

# **Report as of FY2007 for 2007MN215B: "Enhanced Contaminant Remediation: Fermentation as a Method to Enhance Dissolution of Hydrophobic Compounds"**

## **Publications**

Project 2007MN215B has resulted in no reported publications as of FY2007.

## **Report Follows**

## **Enhanced Contaminant Remediation: Fermentation as a Method to Enhance Dissolution of Hydrophobic Compounds**

### **Principal Investigator**

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### **Executive Summary**

The mechanisms of enhanced mass transfer stemming from fermentation of carbon (molasses) were identified through this research. Several partitioning experiments were performed including the use of non-aqueous phase liquid (NAPL) and soil aged with trichloroethene (TCE) to evaluate how both fresh and fermented molasses affect the partitioning of TCE between NAPL, sorbed and aqueous mass. These experiments indicated a >200% increase in TCE solubility could be achieved through addition of fresh molasses solution. Increased solubility leads to increased mass flux of TCE NAPL to aqueous phase liquid. Fermented fluid did not increase solubility of TCE but did interact with the NAPL to form an emulsion. Increased surface area also leads to increased mass transfer from NAPL to aqueous phases. Accordingly, increased mass transfer from NAPL was caused by both fresh and fermented molasses solution, through two separate mechanisms. In addition, soil partitioning appeared to be affected by fermented fluid indicating fermented molasses could also enhance transfer of sorbed mass to the aqueous phase.

### **Introduction**

Recently researchers have been working to develop innovative technologies to address both sorbed phase contaminants and non-aqueous phase liquid (NAPL) source areas *in situ*. Among these technologies are Surfactant-Enhanced Aquifer Remediation and cosolvent flooding. Both of these technologies involve the injection of chemicals into

an aquifer in a manner designed to “flood” the impacted zone, thereby mobilizing sorbed contaminant mass and NAPL (ITRC, 2002). The mobilized contaminant is subsequently extracted and treated *ex situ*. Although effective, these methods depend on our ability to adequately contact the contaminants with surfactants or cosolvents. This can be a challenge, and is typically addressed by adding large quantities of chemical in the hope that this will facilitate contact. A novel, and perhaps better method of cosolvent and/or biosurfactant delivery is through the stimulation of naturally occurring organisms that produce various cosolvents and biosurfactants *in situ* through fermentative processes. These processes can be stimulated by addition of readily degradable carbon sources (i.e. sugars) to the aquifer. Multiple fermentation pathways exist, each yielding a particular set of end products, including alcohols, ketones, volatile fatty acids and gases. Our hypothesis for this research was that the combination of cosolvents and biosurfactants produced *in situ* during fermentation could contribute to the release of sorbed contaminants and aid in the dissolution of NAPL present within the aquifer. We proposed that an accelerated cleanup of lingering source areas could be achieved by optimizing the production of cosolvents and biosurfactants via fermentation processes.

The experiments described within this report were designed to evaluate how fermented carbon (molasses) affects the partitioning of a hydrophobic compound, trichloroethene (TCE), within an aqueous system. Long-term aging of soil to promote sorption of TCE began in February 2007 to prepare for soil partitioning experiments. Two fill and draw reactors were initiated in June 2007, and consisted of one control reactor containing a minimal groundwater media (no carbon amendment) and a fermentation reactor containing minimal groundwater media combined with 10% molasses by volume (vol/vol). In addition, a 20% (vol/vol) molasses-fed culture was also started using a 500-mL batch reactor with approximately 400 g soil and 250 mL of 20% molasses in minimal groundwater media.

Three different types of partitioning experiments were performed. The first set of experiments began in March 2007 and consisted of TCE NAPL and individual cosolvents commonly produced during fermentation, consisting of acetone, ethanol, methanol, 2,3-butanediol, and volatile fatty acids in the protonated form (lactic, acetic, butyric, succinic, formic, propionic, valeric and caprioc) at varying fractions up to 20% vol/vol. These were

initiated to determine whether low levels of cosolvents could individually enhance solubility of TCE. The second set of partitioning experiments were initiated in December 2007, and included the use of the fermentation and control reactors and NAPL to determine whether fermented liquid affected NAPL and TCE solubility. The third set of experiments was initiated in February 2008, and utilized the TCE aged soil (aged approximately 12 months) to determine whether fermented fluid had an affect on sorbed TCE.

Aqueous phase TCE from partitioning and sorption experiments, and alcohols and ketones from the fermentation reactors were analyzed using a Hewlett Packard (HP) 5820 Series II gas chromatograph equipped with a flame ionization detector. For the sorption experiments, TCE was extracted with hexane and analyzed using an HP 5890A GC equipped with an electron capture detector. Extraction efficiencies for TCE in saturated soil averaged 108% with a standard deviation of 7%. Volatile fatty acid analysis was performed using high performance liquid chromatography (Shimadzu LC-19 AT) equipped with a SPD-10A UV-Vis detector (210 nm wavelength) and an Aminex HPX-89H column. Total Organic Carbon from the reactors was analyzed using a HACH High Range (100 to 700 mg/L) Total Organic Carbon Test 'n Tube Reagent set. An Orion Model 420 digital pH meter equipped with a combination electrode was used for pH measurements. Surface tension measurements were obtained using a Kruss DSA10 Drop shape analysis system. Soil was sent to the University of Minnesota Research Analytical Laboratory for textural analysis via sieving and hydrometer analysis, and fraction of organic matter (foc) analysis by the Loss on Ignition method.

## Results

**Table 1** summarizes the results of all tested cosolvents in the NAPL partitioning experiments, and **Figure 1** demonstrates the increases in TCE solubility resulting from the addition of different fractions of selected cosolvents. As shown, fractions of 0.2 or higher were generally required to enhance TCE solubility through cosolvency, if enhancement occurred. Methanol was determined to enhance solubility at a lower fraction of 0.05, and several compounds did not affect TCE solubility at fractions of 0.2 or lower. Cosolvents that enhanced solubility did so at concentrations higher than would

be expected to be produced during fermentation processes (Jones and Woods, 1986; Ren et al., 2007).

In addition to the three-phase NAPL partitioning experiments, the fermented liquid from each reactor were evaluated to determine their effect on TCE solubility. In addition, non-fermented molasses (both 10% and 20% vol/vol) were evaluated for TCE solubility effects. As shown in **Figure 2**, the solutions with the greatest impact on TCE solubility were the fresh molasses solutions. Approximately a 2.3- and 2.7-fold increase in TCE solubility was observed with the fresh 10% and 20% molasses solutions, respectively. The fermented 10% molasses solution (after 234 days of fermentation) did not have an impact on solubility. The fermented 20% molasses solution (after 239 days of fermentation) did, however, increase TCE solubility (**Figure 2**). The solubility enhancement with fresh molasses solutions is similar to that observed by Macbeth et al. (2006), where up to a 6.7-fold increase in TCE solubility was observed when in the presence of a fresh 10% whey solution. Macbeth and coworkers attributed the solubility enhancement to the protein fraction (10 to 13%) present in the whey powder. The blackstrap molasses used in these experiments contained approximately 3% crude protein, which may have resulted in the solubility enhancement with fresh molasses.

Both the liquid in the fermenting reactors and field samples were analyzed for compounds that could potentially serve as cosolvents to determine whether cosolvency was the reason for solubility enhancement in the fermented 20% molasses reactor. High levels of volatile fatty acids (VFAs) and ethanol were detected in the reactors undergoing molasses fermentation (data not shown). To determine whether cosolvency contributed to the solubility enhancement observed in the fermented 20% molasses reactor samples, the highest detected concentration of each cosolvent from the analysis was combined and used in a NAPL partitioning experiment to determine the effect on TCE solubility. The combination of cosolvents did not enhance solubility (**Figure 2**), further supporting that the solubility enhancement shown in the 20% fermented reactor is due to a degradable solubilizing component within the raw molasses.

While solubility was not substantially increased by the fermentation products present in the fermented 10% molasses reactor, the NAPL phase surface area was visibility altered. As shown in **Figure 3A**, the NAPL formed a microemulsion with the

fermented 10% molasses solution (234 days of fermentation) that continued to be stable >100 days after settling, while the control resumed two distinct phases (NAPL and aqueous) immediately after mixing. Fresh molasses (10%) also formed a microemulsion immediately following removal from the rotator, but the emulsion was not stable and returned to two distinct phases after 48 hours of settling (**Figures 3.B1** and **3.B2**).

The effect of fermented molasses on soil partitioning was evaluated by assessing the soil distribution coefficient ( $K_d$ ) of TCE while in the presence of fermented fluid. Data were used to calculate  $K_d$  for each sample by dividing the mass of TCE sorbed to the soil by the equilibrium concentration of TCE in the liquid phase (Eqn 1). An averaged value of triplicates was used for each dataset. Fermented 10% molasses (after 234 days fermentation), fresh 10% molasses and media from the control reactor were used in the experiments.

$$\text{Eqn (1)} \quad K_d = C_{\text{sorb}} / C_{\text{eq}}$$

Where  $C_{\text{sorb}}$  [M/M] is the mass of contaminant sorbed per mass of soil,  $C_{\text{eq}}$  [M/V] is the equilibrium concentration of contaminant present in the liquid phase, and  $K_d$  is an experimentally derived soil distribution coefficient (Watts, 1998).

As shown in **Figure 4**, the fermented molasses sample appeared to result in a decreased  $K_d$  value when compared to the control. This difference, however, was not significant at the 95% confidence level ( $p = 0.12$ ). Accordingly, more work is needed to determine whether the  $K_d$  value is truly affected by the products generated during the fermentation of organic carbon.












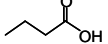
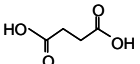
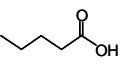
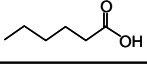
In conclusion, our studies indicate that enhanced dissolution of TCE NAPL can occur through the addition and subsequent fermentation of dilute molasses solutions. The mechanism by which this occurs is different depending on whether the solution is fresh or has been allowed to ferment. Enhanced mass transfer occurs through an increased concentration gradient when fresh molasses is present, as the solubility of TCE is increased greater than 2-fold. Data indicate that cosolvency of TCE does not occur with fermented products; therefore, after the liquid has fermented, solubility enhancement does not contribute to enhanced mass transfer. Biosurfactants appear to be generated

during the fermentation process, as evidenced by the stable emulsion formed when the NAPL and fermented aqueous phases were mixed. The resulting increase in surface area could greatly affect the mass transfer of TCE NAPL into the aqueous phase if mixing could be accomplished in the subsurface. In addition, fermented carbon may have an affect on the soil partitioning coefficient, indicating that fermented liquid may also enhance the dissolution rate of sorbed mass into the aqueous phase.

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**Table 1.** Summary of results from 3-phase cosolvent partitioning runs.

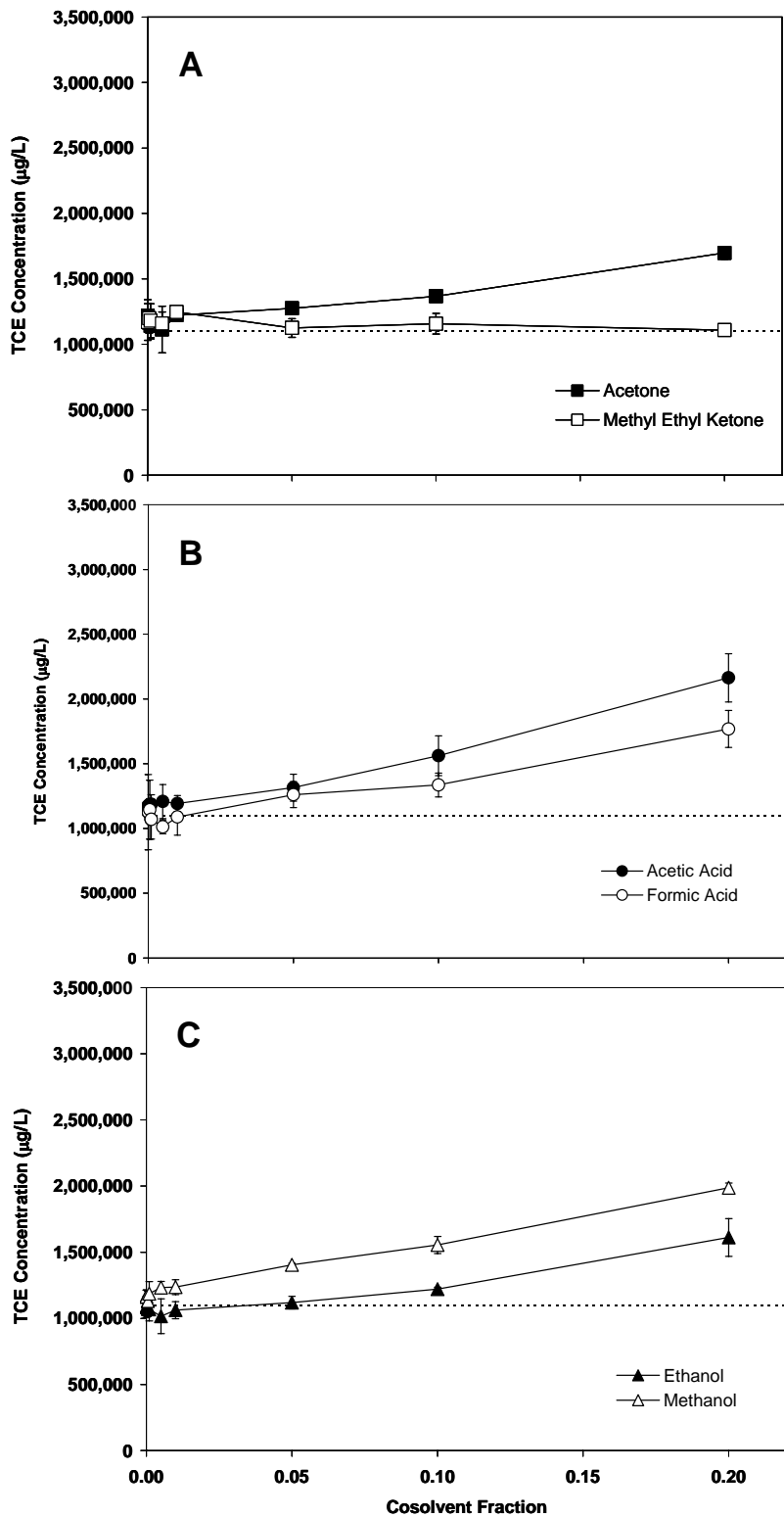
Compound	Molecular Formula	Structure	Effect on TCE Solubility ( $P \leq 0.05$ ) <sup>a</sup>	Normalized Solubility Increase <sup>b</sup>
Acetone	C <sub>3</sub> H <sub>6</sub> O		No change $\leq 10\%$ <sup>c</sup> Increase <b>20%</b>	1.39 (20%) <sup>d</sup>
Methyl Ethyl Ketone	C <sub>4</sub> H <sub>8</sub> O		No change $\leq 20\%$	None
Methyl Isobutyl Ketone	C <sub>6</sub> H <sub>12</sub> O		No change $\leq 2\%$	None
Methanol	CH <sub>4</sub> O		No change $\leq 1\%$ Increase <b><math>\geq 5\%</math></b>	1.21 (5%) 1.34 (10%) 1.71 (20%)
Ethanol	C <sub>2</sub> H <sub>6</sub> O		No change $\leq 10\%$   Increase <b><math>\geq 20\%</math></b>	1.52 (20%)
Butanol	C <sub>4</sub> H <sub>10</sub> O		No change $\leq 20\%$	None
2,3- Butanediol	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>		No change $\leq 20\%$	None
Formic Acid	CH <sub>2</sub> O <sub>2</sub>		No change $\leq 10\%$ Increase <b><math>\geq 20\%</math></b>	1.57 (20%)
Acetic Acid	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>		No change $\leq 10\%$ Increase <b><math>\geq 20\%</math></b>	2.22 (20%)
Lactic Acid	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>		No change $\leq 10\%$ Increase <b><math>\geq 20\%</math></b>	1.64 (20%)
Propionic acid	C <sub>3</sub> H <sub>5</sub> O <sub>2</sub>		No change $\leq 10\%$ Increase <b><math>\geq 20\%</math></b>	1.72 (20%)
Butyric Acid	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>		No change $\leq 20\%$	None
Succinic acid	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>		No change $\leq 20\%$	None
Valeric Acid (Pentanoic)	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>		No change $\leq 20\%$	None
Caproic Acid (Hexanoic)	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>		No change $\leq 20\%$	None

a Change in concentrations considered statistically significant at  $p=0.05$  using the Student's t-test.

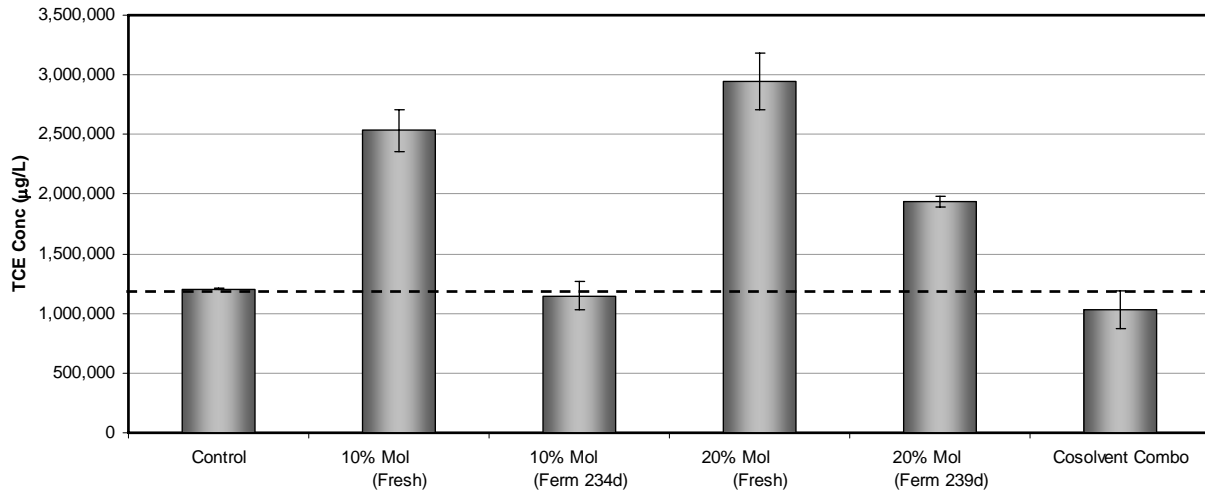
b Solubility increase normalized to average of unamended controls (triplicate run) within same experimental set.

c Percent based on volume per volume (vol/vol) basis

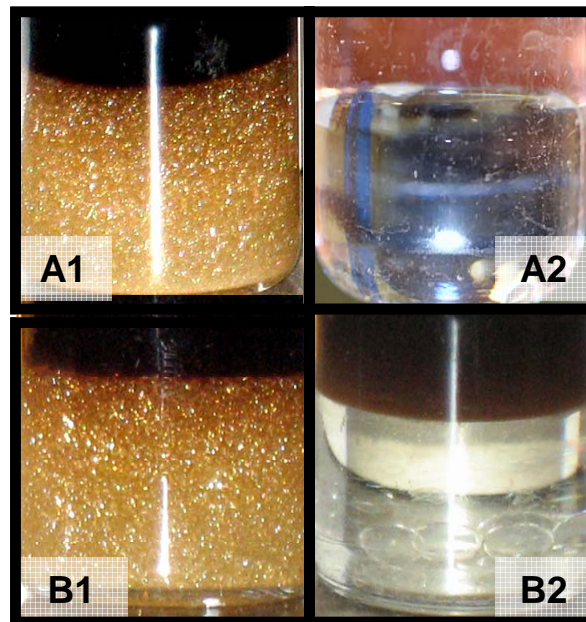
d Cosolvent fraction at which solubility increase observed.



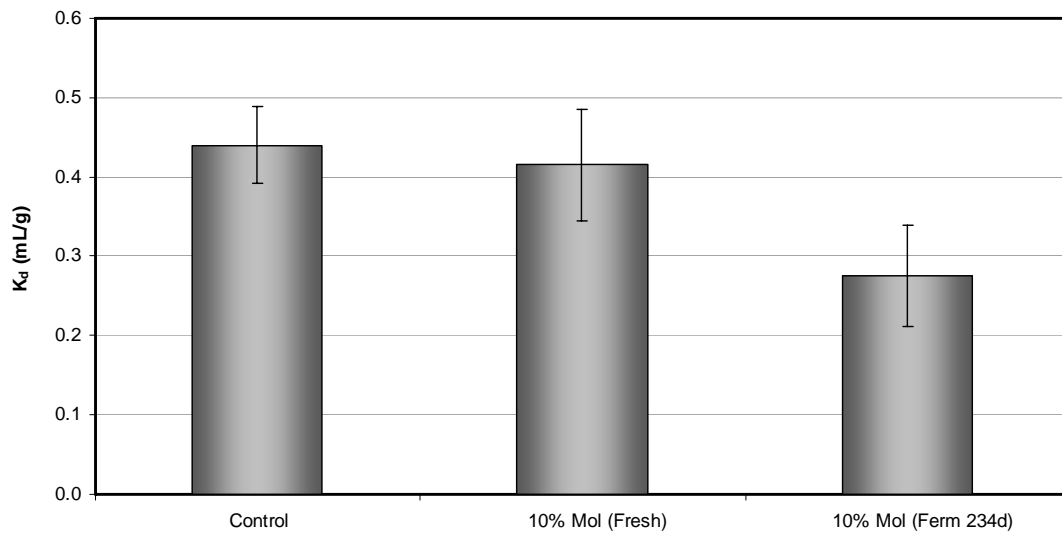
**Figure 1.** TCE solubility in presence of selected (A) ketones, (B) volatile fatty acids, and (C) alcohols. Data points represent average of triplicate runs with error bars showing standard deviation. Dashed line represents TCE solubility (1.1 g/L) in water (Pankow and Cherry, 1996).



**Figure 2.** Concentrations of soluble TCE in vials containing a NAPL phase and media only (control), media amended with unfermented molasses (Fresh), media amended with molasses that was allowed to ferment (Ferm) for 234 days (10% molasses), and 239 days (20% molasses). TCE solubility in water (1.1. g/L) is indicated by the dashed line. Data points represent the average of triplicate runs, with error bars showing the standard deviation.



**Figure 3.** Photographs showing changes in NAPL surface area as affected by fermented versus non-fermented substrate and emulsion behavior over time. (A1) TCE emulsion resulting from mixing NAPL-phase TCE with 10% fermented molasses solution, shown 118 days after mixing, (A2) NAPL surface area after mixing with control reactor media. (B1) TCE emulsion immediately following mixing with fresh non-fermented molasses (<24 hrs), (B2) NAPL >48 hours following mixing with fresh molasses where return to solid state indicates initial emulsion was not stable. Note: Glass beads are visible on the bottom of vials in (A2) and (B2).



**Figure 4.**  $K_d$  values for TCE in soil partitioning experiments with vials containing media only (control), media amended with unfermented 10% molasses (Fresh), and media amended with 10% molasses that was allowed to ferment (Ferm) 234 days. Data points represent the average of triplicate runs, with error bars showing the standard deviation.

**Publications, Presentations, or Published Abstracts:**

Publications

The paper detailing the results of this research has been finalized and will be submitted to the Journal of Environmental Engineering by June 30, 2008.

Presentations (\* indicates student presentation)

Nelson, D.\* and P. Novak. 2007. Fermentation as a Method to Enhance Dissolution of Hydrophobic Compound. Poster Presentation. Association of Environmental Engineering and Science Professors Interactions at the Interface Conference July 28 – August 1, 2007, Blacksburg, VA.

Nelson, D.\* and P. Novak. 2008. Presentation at the Fall 2008 American Chemical Society National Meeting & Exposition. To be held 17- 21 August, 2008, Philadelphia, PA.

**Students and Post-doctoral Research Associates supported by this project:**

None

**Additional Funding:**

Denice Nelson received a Sommerfeld fellowship from the University of Minnesota's Department of Civil Engineering to conduct this research. We have submitted a proposal to the National Science Foundation for follow-up work.

**Awards and Special Recognition:**

None to date