-}, SO_4^{2-}, and S^{2-}. The geochemistry of the bottled water and the cellulose-dialysis sampler came into equilibrium within 4 to 10 hours in the laboratory experiments. In field trials where the results of PD samplers were compared with bailers, DO concentrations obtained using PD samplers were consistently lower by 0.1 to 0.5 milligrams per liter as compared to water collected with disposable bailers. Concentrations of Fe^{2+}, NO_3^{2-}, SO_4^{2-}, and S^{2-} were similar between water collected with dialysis tubing and water collected with bailers in field trials. Geochemical and VOC samples collected with PD samplers did not require filtering before analysis, which was beneficial where geochemical and VOC monitoring was done in wells enriched with molasses, lactic acid and soy milk. One drawback of the cellulose-dialysis PD sampler was that ground-water microbes often generated holes in the membrane when the samplers were placed in wells with aerobic conditions for 4 weeks or longer. However, the cellulose-dialysis samplers maintained integrity in anaerobic conditions over the same time period. There was no observable disintegration in the polyethylene samplers when they were placed in aerobic or anaerobic conditions over a similar time period. In summary, the cellulose-dialysis PD samplers were useful for obtaining accurate geochemical concentrations, and the polyethylene PD samplers were useful for obtaining accurate VOC and DO concentrations.

Published as Namuduri, S., Hileman, G., Chakraborti, K., and Byl, T.D., Evaluation of passive-diffusion samplers to measure dissolved oxygen, geochemistry, and volatile organic compounds in ground water, *in* Tennessee Section, American Water Resources Association, 2003, Proceedings from the Thirteenth Tennessee Water Resources Symposium, April 9–11, 2003, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-21.

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The Value of Single-Well Tracer Studies for Characterizing Karst Sites

Tarra M. Beach[1], Michael W. Bradley[2], Roger Painter[1], and Thomas D. Byl[2]

Water movement, contaminant migration and energy transfer in heterogeneous karst aquifers is challenging to describe quantitatively. Karst ground-water aquifers may be characterized by high velocity, turbulent flow within solution-enhanced fractures, conduits, or stratiform passageways. In other parts of the same aquifer, ground-water flow may be stagnant or very slow. Once a contaminant has entered a karst aquifer, it is often difficult to determine its precise flow-path or residence time in the bedrock. Attempts to characterize a karst site with traditional tracer tests may yield limited information, especially if the tracer cannot be detected at down-gradient monitoring sites. This project applied an integrated approach using local geology, data from fracture mapping, borehole geophysics, and hydraulic testing, as well as geochemical and single-well tracer tests to characterize aquifer hydraulic properties at several karst sites in Middle Tennessee. Two types of wells; existing wells with the characteristics of typical domestic-water wells, and wells constructed to meet project specifications were also evaluated. Single well tracer studies were conducted by injecting a conservative salt tracer of known concentrations into several wells at known depths and measuring the decrease in tracer concentration with time. Changes in tracer concentration were then analyzed to provide information about aquifer advection and dispersion properties in the immediate proximity of the wells. Unfortunately, some of the wells used in this study were drilled approximately 25 years ago and proved to be less than ideal for single-well injection studies. The most difficult problem encountered involved wells characterized by long vertical sections with no bedrock openings for the tracer to enter the aquifer freely. These wells yielded very little useful information. For example, the mean residence time in the water column of bedrock wells with few openings was 65 hours or greater. Conversely, tracer injections in wells with good hydraulic communication with the bedrock aquifer provided useful data with mean residence times ranging from 14 to 45 hours. Single-well tracer studies conducted in properly designed wells provided valuable hydrologic information on the residence-time distribution and dispersion of the tracer in the vicinity of the injection wells. The information collected from single-well injection tests when combined with water chemistry and water level data can be useful for the design of remediation strategies at contaminated karst sites.

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Contaminant Transport and Remediation in Wetlands and Streams

TSU is located in an urban area, and several nearby streams have been adversely affected by sewer overflows and other sources of bacterial contamination. Students in the PEER program have conducted research on the occurrence and fate of bacteria in streams and sediment. The research included studies and model development on the settling, reproduction, and resuspension of fecal bacteria in stream sediment. The PEER program also has a growing program in wetlands research to determine the effect of wetlands on attenuating urban runoff. The research is facilitated by the location of a wetland on the TSU campus. The wetland, adjacent to the main TSU campus, receives urban runoff from adjoining streets, nearby parking lots, and residential and commercial neighborhoods. Students in the PEER program have conducted research at the wetlands and documented the reduction of volatile organic compounds and nutrients as water moved through the wetland. The students deployed water-quality monitors at key points in the wetland and monitored the water-quality changes following storm events and collected and analyzed samples to evaluate the changes due to remediation in the wetland. The results on the research were also utilized within the engineering program to evaluate engineering practices that can be used to reduce the effects of non-point source contaminants in an urban setting.

Survival of Fecal Bacteria in Sediments and Development of a Numerical Model to Predict Storage and Transport in a River

J. Finke[1], R. Graham[1], J. Carpenter[1], T. Rashid[1], L. Sharpe[1], J. Farmer[2] , and T. Byl[2]

Various models, such as CE-QUAL-W2, predict the rate of bacteria removal from the water column based on density, settling rates and water velocity. Such models, however, do not consider survival and reproduction of bacteria in sediments, or re-suspension. Flume experiments are being conducted to measure the survival, reproduction and resuspension of fecal bacteria in sediments. These results will be used to improve numerical models by incorporating survival of bacteria in bed sediments and re-suspension into the water column, in addition to other parameters such as water velocity, initial bacteria concentration, and settling rate. The flume is 10 feet long and 6 inches wide with 2 inches of pre-sterilized sediment (gravel, sand and organic matter) spread on the bottom and 12 inches of water over the sediments. Water was circulated at a velocity of 0.1 meters per second using 2 small pond pumps. Two strains of Escherichia coli (E. coli) and two strains of Klebsiella were introduced into the circulating water at known concentrations and monitored as they settled or remained suspended. Bacteria concentrations were measured in the water column and the sediment along the flume to determine bacterial fate and transport. The model accurately predicted bacteria settling from the water column. Once the bacteria settled onto the sediments, the population declined at an exponential rate over several weeks. The bacteria decomposition in the sediments was described by the equation:

$$B_t = B_i e^{-\mu t}$$

where

B_t = bacteria concentration in the sediment at time t,
B_i = initial bacteria concentration in sediments,
$-\mu$ = exponential decomposition rate (experimental value = 0.2735), and
t = residence time, in days.

This decomposition rate will be coupled to the numerical model after additional tests are done on bacteria re-suspension associated with different velocities to improve the bacterial storage and transport model.

Published as Finke, J.,Graham, R., Carpenter, J., Rashid, T., Sharpe, L., Farmer, J., and Byl,T.D., .Survival of Fecal Bacteria in Sediments and Development of a Numerical Model to Predict Storage and Transport in a River, *in* Tennessee Section, American Water Resources Association, 2004, Proceedings from the Fourteenth Tennessee Water Resources Symposium, March 31–April 2, 2004, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-26.

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Modification of a Numerical Model to Predict Transport and Flux of Fecal Bacteria in a River

James Davis, III[1], Tiffany Hines, John Brew[1], John Finke[1], Lonnie Sharpe[1], and Tom D. Byl[2]

Fecal pollution in surface waters is a serious water-quality problem. As a result, scientists have developed a number of models in an attempt to predict the fate and transport of fecal pollution in riverine systems. Various models predict the rate of bacteria removal from the water column based on density, settling rates and water velocity. Such models, however, do not consider survival and reproduction of bacteria in sediments, or re-suspension. Flume and stream experiments were conducted to measure the survival, reproduction and resuspension of fecal bacteria in sediments. These results will be used to modify a numerical model by incorporating survival of bacteria in bed sediments and re-suspension into the water column, in addition to other parameters such as water velocity, initial bacteria concentration, and settling rate. *E. coli* and *Klebsiella* were introduced into the circulating-water flume at known concentrations and monitored as they settled or remained suspended. Bacteria concentrations were measured in the water column and the sediment along the flume to determine bacterial fate and transport. The model accurately predicted bacteria settling from the water column. The sediment fecal bacteria population declined at an exponential rate over several weeks (experimental decay value = –0.2735). This decomposition rate was coupled to the numerical model and additional tests were done in a small stream contaminated with fecal coliform. Comparison of the model and stream data were mixed due to irregular resuspension of bacteria-contaminated sediments. Additional work is needed on factors that control resuspension of sediments.

Published as Davis III, J., Hines, T., Brew, J., Finke, J., Sharpe, L.,and Byl, T.D., Modification of a numerical model to predict transport and flux of fecal bacteria in a river, *in* Tennessee Section, American Water Resources Association, 2005, Proceedings from the Fifteenth Annual Tennessee Water Resources Symposium, April 2005, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-5.

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A Flux Term to Describe the Movement of Fecal Bacteria between the Sediment and Water Column in a Riverine System

Tiffany Hines[1], James Davis[1], Lonnie Sharpe[1], and Tom Byl[2]

Fecal pollution in surface waters is a serious water-quality problem. As a result, scientists have developed a number of models in an attempt to predict the fate and transport of fecal pollution in riverine systems. Various models predict the rate of bacteria removal from the water column based on density, settling rates and water velocity. Such models, however, do not consider survival and reproduction of bacteria in sediments, or re-suspension. Flume and stream experiments were conducted to measure the survival, reproduction, and resuspension of fecal bacteria in sediments. These results can be used to modify a numerical model by incorporating survival of bacteria in bed sediments and re-suspension into the water column, in addition to other parameters such as water velocity, initial bacteria concentration, and settling rate. Fecal coliform bacteria were introduced into the circulating-water flume at known concentrations and monitored as they settled or remained suspended. Bacteria concentrations were measured in the water column and the sediment along the flume to determine bacterial fate and transport. The model accurately predicted bacteria settling from the water column. The sediment fecal bacteria population declined at an exponential rate over several weeks (experimental decay value = –0.2735). This decomposition rate was coupled to the numerical model, and additional tests were done in a small stream contaminated with fecal coliform. Comparisons of the model and stream data were mixed due to irregular resuspension of bacteria-contaminated sediments. Additional work was done to incorporate resuspension as a function of water velocity, particle size and density into the formula.

Published as Hines, T., Davis, J., Sharpe, L., and Byl, T.D., A flux term to describe the movement of fecal bacteria between the sediment and water column in a riverine system, *in* Tennessee Section, American Water Resources Association, 2006, Proceedings from the Sixteenth Tennessee Water Resources Symposium, April 19–21, 2006, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-8.

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Effect of a Riverine Wetland on Parking Lot Runoff at Tennessee State University

Carlton Cobb[1], Jameka Johnson[1], Tom Byl[2]

A major contributor to non-point source pollution is parking lot runoff during rain storms. Wetlands have been shown to attenuate suspended sediments, bacteria and agricultural pollution. The objective of this project was to determine if a natural riverine wetland located down gradient of a TSU parking lot helped to mitigate the NPS for the runoff. The first phase of the project required walking through the wetlands and the parking lot during a rain storm to observe where the water would flow. This was done to establish sampling points. The second phase was to collect water at the sampling sites during a rainstorm that produced sufficient runoff after a minimum of 3 dry days. These samples were taken back to the lab for analysis. The samples were evaluated for turbidity, specific conductance, pH, and volatile organic compounds (VOC). The most dramatic change in water quality was associated with the VOCs. The water coming off the parking lot–driveway had 62 µg/L benzene, 132 µg/L toluene and 106 µg/L xylenes, as well as, 4 unidentified peaks. As the water moved through the wetland and into a stream, the VOC concentration became undetectable. Additional work is needed to determine if the VOC removal was due to sorption, dilution or biotransformation. However, these preliminary results prove that wetlands are valuable for purifying contaminated runoff from parking lots.

Published as Cobb, C., Johnson, J., and Byl, T.D., Effect of a riverine wetland on parking lot runoff at Tennessee State University, *in* Tennessee Section, American Water Resources Association, 2007, Proceedings from the Seventeenth Tennessee Water Resources Symposium, April 17–19, 2007, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-5.

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Use of Tanks-in-Series Method to Predict Nitrate Removal in Wetlands

Jameka Johnson[1], Carlton Cobb[1], Roger Painter[1], Lonnie Sharpe[1], and Tom Byl[2]

Quantitative tracer studies are frequently conducted to characterize hydrology through non-ideal flow systems and can provide very useful information, such as time of travel, discharge, residence-time distribution, advection and dispersion properties. The objective of this research was to determine if a tanks-in-series numerical model, incorporating residence-time distribution (RTD) coupled to a first-order rate of biodegradation (*k'*), could be used to predict contaminant removal in a series of small, natural, urban wetlands. The study site-wetland system consisted of an upper wetland (200 meters in length) and a lower wetland (400 meters in length) located on the campus of Tennessee State University and was modeled as two non-ideal flows, variable volume tanks-in-series. Flow characteristics used as input to the model were determined by quantitative tracer tests during base-flow and storm-flow conditions. Tracer data established there was an increase in mean residence time during storm-flow conditions due to rising water being diverted through cattails and other vegetation. Dispersion values were also observed to increase during storm-flow. A first-order nitrate removal rate (*k'*) of 0.1748 per hour was derived from a static mesocosm test. The tanks-in-series model using storm-flow conditions (mean residence time of 45 hours) and the *k'* value predicted 93% nitrate removal. Field data to test this model occurred when a leaking sewer provided nitrate-rich inflow to the wetland system during storm-flow conditions. Wetland discharge collected after 45 hours indicated an 83% reduction in nitrate. There was a 10% difference between the measured nitrate concentration and the nitrate concentration predicted using the non-ideal flow tanks-in-series model.

The wetland research site adjacent to the TSU campus. The wetland can be separated into two tanks to model contaminant removal.

Published as Johnson, J., Cobb, C., Painter, R., Sharpe, L., and Byl, T.D.., Use of tanks-in-series method to predict nitrate removal in wetlands *in* Tennessee Section, American Water Resources Association, 2008, Proceedings from the Eighteenth Tennessee Water Resources Symposium, April 2008, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-16.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

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Wetland Removal of Nutrients and Pollution from a Mixed Sewer and Karst Spring System in Nashville, Tennessee

Carlton Cobb[1], Brandon Cobb[1], Patrice Armstrong[2], Jameka Johnson[1], Lonnie Sharpe[1], and Tom Byl[3]

Wetlands have been shown to attenuate suspended sediments and agricultural pollution in rural areas but little work has been conducted regarding the benefits of the wetlands in mitigating urban non-point source pollution (NPS). The objective of this project was to determine if an 80 acre natural wetland located down gradient of bedrock springs, parking lots, city streets and leaky sewer systems in Nashville, Tennessee helped to mitigate urban NPS runoff. Sampling points were selected by reconnaissance during rainfall events to determine general flow paths. Water samples were collected at these sampling points during base-flow and rain runoff events. Water-quality monitors were also placed in the springs and along the flow path during the 12 month period of study. Water samples were analyzed within 48 hours for turbidity, specific conductance, pH, and volatile organic compounds (VOC). Additional analyses were performed for sulfate (SO_4), nitrate (NO_3) ammonia (NH_3) and chemical oxygen demand (COD). It was found that runoff from parking lots and roads during winter storms had relatively high VOC levels (62 µg/L benzene, 132 µg/L toluene, 106 µg/L xylenes, and a number of unidentified compounds). Water samples collected downstream of the wetland, however, had VOC concentrations below detection levels. Water samples collected at the most downstream site also had significantly lower levels of turbidity (90 % lower), NH_3 (99% lower), COD (95% lower), NO_3, (90% lower), and SO_4 (63% lower) on average for the year. The results indicated that routing water through the urban wetland resulted in significant water-quality improvements during the study period.

Published as Hines, T., Davis, J., Sharpe, L., and Byl, T.D., A flux term to describe the movement of fecal bacteria between the sediment and water column in a riverine system, *in* Tennessee Section, American Water Resources Association, 2006, Proceedings from the Sixteenth Tennessee Water Resources Symposium, April 19–21, 2006, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-8.

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3 U.S. Geological Survey

Water-Quality Monitoring—Luminescence

Operators at water and waste-water treatment plants often need to monitor the incoming water stream for the efficient operation of the plant, to prevent contaminants from moving into the drinking-water system or to prevent contaminants from affecting wastewater treatment operations. Traditional methods for real-time or near real-time monitoring of water quality usually rely on easily measured surrogates for contaminants in water, such as turbidity for suspended solids or specific conductance for the concentrations of inorganic compounds or trace metals. The surrogates usually are only applicable for specific contaminants and do not provide broad-spectrum protection. Monitoring water quality by chemical analysis or by periodic bioassays often is too slow in response to avoid problems.

Research into the use of a luminescent response from bioluminescent bacteria or plant oxidase enzyemes has been conducted by TSU students in the PEER program. The luminescent response of an organism or enzyme and the change in the luminescence can represent a direct response to toxicity. The PEER research was conducted to determine if the change in luminescence from biomarkers or bacteria could be used to monitor water quality. The research included two bioluminescent bacteria, Shk1 and PM6, which are gut bacteria from a Tennessee glow worm, *Orfelia fultoni*, and plant oxidase enzymes extracted from watercress and potatoes. Students in the program worked with USGS staff and staff from the Tennessee Wildlife Resources Agency on the field identification and collection of the Tennessee glow worm. Students conducted the laboratory work required to isolate the bioluminescent bacteria from the glow worm, from watercress, and from potatoes.

Evaluating a Bioluminescent Bacteria for Measuring Toxicity of Industrial Wastewaters

Robert Sarfo[1], Tom D. Byl[2] , and Paul Frymier[3]

Toxic compounds can have a negative effect on the performance of activated sludge systems in wastewater treatment systems. Monitoring the influent wastewaters by chemical analysis and periodic bioassays is too slow to avoid problems. The bioluminescent response of bacteria with the luciferase enzyme can provide quick and early toxicity information. Bacteria containing luciferase will bioluminesce under ideal conditions, but bioluminescence decreases as conditions deteriorate. The objective of this project was to evaluate the bioluminescent reporter in the bacterium Pseudomonas fluorescens to determine its suitability as an indicator of industrial toxicants. Bacteria containing the luceferase enzyme and a tetracycline-resistance gene were grown in a nutrient broth amended with 10 parts per million of tetracycline in batch cultures at 25 degrees Celsius. After 48 hours, the bacteria were tested for bioluminescence using a modified fluorometer. The bioluminescent cultures were standardized to 900 fluorescence standard units and used for dose-response bioassays. Toxins were added to the cultures in known concentrations and changes in bioluminescence were measured. Preliminary results found that a 0.02-percent sodium hypochlorite solution elicited an immediate decrease in bioluminescence. Solutions containing less than 1 milligram per liter chlorinated solvents, nickel (2+), and lead (2+), also elicited an immediate decrease in bioluminescence. However, other toxicants such as copper (2+), zinc (2+) and sodium thiosulfate enhanced bioluminescence at low concentrations (0.1 to 1.0 parts per million), but decreased initial bioluminescence by 50 percent at 100 parts per million. Petroleum compounds like toluene and benzene elicited an initial decrease in bioluminescence. However, incubating the bacteria with the toluene for 20 minutes increased the bioluminescence compared to control bacteria, possibly due to induction of toluene di-oxygenase. These preliminary results indicate bioluminescent bacteria might be useful as an early warning indicator of select toxicants.

Published as Sarfo, R., Byl, T. D, and Frymier, P, Evaluating a bioluminescent bacteria for measuring toxicity of industrial wastewaters, *in* Tennessee Section, American Water Resources Association, 2001, Proceedings from the Eleventh Annual Tennessee Water Resources Symposium, April 2001, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association p. P-12.

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Evaluation of Bioluminescent Bacteria as Indicators of Water Quality in Wastewater Treatment Plants

Martin Greene[1], Janique Suber[1], Tom D. Byl[2], and Paul Frymier[3]

Toxic compounds in incoming wastewater can have a negative effect on the performance of activated sludge systems. Monitoring incoming wastewater by chemical analysis and periodic bioassays is often too slow to avoid problems. A more rapid response can be measured by respiration or by using bioluminescent bacteria with the luciferase enzyme. The objective of this study was to compare bioluminescence response with respiration response. The bioluminescence is correlated to metabolic activity and general health of the bacterium. Bacteria containing the luciferase enzyme will bioluminate under ideal conditions, and bioluminescence will decrease as conditions decline. Likewise, healthy sludge bacteria will respire (consume oxygen) at an even rate. During a toxic response, however, the bacteria will rapidly change their rate of respiration and reduce oxygen consumption. In this study, a bioluminescent bacterium, *Pseudomonas sp.* (developed at the University of Tennessee, Knoxville), was compared to the oxygen consumption of sludge bacteria after exposure to varying concentrations of suspected wastewater toxins. Bacteria containing the luciferase enzyme and a tetracycline resistance gene were grown in nutrient broth amended with 10 parts per million of tetracycline in batch cultures at 25°C. After 48 hours the bacteria cultures were tested for bioluminescence using a modified fluorometer. The bioluminescent cultures were standardized to 900 fluorescence standard units, and were used for dose-response bioassays. Toxins, such as sodium hypochlorite or heavy metals, were added to the cultures in known concentrations. Changes in bioluminescence were measured as a response to the toxins. Preliminary results indicate that a 0.02-percent sodium hypochlorite solution elicited an immediate decrease in bioluminescence and oxygen consumption. Nickel (Ni^{2+}) and lead (Pb^{2+}) also elicited rapid decreases in bioluminescence and oxygen consumption. However, sodium thiosulfate enhanced bioluminescence at low concentrations (0.1 to 1.0 parts per million), and increased oxygen consumption at all concentrations. These preliminary results indicate that bioluminescence may be a sensitive and rapid indicator of water quality.

Published as Greene, M., Suber, J., Byl, T. D., and Frymier, P., Evaluation of bioluminescent bacteria as indicators of water quality in wastewater treatment plants, in Tennessee Section, American Water Resources Association, 2003, Proceedings from the Thirteenth Tennessee Water Resources Symposium, April 9–11, 2003, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-16.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

[2] U.S. Geological Survey

[3] University of Tennessee, College of Engineering

Comparison of Bioluminescent Bacteria and Oxygen Consumption as Indicators of Water Quality

Dominic Anako[1], Janique Suber[1], Paul Frymier[2], and Tom D. Byl[3]

Toxic compounds in influent wastewater can have a negative effect on the quality and performance of activated sludge systems. Monitoring the influent wastewater by chemical analysis and periodic bioassays is often too slow in response to avoid problems. Bioluminescent in certain bacteria with a luciferase enzyme can provide quick and early toxicity information. Two bioluminescent bacteria, Shk1 and PM6, were exposed to increasing concentrations of selected chemicals and their bioluminescense monitored to develop dose-response toxicity curves. Likewise, activated sludge bacteria were exposed to increasing concentrations of the same chemicals and their oxygen consumption measured. The bioluminescense and oxygen consumption responses are both indicators of bacteria activity and health. These responses were compared to determine if the bioluminescent responses were comparable to the oxygen consumption response of activated sludge. A control test done with sodium chloride salt did not cause a noticeable change in bioluminescense or oxygen consumption at any of the test concentrations (10 to 100,000 µg/L). Chemicals that elicited a toxic response were zinc (Zn^{2+}), nickel (Ni^{2+}), silver (Ag^{2+}), quaternary ammonia compounds, toluene and sodium hypochlorite. The responses of the two bioluminescent bacteria and the oxygen uptake were found to be similar in most cases with PM6 being the most sensitive and Shk1 being the least. These results indicate that water quality can be monitored using the bioluminescent response in a way that is protective of the activated sludge chamber of the wastewater treatment plant.

Published as Anako, D., Suber, J., Frymier, P., and Byl, T. D., Comparison of Bioluminescent Bacteria and Oxygen Consumption as Indicators of Water Qualityin Tennessee Section, American Water Resources Association, 2004, Proceedings from the Fourteenth Tennessee Water Resources Symposium, March 31–April 2, 2004, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-8.

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Bioluminescent Bacteria as Indicators of Water Quality in a Wastewater-Treatment Plant

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Toxic compounds in influent wastewater can have a negative effect on the quality and performance of activated sludge systems. Monitoring the influent wastewater by chemical analysis and periodic bioassays is often too slow in response to avoid problems. Bioluminescence in certain bacteria with a luciferase enzyme can provide quick and early toxicity information. The two objectives of this project were to determine if a bioluminescent bacterium could serve as a onitor of water quality for incoming water, and, to design a way to apply this technology to provide a continuous toxicity measure. Two bioluminescent bacteria, Shk1 and PM6, were exposed to increasing concentrations of selected chemicals and their bioluminescence monitored to develop dose-response toxicity curves. Likewise, activated sludge bacteria were exposed to increasing concentrations of the same chemicals and their oxygen consumption measured. The bioluminescence and oxygen consumption responses are both indicators of bacteria activity and health. These responses were compared to determine if the bioluminescent responses were comparable to the oxygen consumption response of activated sludge. A control test done with sodium chloride salt did not cause a noticeable change in bioluminescence or oxygen consumption at any of the test concentrations (10 to 100,000 micrograms per liter). Chemicals that elicited a toxic response were zinc (Zn^{2+}), nickel (Ni^{2+}), silver (Ag^{2+}), quaternary ammonia compounds, toluene, and sodium hypochlorite. The responses of the two bioluminescent bacteria and the oxygen uptake were found to be similar in most cases, with PM6 being the most sensitive and Shk1 being the least sensitive. These results indicate that water quality may be monitored using the bioluminescent response in a way that is protective of the activated sludge chamber of the wastewater-treatment plant. Implementing a continuous water-quality monitoring system in a wastewater-treatment plant using bioluminescent bacteria must include consideration of the volume entering the facility, equipment and placement of the monitoring system in-line, potential toxins, and the system's response to a toxin.

Published as Anako, D., Suber, J., Ricks, M., Frymier, P., and Byl, T.D., Bioluminescent bacteria as indicators of water quality in a wastewater-treatment plant, *in* Tennessee Section, American Water Resources Association, 2005, Proceedings from the Fifteenth Annual Tennessee Water Resources Symposium, April 2005, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-3.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

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[3] U.S. Geological Survey

Sensitivity of the Oxydase-Enzyme Induced Chemiluminescent to Water Quality Parameters

Farida Forouzon[1], Lonnie Sharpe[1], and Tom Byl[2]

Biomarkers or enzyme activity are often used to assess the quality of an environment. These indicators are useful because they represent a biological endpoint to toxicity. The objective of this research was to determine if the chemiluminescence from the catalase reaction could be used as a monitor of environmental quality. The primary task to achieve this objective was to evaluate the cause of the chemiluminescence when hydrogen peroxide is added. The second task was to determine whether microbial or plant sources of oxidase enzymes was better. And, the third task was to run assays to determine how water quality parameters affect the chemiluminescence response. This would include tests for optimum pH, optimum temperature, and dose-response tests for various environmental toxins. First a native organism of Tennessee, the glow warm named *Orfelia fultoni* was collected and the gut bacteria were isolated. It was determined that luciferase was not present in the isolated bacteria and that an oxidase reaction in the presence of hydrogen peroxide catalyzed a chemiluminescent response. Additional work is needed to determine which oxidase enzyme is responsible. Since we achieved a chemiluminescent response with the bacteria oxidase enzymes, we tested other sources of oxidase, such as potato, and determined they also gave a chemilumescent response. The experiment shifted to a more available source of catalase, a plant such as potato. A simple experiment using different metals (Pb^{2+}, Ag^{2+}, Ni^{2+}) found that the reaction was microbial and plant oxidase activity was sensitive to the metals at 500 mg/L concentrations. These preliminary tests indicate that plant and microbial sources of oxidase enzymes were capable of producing light. And, that these reactions were sensitive to dissolved metals. Additional work will be done to determine the optimum conditions for the reaction and to describe the dose-response to metals and some organic contaminants. It could be beneficial if this reaction is found to be suitable for monitoring environmental conditions such as water quality and air pollution.

Published as Forouzon, F., Sharpe, L., and Byl , T.D., Sensitivity of the oxydase-enzyme induced chemiluminescent to water quality parameters, *in* Tennessee Section, American Water Resources Association, 2007, Proceedings from the Seventeenth Tennessee Water Resources Symposium, April 17–19, 2007, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-9.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

[2] U.S. Geological Survey

Development of an Aquatic Plant Chemiluminescent Bioassay to Assess Water Quality

Chris Beals[1], Farida Forouzon[2], and Tom Byl[3]

Biomarkers such as enzyme activity from fauna exposed to chemicals in the water column and sediments have been widely used by environmental toxicologists to assess the quality of an environment. Biomarkers are especially useful indicators because they represent a direct biological response to toxicity. The objective of this research was to determine if chemiluminescence from selected plant oxidase enzymes could be used as a biomarker of water quality in aquatic systems. The initial phase of this study included lab determination of optimum pH followed by dose-response assays of various environmental toxins with oxidase enzymes extracted from potato. The optimum pH for the potato oxidase chemiluminescence reaction ranged from 5 to 7. Initial experiments using dissolved metals (Pb^{2+}, Ag^{2+}, Ni^{2+}) found that potato oxidase chemiluminescence was dose sensitive to metal concentrations above 500 mg/L and decreased proportionally with increasing metal concentrations. The chemiluminescent response of watercress collected near a relatively clean spring on Tennessee State University's campus was also investigated. Watercress stems and leaves were macerated with a mortar and pestle and the oxidase enzymes were extracted from the plant material. The crude enzyme extract did provide a chemiluminescent response upon addition of hydrogen peroxide to the assay mixture. The optimum pH for running the watercress chemiluminescence assay was pH of 4. Additional dose-response assays with whole-plant exposure will be needed before this bioassay can be used in water-quality assessments.

Published as Beals, C., Forouzon, F., and Byl, T.D., Development of an aquatic plant chemiluminescent bioassay to assess water quality, *in* Tennessee Section, American Water Resources Association, 2008, Proceedings from the Eighteenth Tennessee Water Resources Symposium, April 2008, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-8.

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Other Research—Degradation and Transport

Research conducted through the PEER program has, at times, included topics that were of specific interest to a student or researcher or were opportune for societal and environmental issues at the time. During a time of high fuel prices and increasing use of ethanol, students in the PEER program conducted research on the rate of contaminant transport for E-85 gasoline. Other research included the application of vitamin B12 to enhance contaminant degradation and measuring the sorption and desorption of fuel to carbonate rocks in karst aquifers. The research was conducted in the TSU environmental laboratory using carbonate rock, soil, and water samples collected from sites in Middle Tennessee and soil samples collected from West Tennessee. The students in the program utilized different methods to collect soil and rock samples, conducted laboratory tests, conducted appropriate QA/QC tests, and documented the results of the experiments.

Desorption Isotherms For Toluene And Karstic Materials And Implications For Transport in Karst Aquifers

Mario Beddingfield[1], Khalid Ahmed[1], Roger Painter[1], and Tom D. Byl[2]

Karst aquifers dominated by conduit flow are extremely vulnerable to fuel contamination such as from leaky underground storage tanks or spills. Direct flow paths through fractures and sinkholes often allow contaminants to move rapidly into the conduit system. Not much is known about how the fuel will interact with the carbonate rock in the conduit system. The objective of this research was to bridge this information gap by measuring sorption and desorption of fuels to karst materials. The first phase of this study involved the dissolution and desorption processes. Initial experiments (n=5) used karst bedrock fragments of known size soaked in toluene for 24 hours. Then the sterile toluene-soaked rocks were placed in sterile distilled water. The concentration of toluene dissolved in the water was measured over increasing time periods. These data were used to derive a first-order exponential rate of desorption [Cw(t)=Ciekt]. The empirical value for k was 0.8958. The toluene concentration in the water reached a maximum carrying capacity in approximately 3 weeks. The second phase of this project involved sorption studies using limestone fragments of known size and water containing a known concentration of dissolved toluene. The empirical value for the sorption k was 1.006. These results show that sorption is faster than desorption and have implications for designing a model that predicts the fate and transport of fuels in karst aquifers.

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Published as Beddingfield, M., Ahmed, K., Painter, R., and Byl, T. D., Desorption isotherms for toluene and karstic materials and implications for transport in karst aquifers, *in* Kuniansky, E.L., ed., 2005, U.S. Geological Survey Karst Interest Group Proceedings, Rapid City, South Dakota, September 2005: U.S. Geological Survey Scientific Investigations Report 2005–5160, p. 188.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

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Reductive Dechlorination of Tetrachloroethylene by Vitamin B12 and Electrokinesis

Tom D. Byl[1] , Tracy Hughes[2], Alishia Miller[2], Pam Franklin[2], and Shannon D. Williams[1]

The reductive dechlorination of aqueous tetrachloroethylene (PCE) by vitamin B12 and a small direct current (DC) was examined in saturated-soil columns and in water columns. A column (24 by 2 inches) filled with saturated soil and subjected to 200 millivolts (mV) DC and 1 micromolar (µM) vitamin B12 lost 10 µM (approximately 10 percent) of the initial PCE volume. There was a concurrent 16 µM increase in trichloroethylene (TCE) in the column. The other columns, lacking the vitamin B12, DC, or both, did not demonstrate a significant loss of PCE or gain of TCE over the 7-day study. In water columns (12 by 0.2 inches) there was a 38 percent decrease of PCE in control columns subjected to no DC or B12. The PCE decreased 55 percent in water columns subjected to DC (250 mV) without B12, and decreased 75 percent in columns subjected to B12 (1 µM) and DC. There was a lack of PCE degradation products in these columns indicating electrokinesis played a major role in removing PCE. In a 4-day experiment, water columns with 0.1 molar (M) sodium chloride, vitamin B12 and 600 mV DC demonstrated a 44 percent loss of PCE (44 µM) and a concurrent gain of 6 µM TCE. There was only a 20 percent loss of PCE in columns without vitamin B12 or DC and no TCE produced. The greater loss of PCE and the subsequent appearance of TCE support the hypothesis that vitamin B12 and a small DC can catalyze the reductive dechlorination of PCE.

Published as Byl, T.D., Hughes, T. H., Miller, A., Franklin, P., and Williams, S.D., Reductive dechlorination of tetrachloroethylene by vitamin B12 and electrokinesis, *in* Crabtree, L.R., Bradley, M.W., Blunt, Tiffany, and Pierre, Salnave, eds., 1999, Proceedings from the Ninth Annual Tennessee Water Resources Symposium, April 1999, Nashville, Tennessee: Tennessee AWRA 1B-43 to 1B-48.

[1] U.S. Geological Survey

[2] Tennessee State University, College of Engineering, Technology, and Computer Science

Are Aquifers at Greater Risk from Alternative Alcohol-Fuel Mixes Compared to Regular Gasoline?

Carlton Cobb[1], Loreal Spear[1], Keyshon Bachus[1], Baibai Kamara[1], Roger Painter[1], Lonnie Sharpe[1], Tom Byl[2]

The United States government is promoting alternative fuels that reduce our dependency on foreign oil. Tennessee is promoting E-85, a fuel that consists of 85 percent ethanol and 15 percent gasoline. The environmental fate of gas-alcohol mixtures, however, has not been investigated. The consequences of an uncontrolled spill of E-85 or a related mixture would, therefore, be very difficult to predict. The objective of this research was to determine if a commercial grade E-85 mixture would dissolve more readily in water and move faster through water-saturated soil than regular gasoline. A better understanding of E-85 mobility in the subsurface is of practical importance if E-85 is to become widely used and stored in underground storage tanks like conventional fuels. Solubility-in-water studies comparing gasoline with E-85 found that the ethanol component in E-85 acted as a co-solvent and enabled aromatic compounds to dissolve five times more rapidly in water than regular gasoline. These enhanced solubility characteristics may allow the aromatic rings to move faster and further through water-saturated soils and karst conduits than regular gasoline. Additional experiments were conducted to determine if regolith soils would affect the dispersal rate of E-85 fuel compounds. Sterile soil-column studies using soils collected from karst regions of Middle Tennessee demonstrated that aromatic compounds, such as benzene, toluene or xylene (BTX), from the E-85 moved 3 to 4 times faster than BTX compounds in regular gasoline when transported by water through the soil. Additional work compared the biodegradation of E-85 with regular gasoline. Using static reactors with karst bacteria, E-85 biodegradation rates were almost 5 times greater than regular gasoline. This is in agreement with previous reports finding that dissolved-phase fuels were more bioavailable and degraded faster. Additional studies are needed to more thoroughly address issues concerning E-85 solubility and biodegradation.

Equipment used to collect soil samples.

Published as Cobb, C., Spear, L., Bachus, K., Kamara, B., Painter, R., Sharpe, L., and Byl, T. D.., Are aquifers at greater risk from alternative alcohol-fuel mixes compared to regular gasoline?, *in* Tennessee Section, American Water Resources Association, 2008, Proceedings from the Eighteenth Tennessee Water Resources Symposium, April 2008, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-10.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

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The Interaction between Calcite Precipitation and Dissolution, Carbon Dioxide, and Perchloroethylene Sorption

Lipi Saha[1] and Tom D. Byl[2]

Calcite ($CaCO_3$) precipitation and dissolution is strongly affected by carbon dioxide (CO_2) concentrations in water. As CO_2 concentrations increase in water, a weak carbonic acid (H_2CO_3) is formed, thereby enhancing dissolution of calcite and, subsequently increasing the concentration of calcium (Ca^{2+}) in solution. Conversely, as CO_2 concentrations decrease, calcite precipitates from solution. Previous studies have shown that metals co-precipitate and dissolve with the calcite. Other studies have shown that nonpolar organic compounds can bind to calcite, but the influence of H_2CO_3 on co-precipitation during calcite precipitation was not studied. The objective of this study was to determine if carbonate-induced calcite dissolution and precipitation influenced perchloroethylene (PCE) sorption to $CaCO_3$. The experiments were conducted using 12-milliliter screw-top test tubes containing 2 grams of calcite (grain size = 0.062 to 0.500 millimeters) and distilled water, along with varying amounts of PCE and H_2CO_3. We used a minimum of three replicates per treatment. The first set of experiments established that $CaCO_3$ dissolved and precipitated as expected under different concentrations of H_2CO_3. The second set of experiments established a linear sorption isotherm for PCE to calcite without the addition of H_2CO_3 (Kd = 1.35). The third set of experiments used calcite that was precontaminated with a known amount of PCE. Clean water containing no PCE and 0.00, 0.01, or 0.10 molar H_2CO_3 was added to the precontaminated calcite and desorption was allowed to occur for 4 hours. Desorption of PCE into solution occurred in all treatments. Approximately 12 percent of the sorbed PCE desorbed into the distilled water. Approximately 28 percent of the PCE desorbed from the calcite when it was placed in the 0.01 and 0.1 molar H_2CO_3 solutions. Thus, H_2CO_3 appears to enhance desorption of PCE 16 percent as it dissolves the calcite. The Ca^{2+}, CO_2, and pH were also measured to establish geochemical conditions in each test.

Published as Saha, L., and Byl, T.D., The Interaction between Calcite Precipitation and Dissolution, Carbon Dioxide, and Perchloroethylene Sorption *in* Tennessee Section, American Water Resources Association, 2001, Proceedings from the Eleventh Annual Tennessee Water Resources Symposium, April 2001, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-11.

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Reductive Dechlorination of Perchloroethylene with a Direct Current, Different Salts, and Vitamin B_{12}

Majharul Alam[1] and Tom D. Byl[2]

Initial tests to reductively dechlorinate perchloroethylene (PCE) by using vitamin B_{12} dissolved in water and a small direct current were successful; however, the transformation process was inefficient (less than 30 percent transformation). If the process is to be utilized in the field, the efficiency must be improved. The objective of this study was to identify environmental conditions that increase PCE transformation into trichloroethylene (TCE) by electrokinesis and vitamin B_{12}. Tests were conducted using 12-inch glass tubes filled with 3.5 milliliters of aqueous solutions containing 10 to 50 milligrams per liter (mg/L) vitamin B_{12}, 0.1 to 25.0 mg/L PCE, and a variety of salts. The ends of the tubes were sealed with silicone gel with a platinum electrode extending through the silicone. A direct current was applied to each test chamber using the platinum electrodes and a constant power supply. Preliminary results show that the salt type and concentration had an effect on PCE-transformation efficiency. For example, test solutions containing 25 mg/L vitamin B12 and 100 mg/L of CaC_{l2} had an 85-percent greater transformation of PCE to TCE than distilled water only containing vitamin B_{12}. Solutions containing NaCl and $CaCl_2$ were twice as effective as solutions with $FeCl_3$ and $Fe(NH_4)_2(SO_4)_2$ at enhancing transformation of PCE to TCE. Addition of the salts NH_4Cl and $FeSO_4$ produced the lowest remaining concentrations of PCE but produced no significant amount of TCE in the test chamber. Whether PCE volatilized or was completely dechlorinated in those tests is not known. Also, a steady low voltage (200 mV) was more effective than higher voltages (greater than 400 mV) at inducing reductive dechlorination. The efficiency of the dechlorination reaction appeared to be unaffected by PCE concentration when PCE concentrations ranged from 0.1 to 25.0 mg/L. This information will be useful for adapting the method from the laboratory to the field.

Published as Alam, M., and Byl, T.D, Reductive Dechlorination of Perchloroethylene with a Direct Current, Different Salts, and Vitamin B_{12}, *in* Tennessee Section, American Water Resources Association, 2001, Proceedings from the Eleventh Annual Tennessee Water Resources Symposium, April 2001, Montgomery Bell State Park, Burns, Tennessee: Tennessee Section of the American Water Resources Association, p. P-1.

[1] Tennessee State University, College of Engineering, Technology, and Computer Science

[2] U.S. Geological Survey

Student Awards

Students in the PEER program are encouraged to actively participate in scientific conferences and to present the results of their investigations in proceedings, oral presentations, and poster sessions. PEER students have presented their research at the following conferences and meetings; American Water Resources Association in Tennessee and Alabama, the USGS Karst Interest Group, the Society of Environmental Toxicology and Chemistry, the Tennessee Louis Stokes Alliance for Minority Participation and the TSU Campus-Wide Research Symposium and Department of Engineering Industrial Cluster. The quality of the research conducted by the PEER students is reflected in the awards they have received.

National Awards

1. Mr. Doug Warden, 2000
2. Ms. Sumedha Namuduri, 2002
3. Ms. Valetta Watson, 2002
4. Mr. Koushik Chakraborti, 2003
5. Ms Tarra Beach, May 2006
6. Ms Tarra Beach, Nov 2006
7. Ms Kelly Ray, Nov. 2006

Regional Awards

1. Mr. Detoy James, 2000
2. Mr. James Murithi, 2000
3. Ms. Shimere Williams, 2001
4. Ms. Kamalah Minor, 2002
5. Ms. Rashaunna Mohmmad, 2002
6. Ms. Sumedha Namuduri, 2002
7. Ms. Tavy Wade, 2002
8. Ms. Valetta Watson, 2002
9. Mr. Koushik Chakraborti, 2003
10. Mr. Khalid Wood. 2003
11. Ms Lashun King, 2004 (twice)
12. Mr. Mario Beddingfield, 2005 (twice)
13. Mr.Carlton Cobb, 2006
14. Ms. LyTreese Hampton, 2006
15. Ms Jameka Johnson, 2006
16. Ms. Lashun King , 2006
17. Mr. Carlton Cobb, 2007
18. Mr. Farida Forouzon, 2007
19. Ms Jameka Johnson, 2007
20. Mr. Derek Lovett, 2007

University Awards

1. Ms. Christa Cruikshank, 1999
2. Mr. Gopu Apo Rao, 1999
3. Mr. Robert Sarfo, 2000
4. Ms. Laltiha Akula, 2001
5. Mr. Majhar Alam, 2001
6. Mr. Allyn Allison, 2001
7. Ms. Amini Boston, 2001
8. Ms. Lipi Saha, 2001
9. Mr. Daniel Agyemang, 2002
10. Ms. Ramona Darlington, 2002
11. Ms. Pam Franklin, 2002
12. Ms. Shaunna Haynes, 2002
13. Ms. Natascha Morris, 2002
14. Ms. Sumedha Namuduri, 2002
15. Ms. Sudeshna Roy, 2002
16. Ms. Valetta Watson, 2002
17. Mr. Koushik Chakraborti, 2003
18. Ms. LeMiracle Hendking, 2004
19. Ms. Lashun King. 2004
20. Mr. Dominic Anako, 2005
21. Mr. John Brew, 2005
22. Mr. James Davis, 2005
23. Ms. Isabelle Ford, 2005
24. Mr. Omar Hamawandi, 2005
25. Ms. Tiffany Hines, 2005
26. Mr. Serge Mondesir, 2005
27. Mr. Carlton Cobb, 2006
28. Mr. Ryan Fitzwater, 2006
29. Ms Jameka Johnson, 2006
30. Ms Charner Rogers, 2006
31. Ms Farida Fourizan, 2007
32. Ms Jameka Johnson, 2007
33. Mr Derek Lovett, 2007

Five PEER presentations by TSU students were made at the international Society of Environmental Toxicology and Chemistry (SETAC) conference in Montreal, November, 2006. (Left to right: Kelly Ray, won 1st place in the Elsevier Poster contest; Tarra Beach, won 2nd place in the student platform; Tom Byl and Tiffany Hines)

Outreach

The PEER program has included a significant outreach componant that includes pre-college students, undergraduate students, and graduate students. As part of the PEER program, ground-water hydrologists with the USGS Tennessee Water Science Center organized educational field trips to hydrogeologic sites in Tennessee. A special field trip to Mammoth Cave National Park for engineering and biology students and faculty was conducted in cooperation with the U.S. National Park Service. A new graduate-level course in ground-water hydrology was developed and taught by Michael Bradley of the USGS Tennessee WSC. The students in the PEER program also conducted outreach activities for local schools. Several classes in earth sciences were taught by students in the PEER program each day at the TSU Summer Camps for pre-college students.

Carlton Cobb (second from left) uses his experiences from the PEER program to teach incoming engineering freshmen about nonpoint-source pollution and water quality.

Brandon Cobb demonstrates contaminant movement in groundwater at the University of Tennessee Agricultural Extension Service Small-Farms Field Day in August, 2008.

Dr. Tom Byl (left photo) and TSU students (Ms. Tarra Beach shown in right photo) visit elementary schools in Nashville to teach them about science and math. The 4th graders learned about earthquakes and then designed houses to withstand a simulated earthquake.

The PEER program helped to sponsor the summer science and technology camps for more than 100 pre-college teens from across the Nation. Small groups of students (four to eight) were instructed in lab safety, environmental engineering, groundwater, and water contamination. These small groups allowed more effective interaction between the students and instructors. TSU provided the camp space, laboratory facilities, and student instructors, and the USGS provided instructors, lessons and experiments, and materials.

TSU students interning at the USGS instructed more than 100 pre-college minority students about lab safety, hydrology, chemistry, stream biomonitoring, nonpoint-source pollution, geology, microbiology, water-quality testing, and fossils at the 2006 summer engineering camps.

Michael Bradley, USGS Tennessee WSC, developed a new graduate course in groundwater hydrology for the College of Engineering. Here Mike discusses well construction and field techniques for the groundwater hydrology class.

Dr. Bill Wolfe, USGS Tennessee WSC, (center) shows TSU students Nkechi Chieko (left) and Derek Lovett (right) how to use the Guelph permeameter on TSU's campus.

The TSU environmental engineering program was recognized at the Southeast U.S. Graduate School Dean's conference in March 2007. (pictured, left to right, Dr. Farouk Mishu, head, Civil & Environmental Engineering; Professor Valetta Watson, Dr. Roger Painter, Dr. Melvin Johnson, President TSU; Ms. Tiffany Hines, (PEER student), and Dr. Tom Byl, USGS & PEER faculty.

Summary

The Partnership for Environmental Education and Research (PEER) is a joint partnership between the Tennessee State University, College of Engineering and Technology (TSU) and the U.S. Geological Survey (USGS) Tennessee Water Science Center (WSC). The partnership was developed to support environmental research at TSU, to expand the environmental research capabilities of the USGS in Tennessee, to conduct environmental research, and to promote environmental education.

Student and faculty research in the PEER program is being conducted to better define the occurrence, fate, and transport of contaminants in groundwater and surface water. Contamination of surface water and groundwater affects the adequacy of water resources to meet ever-increasing demands for municipal, agricultural, and industrial use. Variations in natural water quality, contaminant concentrations, and geochemical, biological, and hydrogeological processes affect the fate and transport of contaminants in surface water and groundwater. Research conducted through the PEER program provides additional information to better understand these processes and help improve strategies for pollution prevention, mitigation, and remediation in karst.

Research in the PEER program has primarily focused on the transport and remediation of organic contamination in karst settings. As the activities in karst areas expanded, PEER research included mathematical modeling for contaminant transport and degradation, the adaptation of residence-time distribution and Lattice-Boltzmann models to karst aquifers, the occurrence and role of bacteria in karst aquifers, and the occurrence and fate of different contaminants in karst aquifers. The PEER program has expanded to apply research to a variety of media and settings. Research has been conducted on new methods to evaluate ground-water contamination, the resuspension of bacteria from sediment in streams, the use of bioluminescence and chemiluminescence to identify the presence of contaminants, and contaminant remediation in wetlands.

The PEER program also supports TSU outreach activities and their efforts to increase minority participation in environmental and earth science programs at the undergraduate and graduate levels. PEER has increased research and education opportunities for the College of Engineering and Technology and provided students with experience in presenting the results of the research. Students in the program have participated in state, regional and national conferences, giving more than 140 presentations since 1998 and have won more than 40 student awards.

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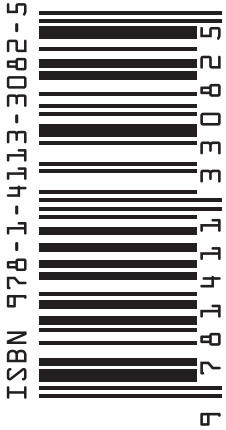

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