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Competitiveness of *Pseudomonas stutzeri* KC

presented by

Mark Lee Sneathen

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of the requirements for

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**THEORETICAL AND EXPERIMENTAL COMPETITIVENESS OF
PSEUDOMONAS STUTZERI KC**

By

Mark Lee Sneathen

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department Of Civil And Environmental Engineering

1996

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ABSTRACT

THEORETICAL AND EXPERIMENTAL COMPETITIVENESS OF *PSEUDOMONAS STUTZERI* KC

By

Mark Lee Sneathen

The competitiveness of *Pseudomonas stutzeri* KC with groundwater flora indigenous to an aquifer impacted by carbon tetrachloride (CT) was evaluated by measuring kinetic growth parameters, predicting competitive outcomes based on those parameters, and testing the predictions in batch experiments. The maximum specific growth rates of KC and the groundwater flora occurred at pH 8.2 and pH 7.5. KC displaced the groundwater flora at pH 8.2 and became the dominant organism when both populations were at similar densities initially. At pH 7.5, if 150 KC colonies existed for every groundwater colony initially, KC was able to remain the dominant species for at least nine days. Simulated groundwater (SGW) medium proved to be the best starter medium of those tested for promoting the dominance of KC. KC displaced the groundwater flora in some regions of an aquifer when it was introduced as part of a bioremediation project.

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S_{min}

μ_{max}

b

K_s

X

k_m

μ

S

t

Y_m

LIST OF SYMBOLS

$S_{\min}, J, R^*$	substrate concentration below which net growth does not occur (mg/L)
$\mu_{\max}$	maximum specific growth rate (days ⁻¹)
b	decay coefficient (days ⁻¹)
K_s	half-velocity coefficient (mg/L)
X	concentration of microorganisms (mg/L)
k_m	maximum specific rate of substrate utilization (days ⁻¹)
μ	specific growth rate (days ⁻¹)
S	rate-limiting substrate concentration (mg/L)
t	time (days)
Y_m	maximum organism yield (mg organisms/mg substrate)

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CHAPTER 1

INTRODUCTION

In-situ Bioremediation with *Pseudomonas stutzeri* KC

In-situ bioremediation of groundwater offers the potential for remediation of many contaminated sites. The two most prominent methods of in-situ bioremediation are biostimulation, the addition of growth limiting factors to increase the concentration of indigenous organisms, and bioaugmentation, the introduction of organisms with desired metabolic capabilities. For situations where indigenous flora are not sufficiently numerous or lack the desired metabolic capabilities, bioaugmentation may prove to be a cost effective clean-up strategy. Many chlorinated solvents are biologically transformed via co-metabolic pathways (Criddle et al. 1991). If an electron donor and acceptor, as well as required growth factors, can be supplied to an organism that transforms the compound(s) of interest co-metabolically, bioaugmentation of sites impacted by this compound may be feasible.

Nutrient delivery presents considerable problems for in-situ bioremediation. Because of the low solubility of oxygen in water, it is difficult to deliver sufficient quantities of oxygen to aerobic organisms using gas delivery. Delivering electron acceptors, such as nitrate, with much higher water solubilities than oxygen should not be as difficult. An aqueous solution with high

nitrate concentrations can be pumped to the desired location. Denitrifying organisms often grow nearly as fast as aerobes. The relative ease of nutrient delivery combined with rapid growth rates may make denitrifiers better suited for in-situ bioremediation applications than aerobes.

Carbon tetrachloride (CT) transformation by indigenous denitrifying microorganisms typically leads to the production of chloroform (CF). In a field scale experiment at Moffett Naval Air Station, for example, CF production resulted when acetate was added to biostimulate denitrifying populations (Semprini et al. 1992). *Pseudomonas stutzeri* KC is a denitrifying organism that rapidly and completely co-metabolizes tetrachloromethane, commonly known as carbon tetrachloride (CT), to carbon dioxide, formate and an unidentified non-volatile product, without the production of chloroform (Criddle et al. 1990, Lewis et al. 1993, Dybas et al. 1995). To transform CT, an actively growing culture of strain KC requires iron limiting conditions (Criddle et al. 1990, Tatara et al. 1993), trace concentrations of copper (Tatara et al. 1993) and an incubation temperature of 4-23° C (transformation is inhibited above 25° C; growth is inhibited above 30° C). Iron solubility (as ferric ions) in water is at a minimum in the pH range of 8.0 to 8.2 (Stumm et al. 1981). Copper is required for CT transformation (Tatara et al. 1993), but inhibits growth at neutral pH (Criddle et al. 1990) when it is more soluble than at alkaline pH. CT transformation by

strain KC is co-metabolic and believed to be linked to an iron-scavenging system (Criddle et al. 1990, Tatara et al. 1993, Dybas et al. 1995). No similar activity has yet been detected in other isolates or indigenous consortia (Criddle et al. 1990, Lewis et al. 1993).

For bioaugmentation, transport of the exogenous organisms and competition with the indigenous microbial community for limiting resources present formidable challenges. This thesis focuses on predicting the most fit competitor, verification of those predictions, methods and effects of habitat modification for promoting the dominance of an exogenous organism, specifically *Pseudomonas stutzeri* KC, and the results of a field-scale bioaugmentation project.

Competition Theory

Microbial growth rate can be modeled using the original formulation by Monod (1942), which was later modified by van Uden (1967) to include the rate of decay:

$$\mu = \mu_{\max} \frac{S}{S + K_s} - b \quad (1)$$

$$\mu_{\max} = k_m Y_m \quad (2)$$

where $\mu = \frac{dX/dt}{X} = \text{specific growth rate (days}^{-1}\text{)}$ (3)

and $b = \text{decay coefficient (days}^{-1}\text{)}$

$k_m = \text{maximum specific rate of substrate utilization (days}^{-1}\text{)}$

$K_s = \text{half-velocity coefficient (mg/L)}$

$\mu_{\max} = \text{maximum specific growth rate (days}^{-1}\text{)}$

$S = \text{rate-limiting substrate concentration (mg/L)}$

$t = \text{time (days)}$

$X = \text{concentration of microorganisms (mg/L)}$

$Y_m = \text{maximum organism yield (mg organisms/mg}$

substrate)

A mechanistic parameter, $S_{\min}$, describing the subsistence concentration of a limiting resource can be derived from equation 1 by setting $dX/dt = 0$ (Rittman and McCarty 1980):

$$S_{\min} = \frac{b K_s}{\mu_{\max} - b} \quad (4)$$

where $S_{\min} = \text{substrate concentration where growth rate equals death rate (mg/L).}$

Experimental field evidence has led to a proliferation of models for interspecific competition for resources (e.g. Stewart and Levin 1973, Lehman et al. 1975, Petersen 1975, Taylor and Williams 1975, Leon and Tumpsen 1975, Hsu et al. 1977, Hansen and Hubbell 1980, Tilman 1977,1980). If numerous species are all limited by the same nutrient and they have similar decay rates, b , and half-velocity coefficients, K_s , for a limited resource, single nutrient competition theory predicts that the species with the highest maximum specific growth rate, μ_{max} , will completely displace all other species at equilibrium in continuous culture (Hsu et al. 1977, Tilman 1977, Hansen et al. 1980). The Monod model describes the nutrient-limited growth of single species. Parameters that are mathematically identical to S_{min} such as J (Hansen and Hubbell 1980) and R^* (Tilman 1981) have been derived and extend to competition between numerous species:

$$J_i = R^*_i = \frac{b_i K_{si}}{\mu_{max} - b_i} \quad (5)$$

where

$$S_{min,i} = J_i = R^*_i$$

$S_{min,i}, J_i, R^*_i$ = substrate concentration where growth rate equals death rate (mg/L) for species i ,

b_i = specific death rate of species i ,

K_{si} = half-velocity coefficient of species i , and

$\mu_{\max,i}$ = maximum specific growth rate of species i .

According to equation 5, the dominant species will have the lowest $S_{\min}$ value. It follows from equation 5 that if competitors have equal death rates and half-velocity coefficients, the competitor with the greatest maximum specific growth rate will be the dominant species (Hsu et al. 1977, Tilman 1977, Hansen and Hubbell 1980). $S_{\min}$ is commonly used in engineering literature and it will be used throughout this thesis to remain consistent with that notation.

EXPERIMENTAL OVERVIEW

This thesis addresses microbial competition issues relevant to engineering the bioaugmentation of a carbon tetrachloride impacted aquifer. Laboratory studies were designed to evaluate the competitiveness of *Pseudomonas stutzeri* KC with a microbial groundwater community. The maximum specific growth rates of *Pseudomonas stutzeri* KC and the groundwater flora were determined over the pH range from 7.0 to 8.5. Single nutrient competition theory was then used to predict whether strain KC or the groundwater consortium will dominate at a given pH. Theoretical predictions

were tested with mixed-strain laboratory experiments. A mixed-strain experiment in which strain KC was grown under similar conditions to a field inoculum (Schoolcraft, MI, Field Experiment) was also conducted to test the outcome in the laboratory prior to field demonstration.

Disinfection could be used as a preemptive habitat modification step to promote the dominance of *Pseudomonas stutzeri* KC. Bleach and hydrogen peroxide were evaluated as groundwater disinfectants.

Aquifer field samples have been collected and analyzed to show that bioaugmentation with strain KC is a viable treatment alternative for carbon tetrachloride impacted aquifers.

CHAPTER 2

MATERIALS AND METHODS

Organisms. *Pseudomonas stutzeri* KC (DSM deposit no. 7136. ATCC deposit number 55595), derived originally from aquifer solids from Seal Beach, CA, (Criddle et al. 1990) is routinely maintained in our laboratory on nutrient agar plates.

Chemicals. All chemicals were American Chemical Society reagent grade (Aldrich or Sigma Chemical Co.). All water used in reagent preparation was deionized 18 M Ω resistance or greater. Tetrachloromethane (CT; 99 % pure) was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin.

Media. Simulated groundwater (SGW) medium (recipe provided by R. Skeen, Battelle Pacific Northwest Laboratory) contained per liter of deionized water: 0.455 g of Na₂SiO₃ • 9H₂O, 0.16 g NaCO₃, 0.006 g of Na₂SO₄, 0.02 g of KOH, 0.118 g of MgCl₂ • 6H₂O, 0.0081 g of CaCl₂ • 2H₂O, 13.61 g of KH₂PO₄, 1.6 g of NaOH, 1.6 g of NaNO₃, 1.6 g of acetate and 1 mL of trace element solution. The

trace element solution contained per liter of deionized water: 0.021 g of LiCl_2 , 0.08 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.106 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.6 g of H_3BO_3 , 0.123 g of $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$, 0.11 g of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.109 g of $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06 g of TiCl_4 , 0.03 g of KBr , 0.03 g of KI , 0.629 g of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.036 g of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, 0.3 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The pH of SGW medium was adjusted to 8.2 with NaOH pellets. The resulting medium was autoclaved at 121°C for 20 minutes.

Medium D contained per liter of deionized water: 2.0 g KH_2PO_4 , 3.5 g of K_2HPO_4 , 1.0 g of $(\text{NH}_4)_2\text{SO}_4$, 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3.0 g of sodium acetate, 2.0 g of sodium nitrate, 1 mL of 0.15 M $\text{Ca}(\text{MO}_3)_3$, and 1 mL of trace nutrient stock TN2. Stock solution TN2 contained per liter of deionized water: 1.36 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.24 g of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.25 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.58 of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.29 g of $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 0.11 g of $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, 35 mg of Na_2SeO_3 , 62 mg of H_3BO_3 , 0.12 g of NH_4VO_3 , 1.01 g of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 1 mL of H_2SO_4 (concentrated). Typically, Medium D is adjusted to pH 8.2 using NaOH pellets followed by 1 M NaOH stock solution. To sterilize Medium D, it is autoclaved for 20 minutes at 121°C . Cultures were grown at 20°C with 150 rpm shaking under aerobic conditions (Lab-Line Orbital Shaker model 3590). Nutrient broth, nutrient agar (Difco) and R2A (Difco) plates were prepared according to manufacturer's instructions.

Groundwater. All groundwater used in experiments was collected from a CT impacted aquifer in Schoolcraft, MI. Groundwater was obtained by manually collecting samples with a Teflon[®] bailer from a 2" steel well screened at 30 feet below the water table. Groundwater samples were stored in pre-sterilized sealed five gallon Nalgene[®] carboys or in Wheaton bottles equipped with Teflon[®] lined caps at 4°C.

Analytical methods. CT was assayed by removing samples from the headspace gas above aqueous samples and injecting them into a gas chromatograph, as described previously (Tatara et al. 1993). External calibration standards were prepared by addition of a primary standard (7.8 ng of CT per μL of methanol) to secondary aqueous standards having the same gas:water ratio, ionic strength, incubation temperature, and speed of shaking as the assay samples. A four-point calibration curve was prepared over a concentration range bracketing that of the assay samples. Measurements of pH were made with an Orion model 720A pH meter. Nitrate, nitrite, and acetate ions were assayed by ion chromatography (Dionex[®] model 2000i/SP ion chromatograph with suppressed ion conductivity detection equipped with a Dionex[®] Ionpak AS4-A anion exchange column and utilizing a 1.8 mM bicarbonate/1.7 mM carbonate mobile phase at 1 mL/min). Chromatograms

were recorded and data integrated using Turbochrom 4[®] software (Perkin Elmer Corp.). External calibration curves which bracketed the concentrations of the test samples were prepared by diluting primary ion standards into deionized water with at least 18 MΩ resistance. Optical density measurements was measured at 660 nm using a Shimadzu UV-160 spectrophotometer.

Bacterial enumeration. To determine the density of strain KC and aquifer flora in competition experiments and aquifer field samples, spread plate counts were used. Serial dilutions of extracted samples were performed in sterile phosphate buffer (50 mM, pH 8.0). Phosphate buffer was prepared by dissolving equimolar quantities of mono and dibasic sodium phosphate in 18 MΩ resistance deionized water. 1.8 mL quantities of phosphate buffer was then dispensed into Kimax cultures tubes (which are subsequently capped and autoclaved for 20 minutes at 121° C). A tenfold dilution can then be achieved by adding 0.2 mL of sample to sterile Kimax tubes containing 1.8 mL of buffer and mixing well. Diluted aliquots (100 µL) were spread on R2A plates. Total colony forming units (C.F.U.) were obtained by counting colonies after 6 days of incubation at 20° C. When grown on R2A plates, a “fried egg” colony morphology characteristic of strain KC is observed.

CHAPTER 3

MAXIMUM SPECIFIC GROWTH RATE DETERMINATION

MATERIALS AND METHODS

Medium preparation. In order to evaluate the maximum specific growth rate of strain KC and the groundwater flora, 500 mL Wheaton bottles, with Teflon[®] lined septa and holes drilled in the bottle caps, containing 250 mL of groundwater (pasteurized at 65° C for 8 hours for strain KC experiments), were amended with 30 mM acetate, 12 mM nitrate, 0.1 mM phosphate and 2 g/L of NaHCO₃ (Knoll 1994). Pasteurization was used to kill indigenous flora because autoclaving produced a precipitate that interfered with growth (Knoll 1994). Filter-sterilization also had an adverse effect on organism growth (Knoll 1994). Gaseous CO₂ was used to reduce pH for experiments below pH 8.2 and 1 N NaOH was added to raise pH to 8.5.

Starter cultures. An individual strain KC colony was transferred from nutrient agar and grown for 60 hours at 20° C with 150 rpm shaking under aerobic

conditions (Lab-Line Orbital Shaker model 3590) in 28 mL sterile serum tubes, capped with crimp tops, that contained 10 mL of Medium D. After 60 hours, strain KC starter cultures were centrifuged at 14,000 rpm for five minutes, re-suspended in pasteurized, amended groundwater and centrifuged again at 8,000 rpm for 10 minutes. Starter cultures undergoing this procedure are considered “washed”. Washing was done to minimize nutrient carryover from starter cultures into experiments.

Removal of oxygen from headspace. Wheaton Bottles (500 mL with loosened caps) were passed through a Coy anaerobic glove box interlock (Coy Laboratory Products, Ann Arbor, Michigan) three times to remove oxygen from the headspace. Thirty (30) milliliters of gas (90% nitrogen, 10% hydrogen) was added to the bottles in the anaerobic glove box so that they were under positive pressure and removing samples would not pull oxygen into the bottles. Strain KC was added as a one percent inoculum (approximately 10^6 CFU/mL) from 60 hour Medium D tubes.

Maximum specific growth rate determination. Microbial growth was followed by optical density measurements at 660 nm. Optical density was measured on a

Shimadzu UV-160 spectrometer from 1.5 mL samples as described above (Chapter 2). The maximum specific growth rate, $\mu_{\max}$, was calculated for cells in log phase growth using optical density measurements and the following relationship: $\mu_{\max} = [\ln (X_f/X_i)] / (t_f - t_i)$, where X_f and X_i represent final and initial optical densities, and t_f and t_i are the final and initial time.

RESULTS AND DISCUSSION

Batch incubations over the pH range of 7.0 to 8.5 were used to determine maximum specific growth rate, $\mu_{\max}$, as a function of pH. Maximum specific growth rates for the groundwater consortium and strain KC were determined independently and are compared in Figure 1. No trace metals were added and incubations were nitrate limited. Because the aquifer water may lack some required nutrients, the batch incubations could be limited by other resources.

Strain KC has a higher maximum specific growth rate than the groundwater flora between approximately pH 8.0 and 8.5 (Figure 1). Net growth must be occurring for maximum specific growth rate to be measured. Death rate,

b , must therefore be less than the maximum specific growth rate. Assuming a death rate of 0.1 days^{-1} and given the half-velocity coefficients, K_s , previously determined for *Pseudomonas stutzeri* KC and the groundwater community (Knoll 1994), strain KC has a lower $S_{\min}$ than the groundwater flora between pH 8.0 and 8.5 (Table 1). It is predicted that KC will outcompete the groundwater flora over this range in continuous culture. Death rate and half-velocity coefficients are functions of pH. To calculate $S_{\min}$ more accurately, b and K_s should be measured as a function of pH over the experimental pH range.

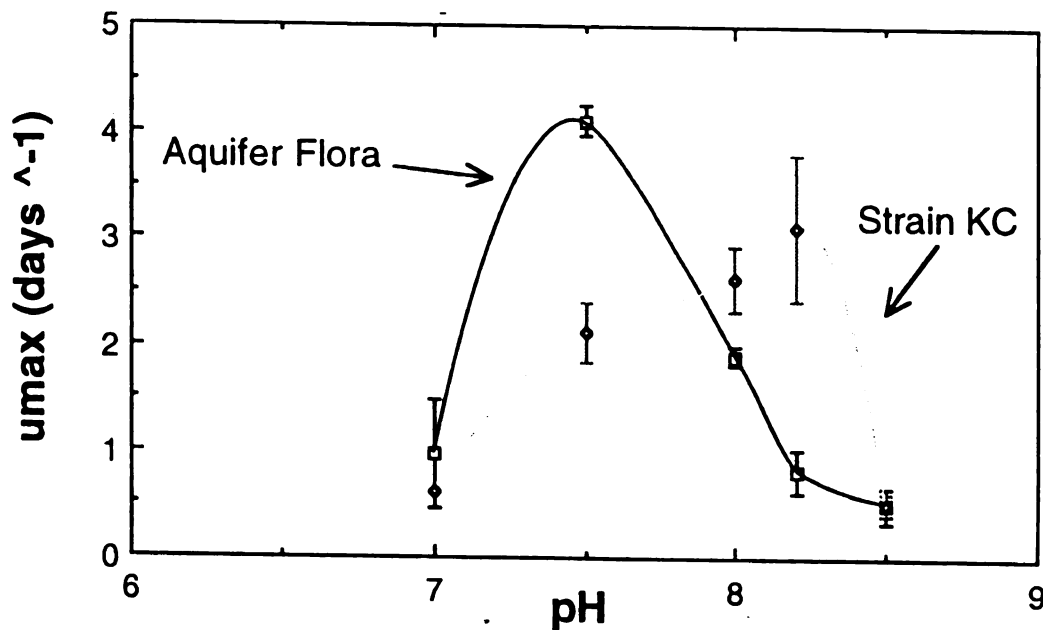


Figure 1: pH dependence of $\mu_{\max}$. Arithmetic mean plotted, error bars represent plus/minus one standard deviation.

TABLE 1: $S_{\min}$ as a Function of pH for the Groundwater Flora and Strain KC

pH	$S_{\min}$ (mg/L)	
	Groundwater Flora ¹	<i>Pseudomonas stutzeri</i> KC ²
7.0	1.08	2.4
7.5	0.23	0.60
8.0	0.60	0.47
8.2	1.32	0.40
8.5	2.35	2.86

1: Assumes nitrate limiting conditions, $b = 0.1 \text{ d}^{-1}$, and

$$K_s = 9.40 \text{ mg NO}_3^- / \text{L}.$$

2: Assumes nitrate limiting conditions, $b = 0.1 \text{ days}^{-1}$, and

$$K_s = 11.97 \text{ mg NO}_3^- / \text{L}.$$

Nutrient concentrations decrease with time in actively metabolizing microbial batch incubations. Because there is no flow out of batch incubations, metabolites accumulate with time unless they decay chemically or biologically. Although single-nutrient competition theory was developed for continuous culture applications, its utility for batch cultures has not been tested and was therefore evaluated experimentally in growth rate and mixed-strain experiments.

Resource-based competition theory was developed for species, not consortia. Growth parameters of the groundwater consortium, as determined by the experiments detailed above, can be attributed to the kinetics of the fastest growing organism(s) in the community. Because death rate is assumed constant, as long as the half-velocity coefficient determined for the community corresponds to the same member of the community for which a maximum specific growth rate was determined, the $S_{\min}$ calculated for the community will actually be for an individual species. Both strain KC's and the groundwater consortium's $S_{\min}$ would then be representative of individual species. Experimental conditions are designed to optimize growth of *Pseudomonas stutzeri* KC. These conditions promote the growth of species that will be most competitive with strain KC. Comparing the $S_{\min}$ parameter of strain KC with that of the groundwater consortium is in effect a comparison between strain KC and the species in the consortium that is most competitive with strain KC. Single nutrient competition theory is intended to facilitate interspecies comparisons and may be a valid predictive tool in this case.

Single nutrient competition theory however was derived for continuous culture. In continuous culture, once steady-state is reached, nutrient and cell concentrations do not vary temporally and if the vessel is well-mixed, are constant spatially as well. Nutrient and cell densities are time dependent in batch

culture. Due to the physical and therefore mathematical differences between batch and continuous cultures, single nutrient competition theory probably has limited applicability to batch cultures.

CHAPTER 4

MIXED-STRAIN INCUBATIONS

MATERIALS AND METHODS

To determine if single nutrient competition theory can be applied to batch experiments and to determine the conditions where strain KC is able to displace the groundwater flora, 10 mL of amended groundwater was put in 30 mL serum tubes. The groundwater was amended with 60 mg of sodium acetate, 13.6 mg of KH_2PO_4 , and 250 mg of NaHCO_3 per liter. The pH was increased by 1 N NaOH addition and decreased by 1 N HCl addition. After the groundwater was added to the serum tubes they were passed through the Coy glove box interlock chamber to remove oxygen from the headspace (Chapter 3). The tubes were then capped in the Coy glove box (Chapter 3) with Teflon coated septa under anaerobic conditions (90% nitrogen, 10% hydrogen). Strain KC starter cultures, which were grown as described below, were used to inoculate the anaerobic serum tubes. CT concentration, strain KC and groundwater flora densities were recorded with time.

Starter cultures. An individual KC colony was transferred from nutrient agar and grown for 48 hours at 20° C with 150 rpm shaking under aerobic conditions (Lab-Line Orbital Shaker model 3590) in 10 mL sterile serum tubes with crimp tops that contained 10 mL of Medium D (pH 8.2). After 24 hours, a 100 mL Erlenmeyer flask containing 50 mL of sterile SGW medium (pH 8.2, aerobic) was inoculated with 1 mL of the Medium D grown KC culture and placed on a stir plate. Flasks were loosely covered with aluminum foil to prevent contamination and to allow oxygen into the headspace. Strain KC was grown for 48 hours in SGW medium and used to inoculate anaerobic serum tubes at the desired density.

Bacterial enumeration. *Pseudomonas stutzeri* KC and the groundwater flora were enumerated with plate counts on R2A agar. Samples were diluted (Chapter 2) and a range of dilutions was selected for plating that would produce between thirty and three hundred colonies per plate. R2A was chosen because higher plating efficiencies were achieved with it than with nutrient agar or aquifer water agar (aquifer water amended with 10% nutrient broth and noble agar). As mentioned above strain KC has a distinctive “fried egg” morphology. To determine if strain KC could be reliably differentiated from the groundwater flora by morphology, twenty-five (25) suspected KC colonies and twenty-five (25)

suspected non-KC colonies were sampled and placed in medium D vials containing CT. Vials were incubated for 6 days at 20° C. All twenty-five (25) KC colonies degraded CT to non-detectable levels on a gas chromatograph (CT assays performed as described in Chapter 2), whereas the non-KC colonies had not significantly reduced the concentration of CT in the headspace.

RESULTS AND DISCUSSION

Pseudomonas stutzeri KC and the groundwater flora have previously been incubated separately in competition experiments. In those experiments, kinetic parameters were measured and used to predict competitive outcomes. To experimentally evaluate the application of single nutrient competition theory to batch cultures, mixed-strain batch incubations (strain KC and the groundwater flora incubated in the same vessel) were prepared. Mixed-strain batch incubations were also used to estimate the inoculum density required for domination of the groundwater community by strain KC. The effectiveness of two starter culture growth media were also compared. Mixed-strain results will help to determine if the maximum specific growth rate of a community is a quantitative measure of its competitiveness in batch experiments.

Competitive interactions between species have been studied by additive design, in which the density of one species is held constant while the other is varied, or by substitutive design, in which the density of two species is varied while total density is held constant (Firbank et al. 1990, Harper 1977, Snaydon 1991, Wilson et al. 1994). The substitutive design or replacement series, introduced by de Wit (1960), has the advantage that the effects of proportion and density are not confounded. When strain KC is introduced into an aquifer, the background flora are present at a fairly constant natural density. In order to closely model field conditions and to avoid the complexity of maintaining a constant total density, additive experiments were conducted with constant groundwater flora density and varying densities of strain KC.

Mixed-strain experiments test the predictive capabilities of single nutrient competition theory. As discussed in Chapter 3, nutrient and cell concentrations vary with time in batch incubations, but are constant in continuous culture once steady-state has been achieved. This mathematical inconsistency is one of the reasons that competition theory predictions may not be validated in batch culture. Also, if more than one nutrient is limiting the growth of the competing organisms or complex ecological relationships develop, theoretical predictions may not be verified in mixed-strain incubations.

Mixed-strain experiments will help determine if single-nutrient competition theory can be applied to batch incubations. As nutrients are consumed in batch incubations, the community structure will change. Initially, when nutrient concentrations are high, an *r* strategist (a fast growing organism that prevails when nutrients are not severely limiting) may be dominant and as the nutrients are consumed a *K* strategist (an organism that reproduces more slowly than an *r* strategist, but prevails under resource limiting conditions) may displace it. Both types of strategists could also coexist, but proliferate under different conditions.

Initial $\mu_{\max}$ experiments indicated that the minimum $S_{\min}$ for *Pseudomonas stutzeri* KC (under the specified experimental conditions) occurs at pH 8.2 (Table 1). Strain KC is predicted to outcompete the groundwater flora at this pH because it has a lower $S_{\min}$. Mixed-strain batch incubations near pH 8.2 were used to test this prediction. Support for single nutrient competition theory was obtained at pH 8.0 and 8.2 when strain KC starter cultures were grown in SGW medium. At pH 8.0 (Figure 2) and pH 8.2 (Figures 3 and 4), strain KC replaced the groundwater flora as the dominant population and readily transformed CT (Figures 5 and 6). Additional experiments, over a longer time frame, might help to answer the question of whether single nutrient competition theory can be used to reliably predict batch incubation results.

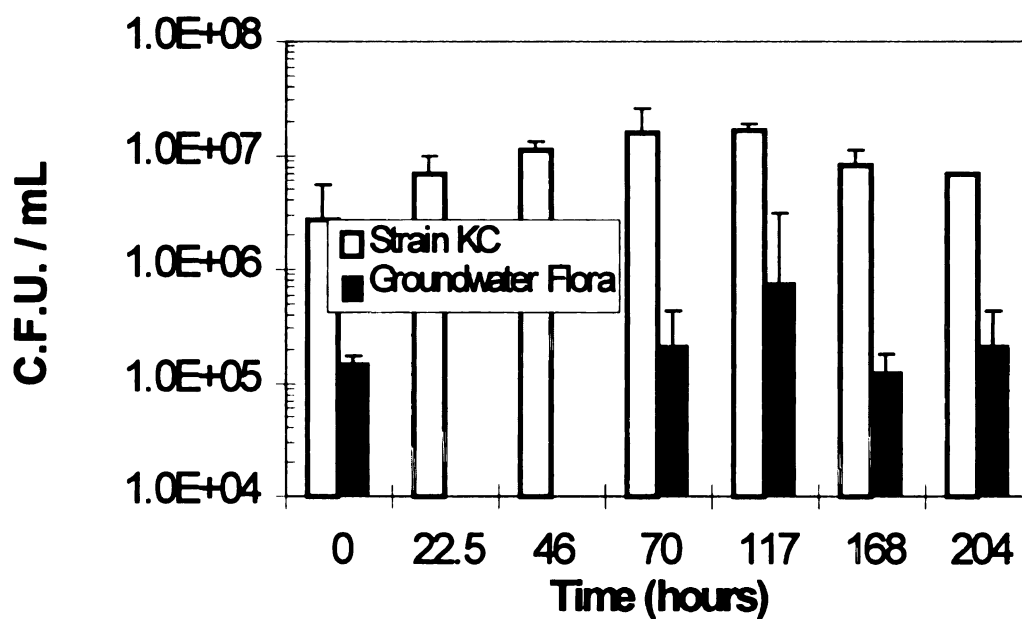


Figure 2: Mixed-strain plate count results. Initial pH 8.0, 18:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

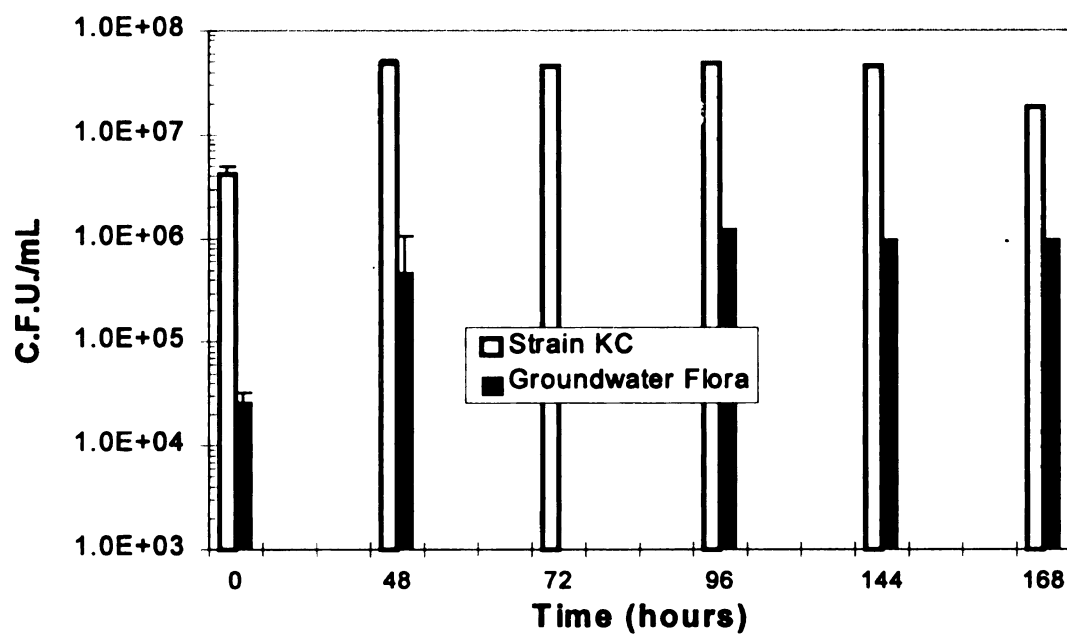


Figure 3: Mixed-strain plate count results. Initial pH 8.2, 160:1 (strain KC: groundwater flora), SGW medium as the starter medium.

Geometric mean plotted, error bars represent one standard deviation.

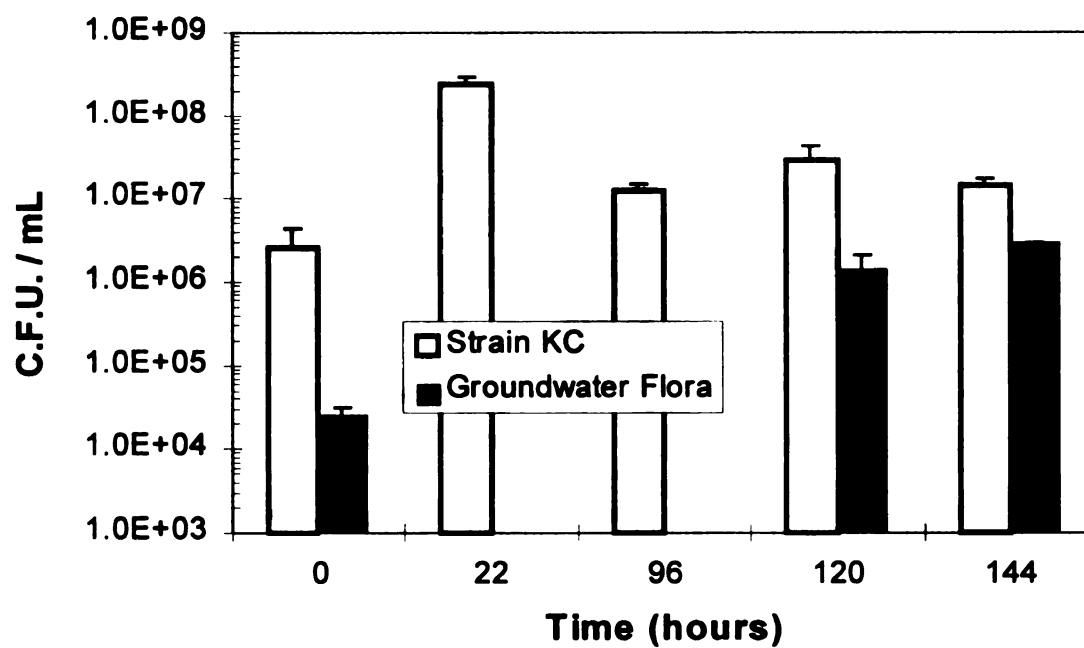


Figure 4: Mixed-strain plate count results. Initial pH 8.2, 100:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

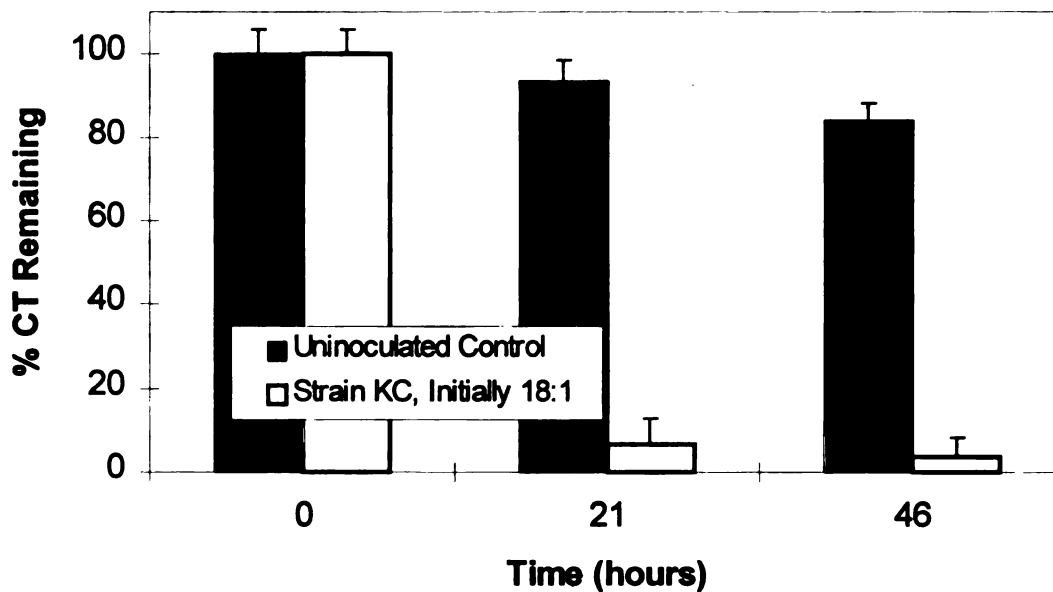


Figure 5: Carbon tetrachloride transformation in the presence of groundwater flora. Mixed-strain experiment, initial pH 8.0, 18:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent standard deviation.

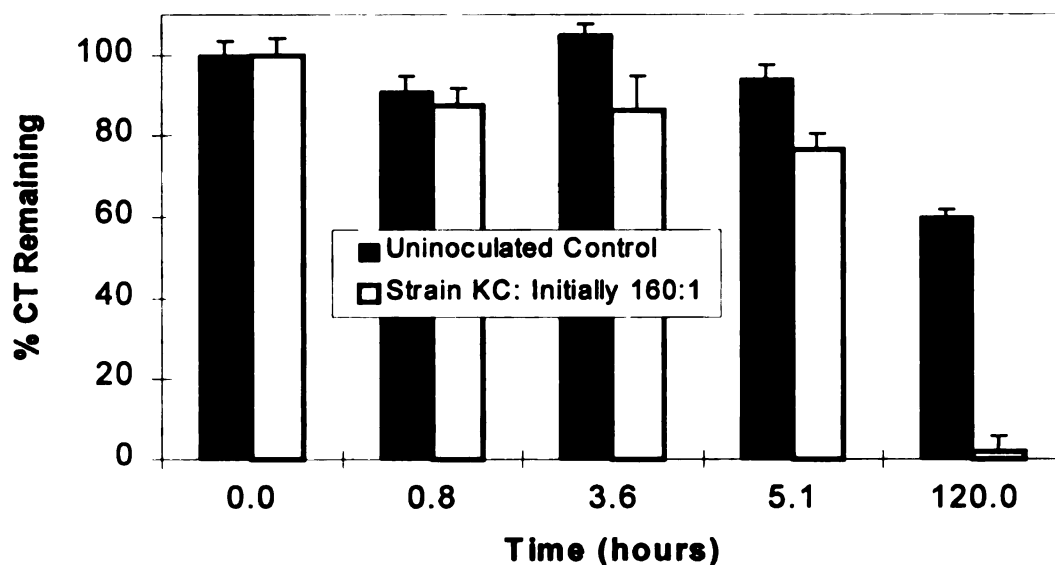


Figure 6: Carbon tetrachloride transformation in the presence of groundwater flora. Mixed-strain experiment initial pH 8.2, 160:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

To verify that the groundwater consortium would be the dominant population when it has a lower S_{min} than KC, a mixed-strain experiment was conducted at pH 7.5 with SGW medium as the starter medium (Figure 7). Strain KC maintained higher cell densities than the groundwater consortium throughout the experiment. Strain KC concentration however did not increase above the its initial density. Groundwater flora concentrations increased more

than one order of magnitude (from 10^5 to 10^6) between 26 and 168 hours after inoculation. These results suggest that single nutrient competition theory may have limited utility for predicting the outcome of batch experiments. In batch experiments, initial conditions such as inoculum size have a strong influence on the outcome. The groundwater flora would probably displace strain KC in continuous culture at pH 7.5, as predicted. The groundwater flora would also most likely displace strain KC in a batch experiment (at pH 7.5) that was repeatedly spiked with microorganisms (strain KC and groundwater flora) and nutrients. As the spiking frequency increases, batch experiments look more and more like continuous cultures. In this batch experiment however, strain KC and the groundwater organisms coexisted.

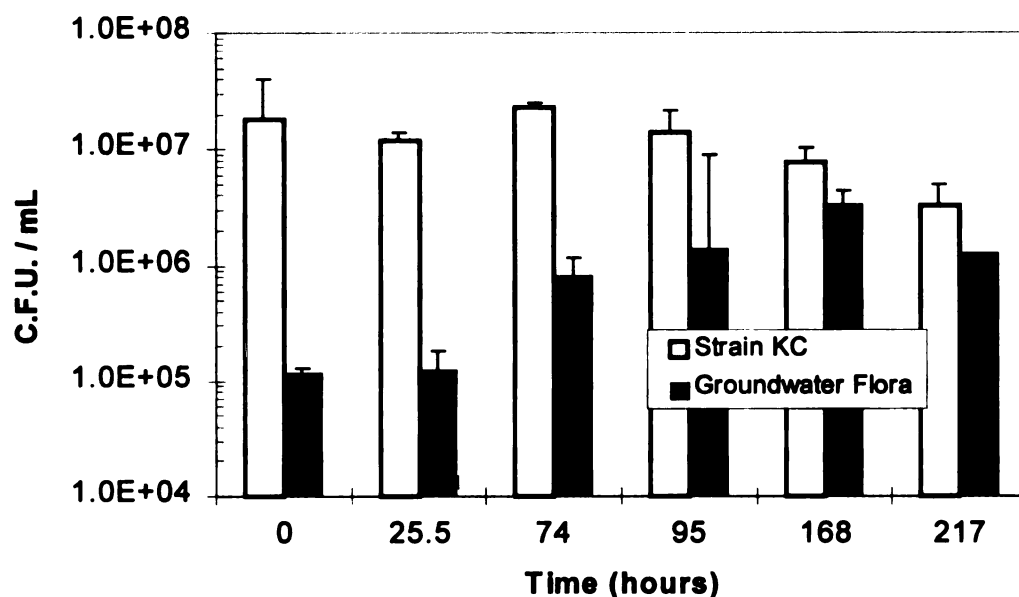


Figure 7: Mixed-strain plate count results. Initial pH 7.5, 150:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

Mixed-strain competition experiments under denitrifying conditions were also used to compare prospective starter culture media. Prior to inoculating the aquifer, strain KC must be grown to the cell density required for competitive domination. Pasteurized aquifer water amended with acetate, nitrate, and phosphate, as described above, was tested as a prospective starter-culture

medium. Cells pre-adapted to an environment similar to one in which they will be introduced should experience a minimal lag phase after inoculation (Brock and Madigan 1991). When pre-grown in amended groundwater, acclimated strain KC cells did not displace the groundwater species in mixed-strain experiments at pH 8.2 (Figures 8 and 9) and did not generate a population with high levels of CT transformation activity (Figure 10). Carbon tetrachloride losses in incubations that did not contain KC (Figure 10) could be due to abiotic losses (leaking septa) or biological transformations by the groundwater flora. SGW medium proved to be a superior starter medium because strain KC was able to displace the groundwater flora in mixed-strain experiments at pH 8.2 when the initial strain KC to groundwater flora ratio was approximately equal (Figures 3 and 4).

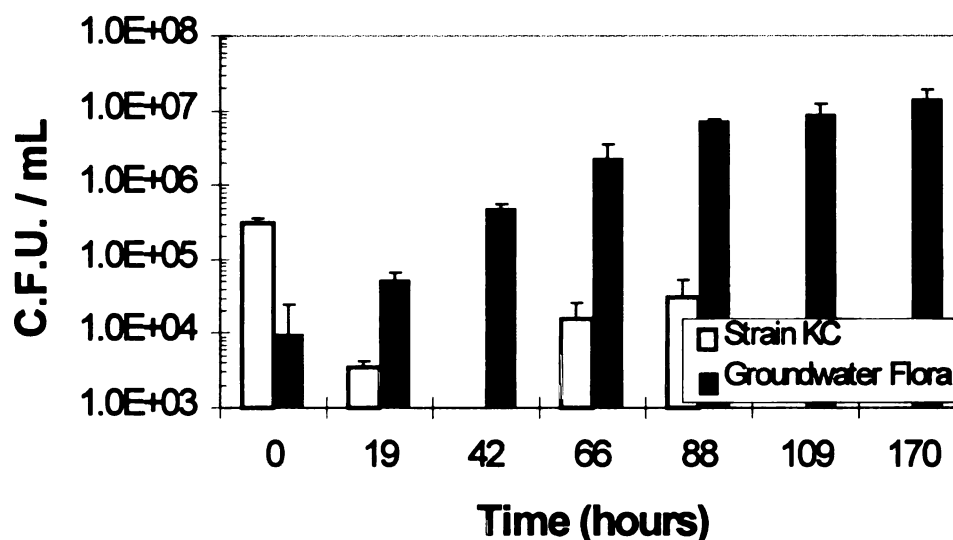


Figure 8: Mixed-strain plate count results. Initial pH 8.2, 32:1 (strain KC: groundwater flora), groundwater medium as the starter medium.

Geometric mean plotted, error bars represent one standard deviation.

Presumably, SGW medium provides nutrients in early growth stages that make strain KC more competitive. Cell growth rates depend on the growth medium. Rapidly growing cells produce more proteins and copies of genes than cells growing slowly. Increases in cell protein density and quantity of gene copies increase competitiveness (Bailey and Ollis 1986). Because it is growing

much more rapidly, an inoculum in log phase growth should be more competitive than one in the stationary phase.

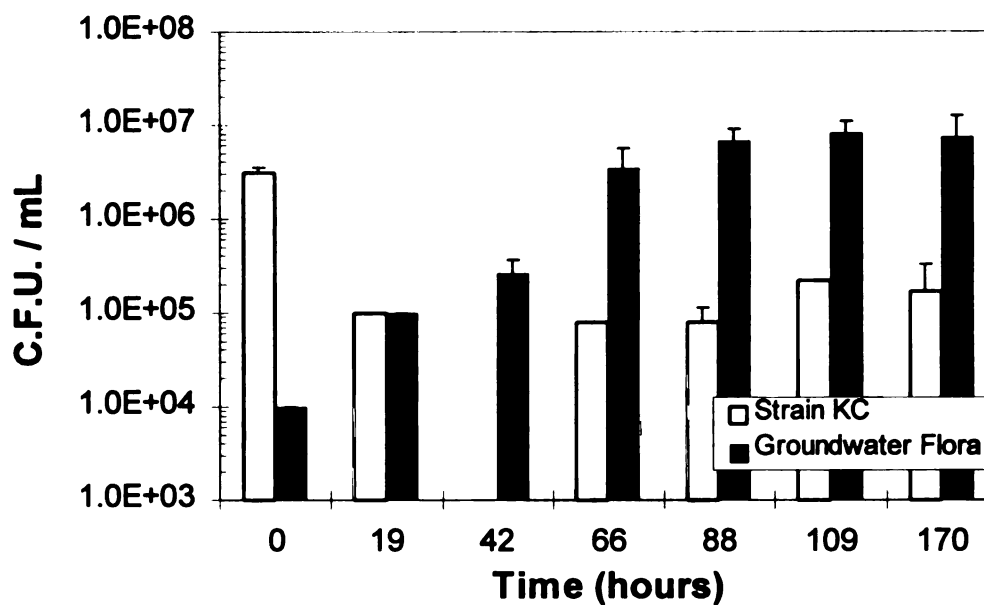


Figure 9: Mixed-strain plate count results. Initial pH 8.2, 308:1 (strain KC: groundwater flora), groundwater medium as the starter medium. Geometric mean plotted, error bars represent plus one standard deviation.

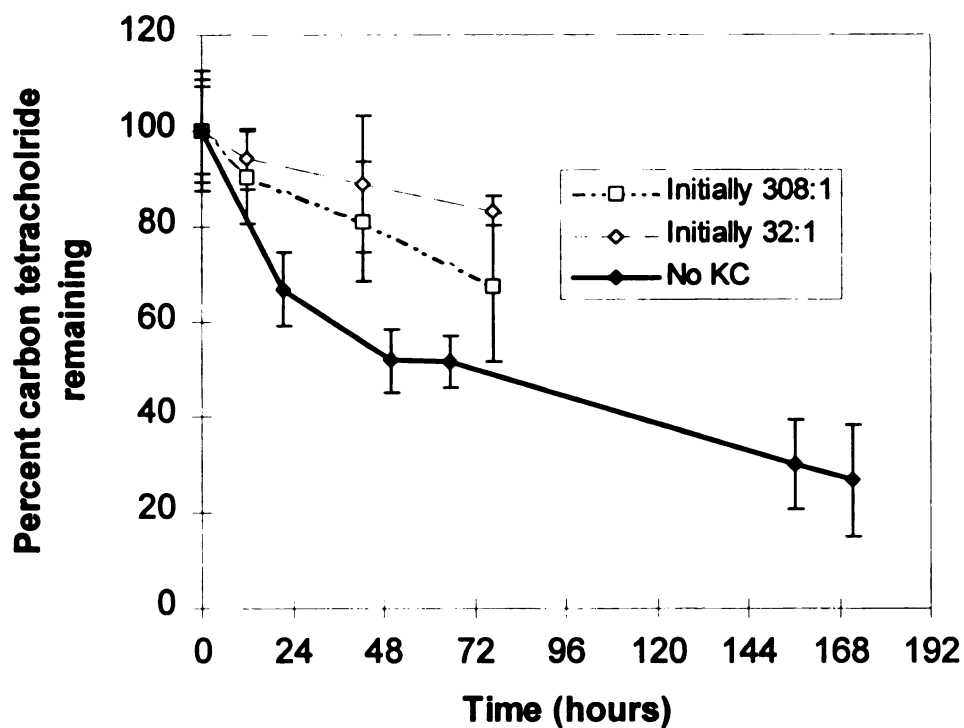


Figure 10: Carbon tetrachloride transformation in the presence of groundwater flora. Mixed-strain experiment initial pH 8.2, 308 and 32:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

Although inoculum growth rate was not quantified, it probably varies in each medium and could contribute to differences in inoculum competitiveness. All starter cultures regardless of growth medium were incubated for 48 hours

prior to inoculation. Starter cultures from different starter media could therefore be in different growth phases at inoculation. The composition of SGW medium is known. A chemical analysis of the groundwater is recommended so that the two media can be compared and the critical component(s) determined.

Stressing an inoculum could decrease its colonization efficiency.

Washing cells may cause them to lyse. When strain KC was centrifuged and washed prior to inoculation (to minimize the amount of starter medium added to experiments at inoculation), strain KC was not able to displace the groundwater flora regardless of the starter medium or inoculum size (Figures 11, 12, 13, 14 and 15). This suggests that the stress on the inoculum due to washing significantly reduced the competitive fitness of strain KC. Strain KC starter cultures used in maximum specific growth rate determination experiments (Chapter 3) were always washed prior to inoculation. Thus the $\mu_{\max}$ data may have been affected by the washing. Strain KC could actually have higher maximum specific growth rates (and lower $S_{\min}$) than were measured experimentally. To determine the maximum specific growth rate of the groundwater organisms, nutrients were added to groundwater and optical density was measured. Because strain KC cultures were more stressed than the groundwater flora, strain KC may be an even better competitor with the groundwater flora than single nutrient theory predictions suggest.

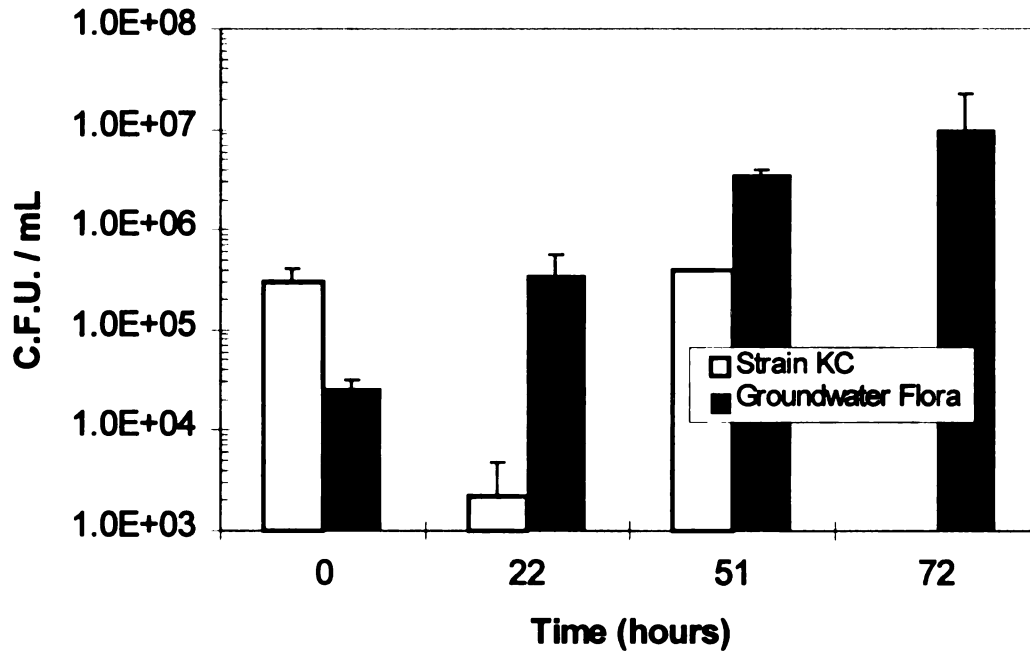


Figure 11: Mixed-strain plate count results. Initial pH 8.2, 11:1 (strain KC: groundwater flora), SGW medium as the starter medium, washed inoculum. Geometric mean plotted, error bars represent one standard deviation.

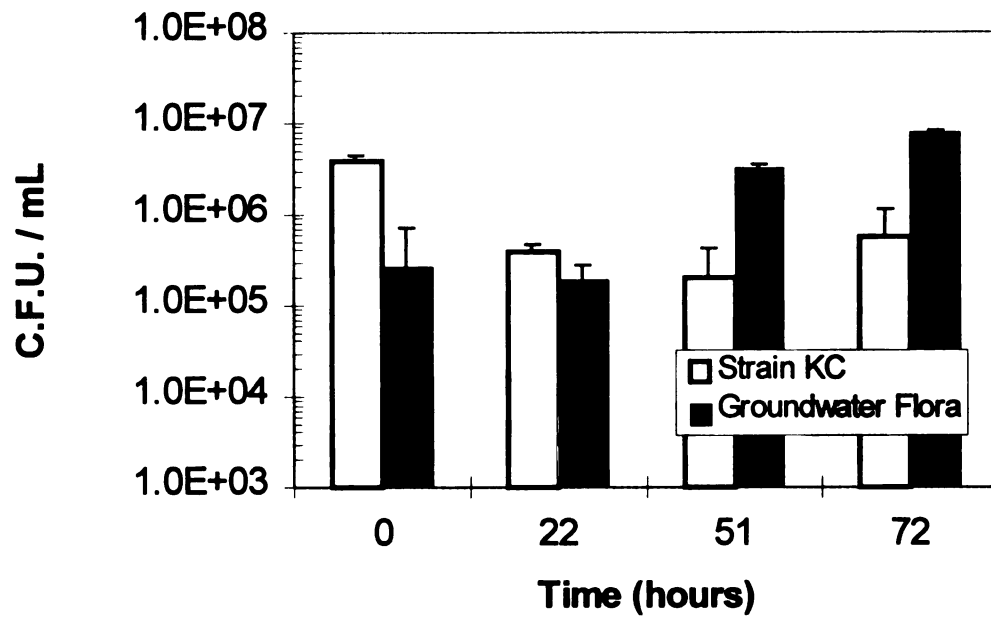


Figure 12: Mixed-strain plate count results. Initial pH 8.2, 15:1 (strain KC: groundwater flora), SGW medium as the starter medium, washed inoculum. Geometric mean plotted, error bars represent one standard deviation.

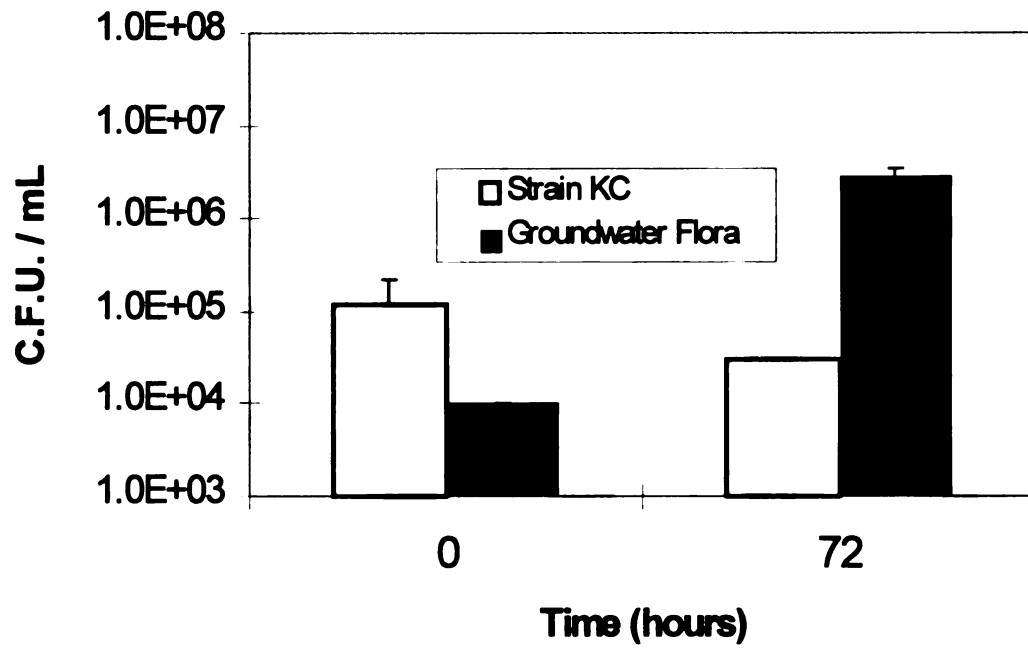


Figure 13: Mixed-strain plate count results. Initial pH 8.0, 10:1 (strain KC: groundwater flora), Medium D as the starter medium, washed inoculum. Geometric mean plotted, error bars represent one standard deviation.

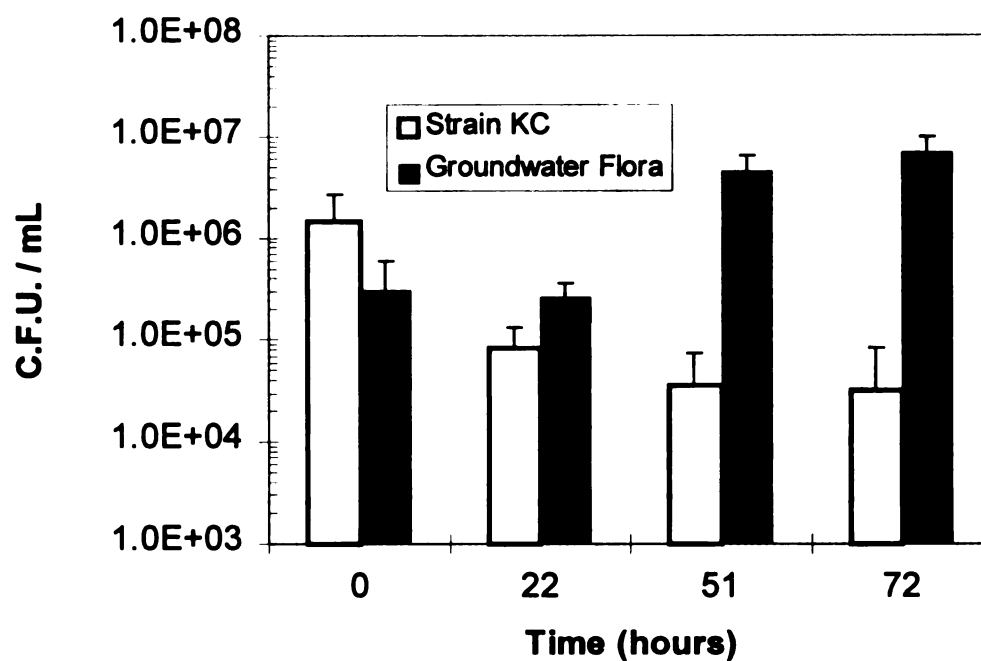


Figure 14: Mixed-strain plate count results. Initial pH 8.0, 6:1 (strain KC: groundwater flora), Medium D as the starter medium, washed inoculum. Geometric mean plotted, error bars represent one standard deviation.

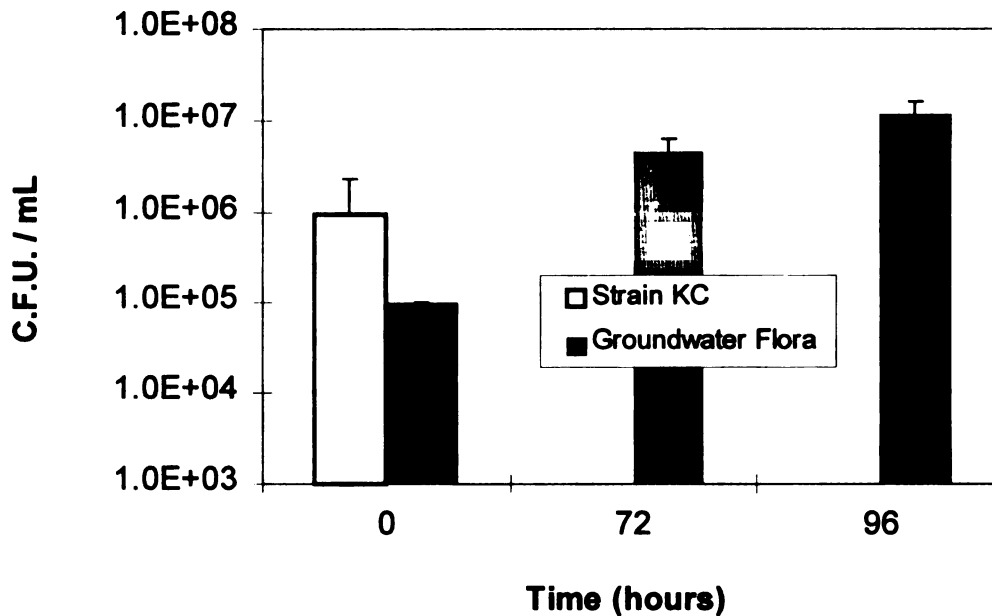


Figure 15: Mixed-strain plate count results. Initial pH 8.0, 12:1 (strain KC: groundwater flora), Medium D as the starter medium, washed inoculum. Geometric mean plotted, error bars represent one standard deviation.

Although inoculum density was varied in the mixed-strain experiments, the minimum dose of strain KC required to displace the groundwater flora has not been determined. A 0.1% inoculum (10^5 C.F.U. / mL) yields a ratio of approximately 7.2:1 (strain KC:groundwater Flora) (Figure 16). Strain KC was able to displace the aquifer flora under these conditions, but the smallest density difference (for unwashed SGW medium cultures) between the two

competitors was observed. A 0.1% inoculum (10^5 C.F.U. / mL) is probably close to the minimum required dose when native flora are present at concentrations less than 10^5 C.F.U. / mL.

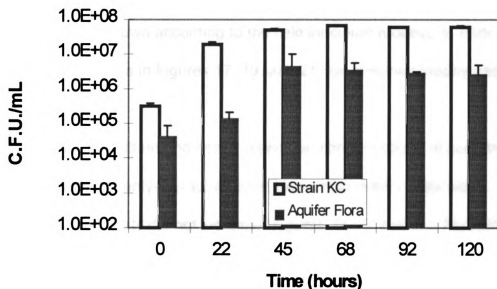


Figure 16: Mixed-strain plate count results. Initial pH 8.2, 7.2:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

To better understand nutrient consumption in the mixed-strain incubations, cell densities and nutrient concentrations were measured at time points throughout experiments with initial pHs of 7.5, 8.0, and 8.2. To test the competitive fitness of the KC inoculum used in the field demonstration, a colony collected during field inoculation was used as the starter culture for these experiments and grown according to the field inoculum procedure. Plate count results are presented in Figures 17, 19, and 21; ion chromatography data in Figures 18, 20, 22.

Most of the nitrate and nearly equimolar concentrations of acetate were consumed within twenty-four hours after inoculation. After nitrate was consumed, KC density did not increase (Figures 17, 19, and 21). No nitrite concentrations above detection limits were measured in the experiment at pH 7.5 (Figure 18). In the incubations at pH 8.0 and pH 8.2, as nitrate was utilized, low nitrite concentrations were measured (Figures 20 and 22). No nitrite concentrations above detection limits were recorded in any of the incubations after 48 hours. KC remained at greater densities than the groundwater flora in all three experiments (Figures 17, 19, and 21). KC also transformed CT to non-detectable levels during the initial twenty-four hours after inoculation.

Significant quantities of acetate were not metabolized after all the available nitrate was consumed. It is possible that the groundwater flora does not contain very many denitrifiers and may not be directly competitive with KC. In earlier mixed-strain experiments, groundwater flora densities increased after a four or five day lag period. The previously observed increase may not have occurred in these experiments because of their reduced duration. The long lag period for the groundwater flora could be explained if the consortia has significant numbers of fermentative organisms. Strain KC may outcompete other denitrifiers in the groundwater consortia and at later time points the fermentative groundwater organisms begin to grow. An experiment with increased nitrate concentrations and a longer duration could provide more information about the relationship between KC and the groundwater flora.

In previous experiments where KC was grown in pure culture, it was shown that nitrite concentrations increased as nitrate was used and then nitrite concentrations gradually decreased (Knoll 1994). In those same experiments, strain KC decayed rapidly after growth stopped. Knoll observed a decay rate of 8.92 days^{-1} for strain KC growing as a pure culture in Medium D. The maximum decay rates calculated, from the data presented in Figures 17, 19, and 21, at pHs 7.5, 8.0, and 8.2 are 1.3 days^{-1} , 1.7 days^{-1} , and 1.6 days^{-1} respectively. The lower nitrite concentrations and smaller decay rates in mixed culture versus

pure culture may be related. A plausible explanation is that the groundwater flora are utilizing the nitrite produced by KC. By keeping the nitrite concentration low, the groundwater flora protect KC from nitrite toxicity and help to stabilize the KC population.

Samples were saved from the final mixed-strain experiments for some type of genetic analysis. Genetic assays could provide valuable information about the types of organisms present and the changes in community structure occurring after nutrient addition and inoculation.

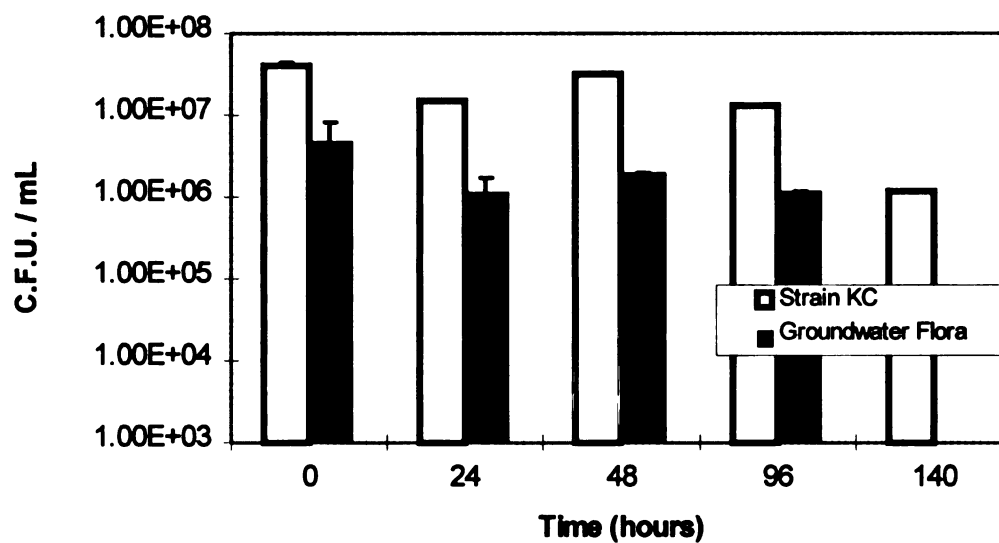


Figure 17: Mixed-strain plate count results. Initial pH 7.5, 8.5:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

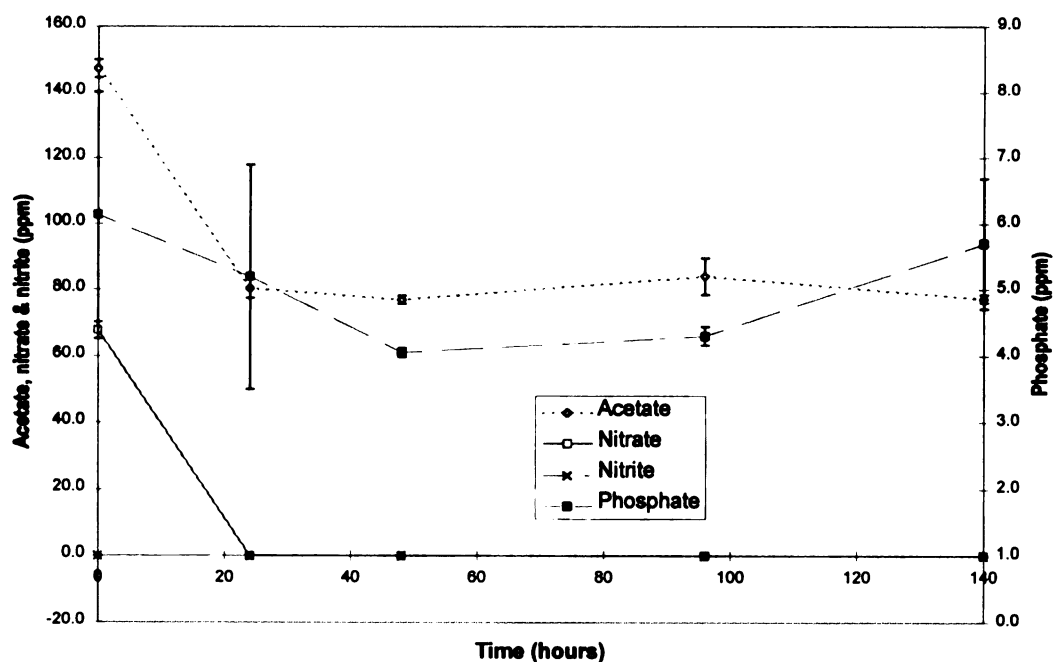


Figure 18: Ion analysis from a mixed-strain experiment. Initial pH 7.5, 8.5:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

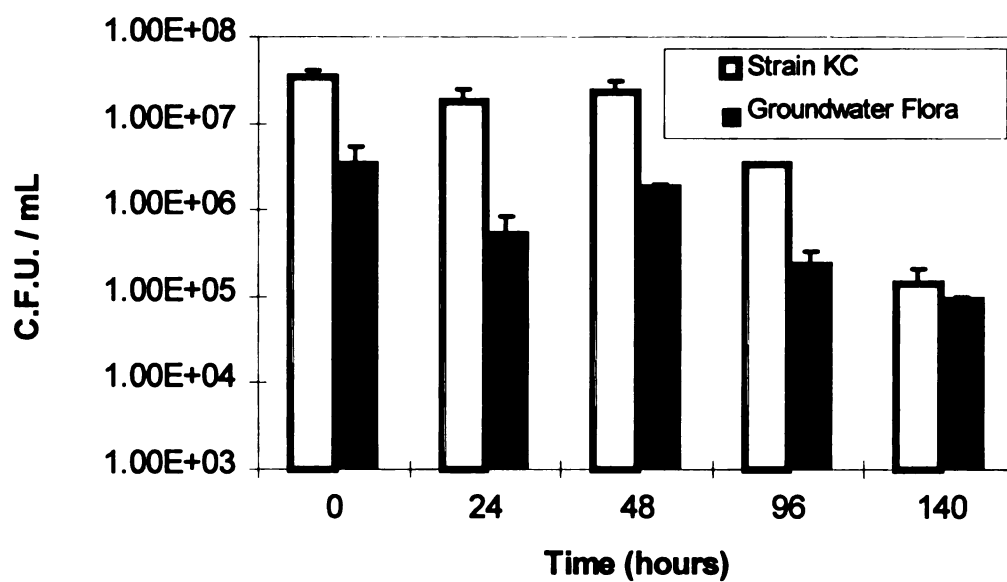


Figure 19: Mixed-strain plate count results. Initial pH 8.0, 9.2:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

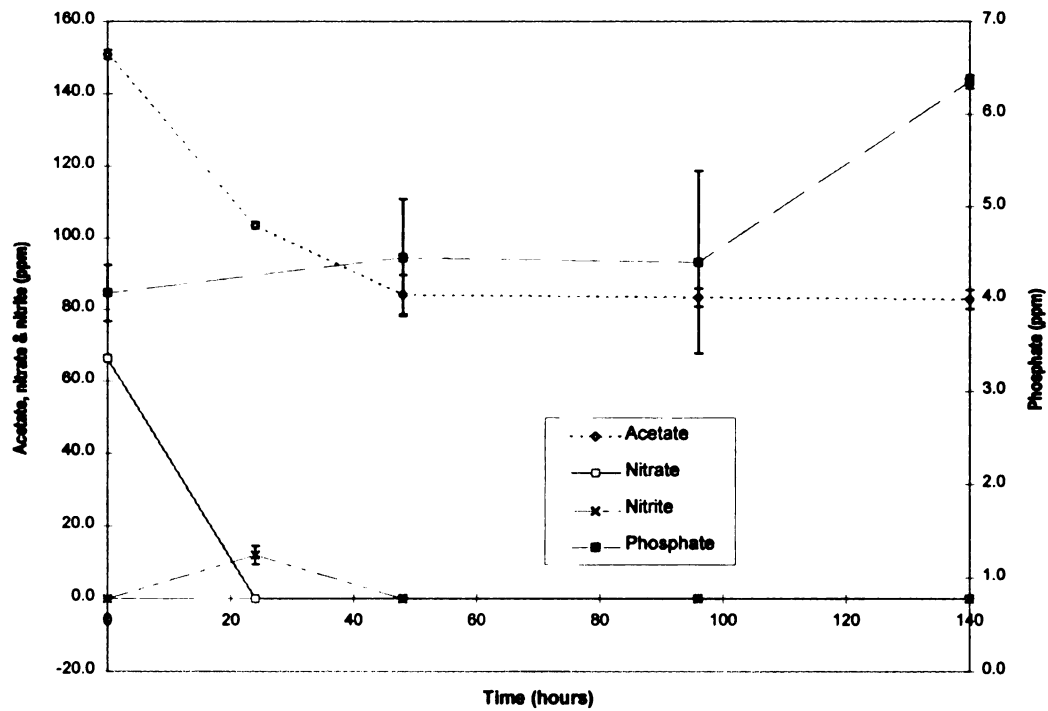


Figure 20: Ion analysis from a mixed-strain experiment. Initial pH 8.0, 9.2:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

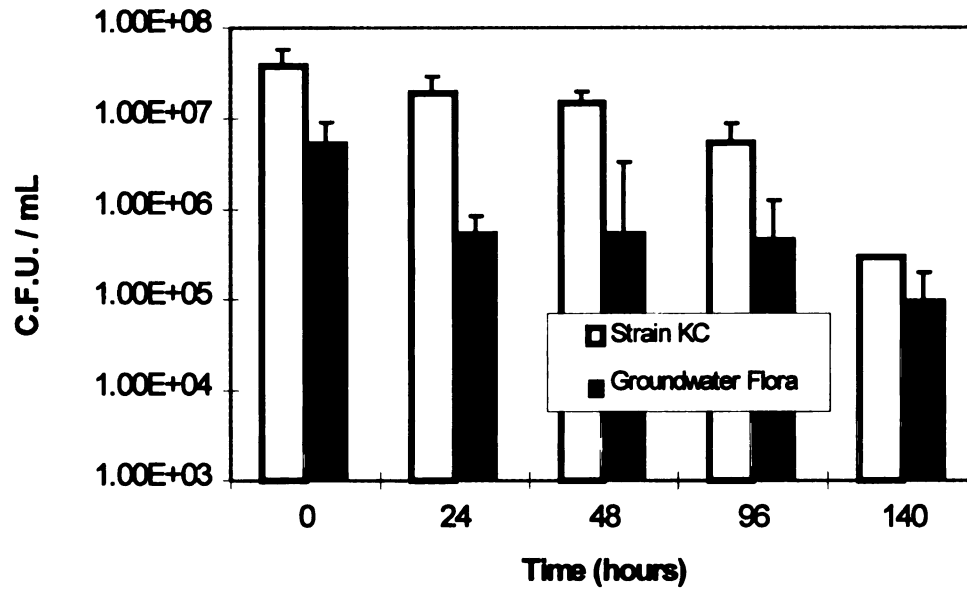


Figure 21: Mixed-strain plate count results. Initial pH 8.2, 6.8:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

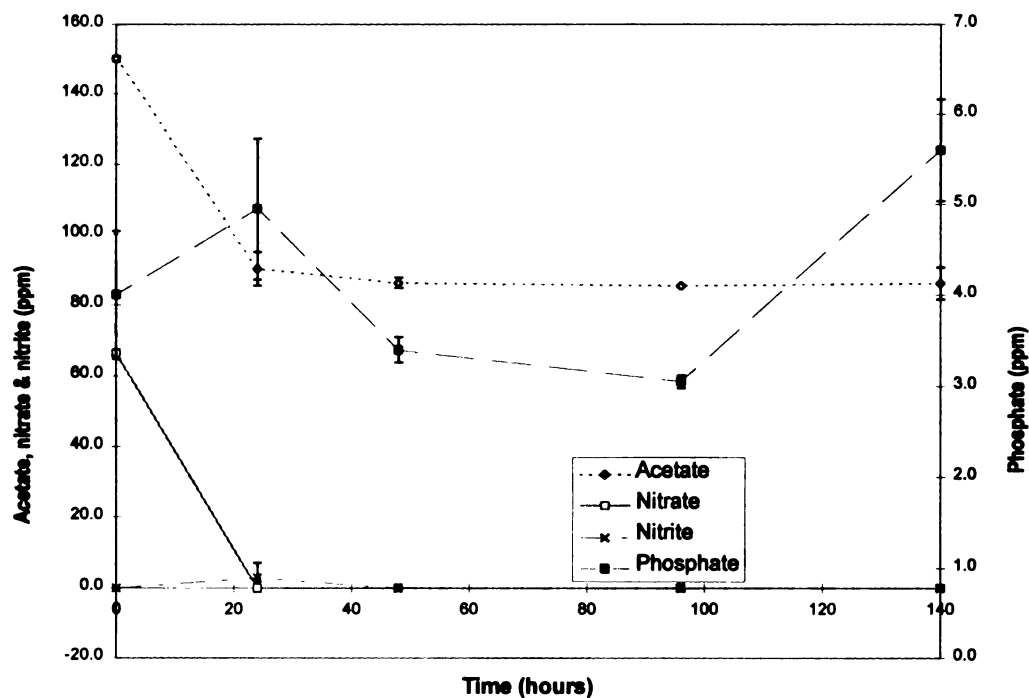


Figure 22: Ion analysis from a mixed-strain experiment. Initial pH 8.2, 6.8:1 (strain KC: groundwater flora), SGW medium as the starter medium. Geometric mean plotted, error bars represent one standard deviation.

CHAPTER 5

HABITAT MODIFICATION

NICHE ADJUSTMENT

To enhance the competitiveness of exogenous organisms, a temporary niche can be created in aquifers selected for bioaugmentation. Colonization of this niche by the introduced organism would facilitate remediation. Initial maximum specific growth experiments revealed that the optimum pH for growth of strain KC is 8.2 (Figure 1). Schoolcraft groundwater (Schoolcraft, MI, Field Project) has a natural pH of 7.5 which, as expected, is the optimum pH for groundwater flora growth (Figure 1). Alkalinity addition was used to raise the groundwater pH to 8.2. Subsequently, the aquifer was inoculated with strain KC. The favorable niche created by alkalinity addition may have helped establish the *Pseudomonas stutzeri* KC population in the aquifer.

If strain KC outcompetes the indigenous flora and transforms CT at a pH less than 8.2, less niche adjustment will be required and costs will be reduced. Ideally, the treatment zone will have a pH near 8.2. Near the boundary of the treatment zone, pH will begin to decrease and as the distance from the

treatment zone increases, groundwater pH will return to the background value (pH 7.5). If strain KC can displace the groundwater flora in regions where the pH is less than 8.2, a larger than anticipated treatment zone will be created.

Mixed-strain experiments were used to evaluate the competitiveness of strain KC at pH 8.0. Strain KC has a lower $S_{\min}$ than the groundwater consortium at pH 8.0 and the mixed-strain experiment verified its predicted dominance (Figure 2). The treatment zone should therefore extend to regions where the pH is less than 8.2.

PRE-INOCULATION DISINFECTION

MATERIALS AND METHODS

To determine the effects of disinfection on the groundwater flora, 50 mL of groundwater was put in 125 mL Erlenmeyer flasks. Bleach (HOCl) and hydrogen peroxide (H₂O₂) were added to the flasks at the desired concentrations. Flasks were incubated at 20° C on a rotary shaker at 150 rpm (Lab-Line Orbital Shaker model 3590) and samples were plated on R2A agar.

Organism density was determined according to the enumerative procedure in Chapter 2.

RESULTS AND DISCUSSION

Aquifer solids at the field site (Schoolcraft, MI) have been colonized by indigenous organisms (Chapter 6). In a model aquifer experiment, strain KC was no longer detected in the liquid phase 4 weeks after inoculation, but was found on the solid phase (Zhu 1994). Pumping bleach or ozone into the aquifer prior to inoculation may remove many of the indigenous flora from the solids and create available surface sites for colonization by *Pseudomonas stutzeri* KC.

Disinfection with hydrogen peroxide and bleach was tested in liquid phase batch experiments. Hydrogen peroxide proved to be an excellent disinfectant (Figure 23), but it is not commonly used for well disinfection. Bleach is also an effective disinfectant and it is commonly used as a well disinfectant. Bleach was most effective at 100 ppm (Figure 24).

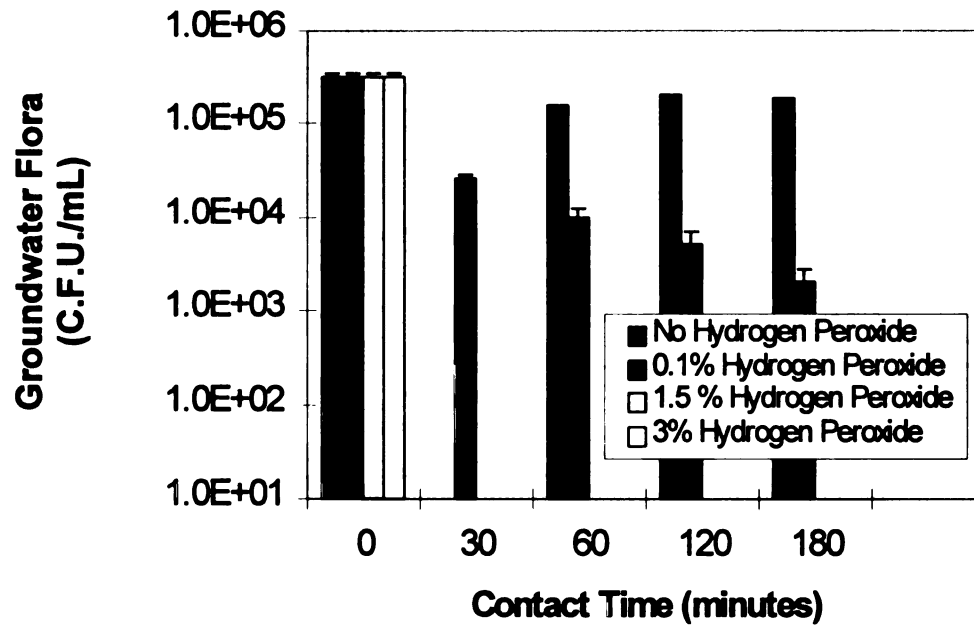


Figure 23: Plate count results from disinfection of Schoolcraft groundwater with hydrogen peroxide. Geometric mean plotted, error bars represent one standard deviation.

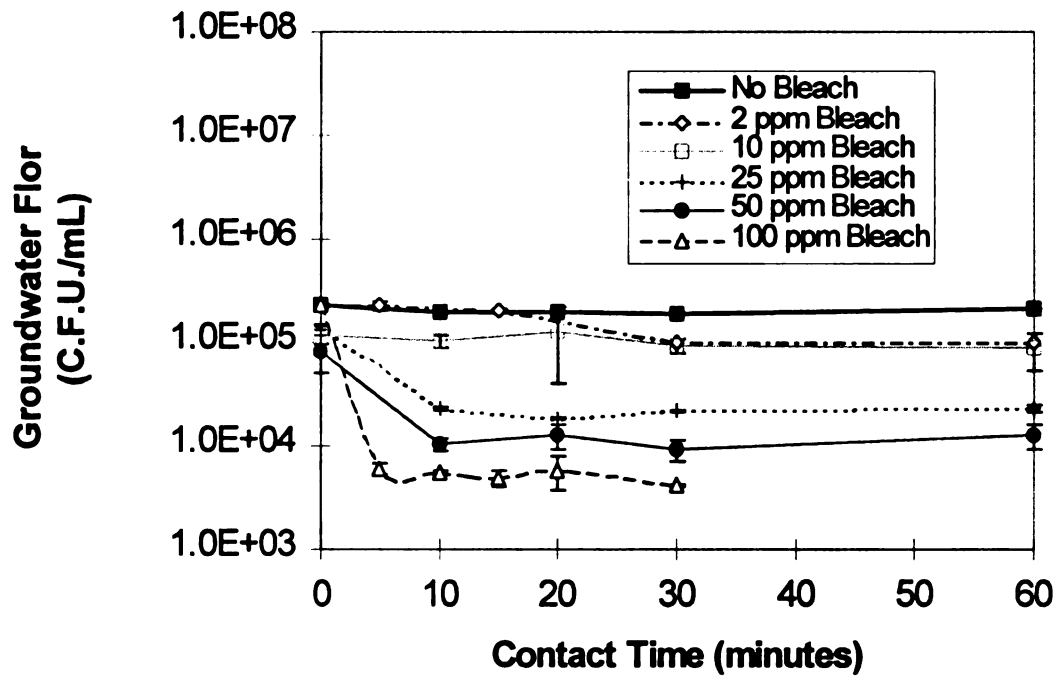


Figure 24: Plate count results from disinfection of Schoolcraft groundwater with bleach. Geometric mean is plotted, error bars represent plus or minus one standard deviation.

CHAPTER 6

FIELD DATA

MATERIALS AND METHODS

Extraction buffer. As previously reported by Warren et. al 1992, extraction buffer can be used to remove microorganisms from solid media. To prepare 1000 mL of 20 mmol phosphate buffer solution add 400 mL of 50 mmol phosphate buffer (Chapter 2) to 600 mL of DI water. Add KOH to adjust to pH 8.0, if necessary. Add the following chemicals to a 1 L Wheaton bottle: 1000 mL of 20 mmol phosphate buffer solution, 1000 μ L of 400 mg/L Tween, 380 mg of Ethylene bis N,N,N',N' tetraacetic acid, and 70 mg of yeast extract. Autoclave the solution in the Wheaton bottle with the lid on loosely at 250° F at 17 psi.

Plate counts. To determine the density of strain KC and aquifer flora in groundwater and solids samples, spread plate counts were used. Groundwater samples were diluted (Chapter 2) and a range of dilutions was selected for plating that would produce between thirty and three hundred colonies per plate.

To determine bacterial density in solid samples, approximately 0.1 gram of soil was placed in sterile 1.5 mL microcentrifuge tubes. To extract organisms from the soil into the liquid phase, 1 mL of extraction buffer was added to the tubes which were then sealed and shaken for approximately two minutes (at high speed on a vortexer). Extraction buffer was then diluted in the same manner as groundwater samples. Soil from which samples had been extracted was dried so that the solid phase plate count data could be normalized to colony forming units per gram of dry soil. Serial dilutions of samples were performed in sterile phosphate buffer (50 mM, pH 8.0). Diluted aliquots (100 μ L) were spread on R2A plates. Total colony forming units were counted after 6 days of incubation at 20° C.

Denitrifier quantification procedure. The most reliable and convenient method to measure denitrifier populations is to measure the denitrification process in most probable number (MPN) tubes by screening for nitrate and nitrite disappearance as the presumptive test (Tiedje 1994). The following description summarizes the method previously published by Tiedje (1994). Culture medium is prepared by adding 8.0 g of nutrient broth and 0.5 g of potassium nitrate to one liter of ultra-pure deionized water. Culture medium is added in 10 mL aliquots to 16 mm by 125 mm Hungate tubes (have butyl rubber

septa in screw caps, Bellco Glass Inc., Vineland, NJ), inverted Durham tubes are also added and sealed. Hungate tubes are autoclaved for 20 minutes at 121° C. For the nitrate and nitrite presumptive test, 0.2 g of diphenylamine $[(C_6H_5)_2NH]$ should be dissolved in 100 mL of concentrated sulfuric acid. The reagent bottle containing the diphenylamine solution should be wrapped in foil and stored in a refrigerator. To extract organisms from the liquid to solid phase, approximately 0.1 gram of soil is placed in sterile 1.5 mL microcentrifuge tubes. Extraction buffer, 1 mL, is added to the tubes which are then sealed and shaken for approximately two minutes (at high speed on a vortexer). Serial dilutions of the extraction solution are performed in autoclaved Hungate tubes (three per dilution), final dilutions of 10^{-3} to 10^{-8} should suffice. Tubes are incubated at 12° C for fourteen days. At that time, 0.1 to 1.0 mL of medium is withdrawn by syringe and tested for nitrate and nitrite by adding drop-wise up to six drops of the diphenylamine reagent. A blue color indicates the presence of nitrate or nitrite; a colorless response is considered presumptive evidence of denitrification. The denitrifier population can be estimated using the MPN procedure outlined below. For confirmation of the presence of denitrifiers, visually inspect the inverted (Durham) tubes for bubble formation.

Most probable number determination. The most probable number (MPN) method permits estimation of population density without an actual count of single cells or colonies (Alexander 1982). On the basis of probability theory, it is possible to combine the results from successive serial dilutions in such a way that a single value is obtained for the most probable number of microorganisms. As previously described by Halvorson and Ziegler (1933), an equation can be solved for its one unknown variable which is the most probable number of organisms.

RESULTS AND DISCUSSION

The field-scale aspect of this project involves collection of field data and comparison with laboratory data. A grid of monitoring wells has been placed at a field site (Schoolcraft, MI, Field Experiment) to allow sampling throughout the bioaugmentation zone. Prior to inoculation of the aquifer with strain KC, groundwater was collected from the sampling wells and soil cores were removed from the aquifer strata to provide baseline natural flora data. At the time of inoculation and throughout the treatment process, groundwater samples were collected so that strain KC and aquifer flora colonies could be quantified. A

statistically significant number of the samples were analyzed with a polymerase chain reaction (PCR) gene probe as a confirmatory assay and to verify the reliability of the morphology differentiation technique. Use of mixed-strain liquid batch incubations as a predictive tool for field applications will also be discussed.

An initial microbial site characterization should be conducted to evaluate the effects, of introducing *Pseudomonas stutzeri* KC into a carbon tetrachloride impacted aquifer (Schoolcraft, MI, Field Experiment), on the indigenous flora. Microbial habitat in an aquifer consists of two phases: liquid and solid. To sufficiently quantify the density of the indigenous microbial population, enumerative procedures should be conducted on both of these phases.

Total platable colonies and denitrifier density were identified as important microbial indicators. Plate counts have proven to be a simple and reliable method for enumerating both strain KC and the aquifer flora. Liquid phase plate count data from before niche adjustment and after niche adjustment prior to inoculation are shown in Table 2. Baseline platable colony density of groundwater flora was approximately 10^5 . The data from after niche adjustment show higher platable colony densities than prior to niche adjustment. Niche adjustment may have increased indigenous groundwater flora concentrations, but only one of the sample locations (3.6) was assayed during both sampling

events. A larger data set is needed to draw conclusions about the effects of niche adjustment on the indigenous population density. Baseline solid phase plate counts were between 10^5 and 10^7 (Table 3). As expected, solid phase densities are greater than liquid phase densities, but only by about one order of magnitude. Plate count results underestimate the actual population density because only platable organisms are quantified.

Table 2: Pre-inoculation Liquid Phase Field Results, Before and After Niche Adjustment

Sample: MSU-GW-MLS-3-6-Baseline (pre-niche adjustment, all depths)

<u>Well</u>	<u>Geometric Mean (C.F.U./mL)</u>
2	5.45E+05
3	2.96E+05
4	2.01E+05
5	6.71E+05
6	5.38E+05
7	6.50E+05

Sample: MSU-GW-MLS-2,3-14 (post-niche adjustment)

<u>Cell Number</u>	<u>Well</u>	<u>Depth (feet)</u>	<u>Geometric Mean (C.F.U./mL)</u>
2.8.14	2	8	1.63E+06
3.6.14	3	6	1.26E+06
3.8.14	3	8	9.79E+05

Table 3: Plate Count Results from Schoolcraft Aquifer Solids Before Inoculation with *Pseudomonas stutzeri* KC

Boring #	Core #	Depth (feet)	Geometric Mean of Groundwater flora (C.F.U. / gram of dry soil)	Standard Deviation of Groundwater flora (C.F.U. / gram of dry soil)
MSU-SO-MLS-3	2	60.5	5.44E+06	3.45E+06
MSU-SO-MLS-3	2	61.5	2.53E+07	1.01E+07
MSU-SO-MLS-3	2	62.5	6.32E+06	2.10E+06
MSU-SO-MLS-3	2	64.0	5.49E+05	1.92E+05
MSU-SO-MLS-3	2	65.5	3.81E+06	1.27E+06
MSU-SO-MLS-6	1	61.5	1.29E+07	2.93E+06
MSU-SO-INW-1	2	61.5	3.50E+06	3.34E+05
MSU-SO-INW-1	2	62.5	2.33E+06	5.95E+05
MSU-SO-INW-1	2	63.5	4.79E+06	1.54E+06

Pseudomonas stutzeri KC is a denitrifying organism (Criddle et al. 1990).

Of the many types of microorganisms in the indigenous population, denitrifiers could prove to be the most competitive with strain KC for resources. Baseline denitrifier Most Probable Number (MPN) assays were conducted on soil samples to quantify this segment of the indigenous community. Results of MPN assays are shown in Table 4. Approximately 10^3 to 10^5 denitrifiers per gram of soil existed on the aquifer solids prior to niche adjustment. Solid phase assays (both plate counts and MPN tubes) may underestimate the denitrifier population

due to less than one hundred percent extraction of the organisms from the solid to liquid phase.

Table 4: Most Probable Number (MPN) Denitrifier Assay Results from Schoolcraft Aquifer Solids Before Inoculation with *Pseudomonas stutzeri* strain KC

Boring #	Core #	Depth (feet)	Geometric Mean of Groundwater flora (denitrifiers / gram of dry soil)	Standard Deviation of Groundwater flora (denitrifiers / gram of dry soil)
MSU-SO-MLS-3	2	60.5	3.22E+04	3.13E+04
MSU-SO-MLS-3	2	61.5	1.87E+05	4.01E+05
MSU-SO-MLS-3	2	62.5	7.66E+03	1.19E+04
MSU-SO-MLS-3	2	64.0	3.85E+04	4.11E+04
MSU-SO-MLS-3	2	65.5	1.22E+05	1.08E+05
MSU-SO-MLS-6	1	61.5	6.25E+05	5.16E+05
MSU-SO-INW-1	2	61.5	5.07E+05	1.96E+06
MSU-SO-INW-1	2	62.5	8.32E+04	1.24E+04
MSU-SO-INW-1	2	63.5	6.84E+05	3.36E+06

Plate counts have proven to be a relatively simple and reliable method for enumerating both *Pseudomonas stutzeri* KC and the aquifer flora. Results from plate count assays on liquid phase samples collected at the field site (Schoolcraft, MI, Field Experiment) are shown in Table 5. Liquid phase

concentrations of strain KC varied greatly, both spatially and temporally. Some test wells (#2 and #3) consistently showed approximately 10^3 to 10^4 colony forming units of strain KC per milliliter. Other wells (#4, #6, and #7) showed little evidence of strain KC. Groundwater flora densities remained between 10^4 and 10^5 colony forming units of groundwater flora per millimeter during the period of nutrient addition to the aquifer.

In wells (#2 and #3) where strain KC was present in sufficient quantities to allow successful enumeration in the first sampling event after inoculation (#15), it appears that between sampling events 16 and 17, liquid phase KC densities decreased by approximately two orders of magnitude. It was previously shown in a model aquifer study that four weeks after inoculation, strain KC was no longer detected in the liquid phase, but was detectable on the solid phase (Zhu 1994). The decreased density of strain KC in the liquid phase could therefore be due to preferential partitioning to the solid phase. Analysis of aquifer solids at the completion of the field study will help to confirm whether or not strain KC migrated to the solid phase.

**Table 5: Liquid phase plate counts from Schoolcraft Aquifer After
Inoculation (C.F.U. / mL)**

MLS Sampling Port	Event #15		Event #16		Event #17	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
2.1	0 @ 10 ⁻³	1.88E+05	5.76E+06	6.80E+05	0 @ 10 ⁻⁴	3.50E+05
2.2	0 @ 10 ⁻⁴	3.30E+05			4.00E+04	5.20E+05
2.3	0 @ 10 ⁻⁴	1.51E+06	2.56E+06	2.00E+05	3.00E+04	3.80E+05
2.4	0 @ 10 ⁻⁴	4.70E+05			1.00E+04	4.60E+05
2.5	6.70E+05	4.00E+04	3.08E+06	6.00E+05	3.00E+04	3.00E+05
2.6	4.70E+05	5.00E+04			3.00E+04	3.90E+05
2.7	1.40E+06	0 @ 10 ⁻⁵	4.80E+06	7.00E+05	1.00E+04	6.70E+05
2.8	4.60E+05	1.20E+05			3.00E+04	1.21E+06

MLS Sampling Port	Event #18		Event #19		Event #20	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
2.1	3.00E+03	6.80E+05	1.00E+03	2.00E+05	2.00E+03	5.00E+05
2.2	4.00E+03	7.90E+05	1.10E+04	4.00E+05	1.50E+04	1.01E+06
2.3	7.00E+03	8.30E+05	1.00E+03	4.30E+05	2.00E+03	3.60E+05
2.4	6.00E+03	7.30E+05	6.00E+03	5.00E+05	3.00E+03	8.80E+05
2.5	4.00E+03	5.60E+05	0 @ 10 ⁻³	3.00E+05	1.00E+03	3.80E+05
2.6	1.00E+04	4.00E+05	3.00E+03	3.50E+05	4.00E+03	4.80E+05
2.7	6.00E+03	7.60E+05	5.00E+03	7.20E+05	4.00E+03	3.80E+05
2.8	4.00E+03	2.24E+06	4.00E+03	9.80E+05	7.00E+03	7.00E+05

Table 5 (cont'd)

MLS Sampling Port	Event #15		Event #16		Event #17	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
3.1	0 @ 10 ⁻³	1.80E+05	4.76E+06	7.60E+05	0 @ 10 ⁻⁴	5.60E+05
3.2	0 @ 10 ⁻³	1.58E+05			0 @ 10 ⁻⁴	6.20E+05
3.3	0 @ 10 ⁻³	4.80E+05	3.32E+06	4.40E+05	1.00E+04	7.20E+05
3.4	0 @ 10 ⁻³	1.55E+05			0 @ 10 ⁻⁴	1.16E+06
3.5	2.27E+06	1.50E+05	1.72E+06	4.10E+05	0 @ 10 ⁻⁴	3.20E+05
3.6	5.00E+05	9.00E+04			0 @ 10 ⁻⁵	2.80E+05
3.7	2.60E+05	9.00E+04	2.88E+06	1.60E+05	2.00E+04	3.80E+05
3.8	1.80E+05	1.10E+05			0 @ 10 ⁻⁴	1.40E+06

MLS Sampling Port	Event #18		Event #19		Event #20	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
3.1	0 @ 10 ⁻³	1.48E+05	1.00E+03	1.48E+05	1.00E+03	1.18E+06
3.2	3.00E+03	1.48E+05	2.00E+03	4.00E+05	0 @ 10 ⁻³	5.60E+05
3.3	9.00E+03	5.60E+05	2.00E+03	6.00E+05	3.00E+03	7.70E+05
3.4	1.00E+03	1.36E+06	0 @ 10 ⁻³	2.32E+05	1.20E+04	2.34E+06
3.5	1.70E+04	1.12E+06	0 @ 10 ⁻³	8.00E+05	1.00E+04	1.82E+06
3.6	6.40E+04	1.56E+06	0 @ 10 ⁻³	8.40E+05	2.70E+04	1.82E+06
3.7	2.00E+03	9.60E+05	0 @ 10 ⁻³	9.20E+05	1.80E+04	2.31E+06
3.8	0 @ 10 ⁻⁵	2.00E+06	3.00E+04	2.00E+06	1.00E+04	5.10E+06

Table 5 (cont'd)

MLS Sampling Port	Event #15		Event #16		Event #17	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
4.1	0 @ 10 ⁻³	8.50E+04	1.60E+04	2.56E+05		
4.2	0 @ 10 ⁻³	1.24E+05			5.00E+03	8.60E+04
4.3	0 @ 10 ⁻³	8.90E+04	8.00E+03	1.72E+05	0 @ 10 ⁻³	1.12E+05
4.4	0 @ 10 ⁻³	7.90E+04			0 @ 10 ⁻³	6.40E+04
4.5	0 @ 10 ⁻³	7.60E+04	0 @ 10 ⁻⁴	4.50E+05	0 @ 10 ⁻³	9.60E+04
4.6	1.00E+03	9.60E+04			0 @ 10 ⁻³	1.51E+05
4.7	0 @ 10 ⁻³	1.63E+05	0 @ 10 ⁻⁴	1.60E+06	0 @ 10 ⁻³	1.60E+05
4.8	0 @ 10 ⁻³	1.29E+05			0 @ 10 ⁻⁴	1.53E+06

MLS Sampling Port	Event #18		Event #19		Event #20	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
4.1	0 @ 10 ⁻³	1.20E+04	0 @ 10 ⁻³	2.36E+05	0 @ 10 ⁻³	4.90E+05
4.2	0 @ 10 ⁻³	9.20E+04	0 @ 10 ⁻³	1.28E+05	0 @ 10 ⁻³	5.90E+05
4.3	0 @ 10 ⁻³	8.60E+04	0 @ 10 ⁻³	3.24E+05	0 @ 10 ⁻³	6.50E+05
4.4	0 @ 10 ⁻³	9.20E+04	0 @ 10 ⁻³	1.36E+05		
4.5	0 @ 10 ⁻³	1.20E+05	0 @ 10 ⁻³	2.68E+05	0 @ 10 ⁻³	5.70E+05
4.6	0 @ 10 ⁻³	1.16E+05	0 @ 10 ⁻³	2.76E+05	0 @ 10 ⁻³	3.80E+05
4.7	0 @ 10 ⁻³	5.20E+04			0 @ 10 ⁻³	4.30E+05
4.8	0 @ 10 ⁻³	2.28E+05	0 @ 10 ⁻³	6.40E+04	0 @ 10 ⁻³	2.14E+05

Table 5 (cont'd)

MLS Sampling Port	Event #15		Event #16		Event #17	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
5.1	0 @ 10 ⁻⁴	2.40E+05	1000	1.38E+05	0 @ 10 ⁻⁴	1.07E+05
5.2	0 @ 10 ⁻⁴	1.36E+05			0 @ 10 ⁻⁴	6.10E+06
5.3	0 @ 10 ⁻⁴	1.40E+05	0 @ 10 ⁻³	1.98E+05	0 @ 10 ⁻⁵	6.80E+06
5.4	0 @ 10 ⁻⁴	1.92E+05			0 @ 10 ⁻⁵	1.31E+06
5.5	0 @ 10 ⁻⁴	8.10E+04	0 @ 10 ⁻³	1.61E+05	0 @ 10 ⁻⁴	2.80E+05
5.6	0 @ 10 ⁻⁴	9.10E+04			0 @ 10 ⁻⁴	1.52E+06
5.7	0 @ 10 ⁻⁴	1.17E+05	0 @ 10 ⁻³	1.59E+05	0 @ 10 ⁻⁴	2.80E+05
5.8	0 @ 10 ⁻⁴	7.20E+04			0 @ 10 ⁻⁴	3.80E+05

MLS Sampling Port	Event #18		Event #19		Event #20	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
5.1	0 @ 10 ⁻⁴	8.70E+05	3.00E+03	4.70E+04	0 @ 10 ⁻³	2.39E+05
5.2	0 @ 10 ⁻⁴	5.10E+05	4.00E+03	1.70E+04	0 @ 10 ⁻³	2.96E+05
5.3	0 @ 10 ⁻⁴	1.02E+06	7.00E+03	1.40E+04	0 @ 10 ⁻³	1.54E+05
5.4	0 @ 10 ⁻⁴	8.20E+05	6.00E+03	2.30E+04	0 @ 10 ⁻³	4.96E+05
5.5	0 @ 10 ⁻⁴	2.64E+06	4.00E+03	2.00E+04	0 @ 10 ⁻³	1.88E+05
5.6	0 @ 10 ⁻⁴	3.16E+06	1.00E+04	3.20E+05	0 @ 10 ⁻³	4.20E+04
5.7	0 @ 10 ⁻⁴	1.04E+06	6.00E+03	2.50E+05	0 @ 10 ⁻³	2.26E+05
5.8	0 @ 10 ⁻⁴	1.32E+06	4.00E+03	1.50E+04	0 @ 10 ⁻³	3.08E+05

Table 5 (cont'd)

MLS Sampling Port	Event #15		Event #16		Event #17	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
6.1	0 @ 10 ⁻³	6.80E+04	0 @ 10 ⁻³	1.83E+05	0 @ 10 ⁻⁴	3.20E+05
6.2	0 @ 10 ⁻³	1.02E+05			0 @ 10 ⁻⁴	3.80E+05
6.3	0 @ 10 ⁻³	1.68E+05	0 @ 10 ⁻³	1.52E+05	0 @ 10 ⁻⁴	3.00E+05
6.4	0 @ 10 ⁻³	7.20E+04			0 @ 10 ⁻⁴	3.50E+05
6.5	0 @ 10 ⁻³	4.10E+04	0 @ 10 ⁻³	1.07E+05	0 @ 10 ⁻⁴	2.23E+06
6.6	0 @ 10 ⁻³	2.70E+04			0 @ 10 ⁻⁴	2.48E+06
6.7	0 @ 10 ⁻³	8.20E+04	1000	1.55E+05	0 @ 10 ⁻⁴	
6.8	0 @ 10 ⁻³	2.70E+04			0 @ 10 ⁻⁴	3.64E+06

MLS Sampling Port	Event #18		Event #19		Event #20	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
6.1	2.00E+04	2.08E+06	0 @ 10 ⁻³	2.64E+05	0 @ 10 ⁻³	7.70E+04
6.2	0 @ 10 ⁻⁴	3.48E+06	0 @ 10 ⁻³	1.16E+05	0 @ 10 ⁻³	4.60E+04
6.3	1.00E+04	6.50E+05	0 @ 10 ⁻³	3.40E+05	0 @ 10 ⁻³	6.70E+04
6.4	0 @ 10 ⁻⁴	4.50E+08	0 @ 10 ⁻³	2.12E+05	0 @ 10 ⁻³	4.10E+04
6.5	0 @ 10 ⁻⁴	2.80E+07	0 @ 10 ⁻³	2.00E+05	0 @ 10 ⁻³	1.88E+05
6.6	2.00E+04	1.44E+06			0 @ 10 ⁻³	1.47E+05
6.7	0 @ 10 ⁻⁴	9.60E+05	0 @ 10 ⁻³	3.00E+05	0 @ 10 ⁻³	1.02E+05
6.8	0 @ 10 ⁻⁴	1.48E+06	0 @ 10 ⁻³	1.64E+05	0 @ 10 ⁻³	5.40E+04

Table 5 (cont'd)

MLS Sampling Port	Event #15		Event #16		Event #17	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
7.1	0 @ 10 ⁻³	3.50E+05	0 @ 10 ⁻³	2.60E+05	0 @ 10 ⁻⁵	4.90E+06
7.2	0 @ 10 ⁻³	3.00E+05			0 @ 10 ⁻⁵	3.00E+06
7.3	0 @ 10 ⁻³	4.10E+05	0 @ 10 ⁻³	1.99E+06	0 @ 10 ⁻⁵	5.30E+06
7.4	0 @ 10 ⁻³	2.08E+05			0 @ 10 ⁻⁵	3.50E+06
7.5	0 @ 10 ⁻³	3.20E+05	0 @ 10 ⁻³	1.41E+06	0 @ 10 ⁻⁵	4.60E+06
7.6	0 @ 10 ⁻³	2.90E+05				8.20E+06
7.7	0 @ 10 ⁻³	9.50E+05	0 @ 10 ⁻³	1.80E+06		8.80E+06
7.8	0 @ 10 ⁻³	1.18E+06			1.00E+05	7.60E+06

MLS Sampling Port	Event #18		Event #19		Event #20	
	PKC	Aquifer Flora	PKC	Aquifer Flora	PKC	Aquifer Flora
7.1	1.00E+05	4.40E+06	0 @ 10 ⁻³	3.20E+04	0 @ 10 ⁻²	1.20E+04
7.2	2.00E+05	3.90E+06	0 @ 10 ⁻³	7.00E+03	0 @ 10 ⁻³	9.20E+04
7.3	6.00E+04	1.60E+05	0 @ 10 ⁻³	5.70E+04	0 @ 10 ⁻³	8.60E+04
7.4	2.00E+04	5.10E+06	0 @ 10 ⁻³	1.40E+04	3.00E+04	3.88E+06
7.5	0 @ 10 ⁻⁴	3.90E+05	0 @ 10 ⁻⁴	3.30E+05	0 @ 10 ⁻³	5.00E+05
7.6	0 @ 10 ⁻⁴	7.00E+05	0 @ 10 ⁻³	1.92E+05	0 @ 10 ⁻³	4.20E+05
7.7	0 @ 10 ⁻⁴	4.50E+05	0 @ 10 ⁻³	6.10E+05	6.00E+04	3.40E+05
7.8	0 @ 10 ⁻⁴	1.52E+06	0 @ 10 ⁻³	7.70E+05	0 @ 10 ⁻³	2.06E+06

CHAPTER 7

ENGINEERING APPLICATION

Bioaugmentation is emerging as a promising technology for groundwater applications. If an introduced species cannot coexist with or displace an indigenous population, bioaugmentation may not be a plausible option. Addition of alkalinity can be used to create a favorable niche for colonization by an exogenous population. Strain KC multiplies rapidly at pH 8.2, but not at pH 7.5. For successful remediation with KC, it is therefore imperative to maintain pH within the remediation zone near 8.2. When remediation activities are complete, niche adjustment provides another advantage. If alkalinity is no longer added, the pH of the remediation zone should return to its natural level. With the return of natural conditions, indigenous flora should displace the exogenous population.

If pH adjustment is not sufficient to provide the exogenous population with a competitive advantage, pre-inoculation disinfection could be used to reduce the indigenous flora concentration. After disinfection, exogenous organisms would have a better chance of successfully colonizing an aquifer. Further experimental investigations are necessary if in-situ bioremediation is to become a viable alternative for remediating chemically impacted sites, but niche

adjustment and pre-inoculation disinfection should provide competitive advantages to exogenous populations.

Although single nutrient competition theory was derived for continuous culture experiments, it was used to predict the outcome of batch experiments described in earlier chapters. Some of the batch experiment results at pH 7.5, where the groundwater flora was predicted to outcompete KC, seemed to contradict single nutrient theory predictions. At pH 7.5, although KC did not appear to growing, it maintained a higher population density than the groundwater flora. In continuous culture however, KC would probably be displaced by the groundwater flora at pH 7.5. Although single nutrient theory predictions do not strictly apply to batch incubations, they do indicate which species would dominate as the incubations became less like batch experiments and more like continuous cultures.

One of the concerns that arose in the planning stages of the associated bioaugmentation project was the problem of conducting laboratory experiments using only the liquid phase when in reality the introduced organism would have to compete for nutrients with organisms present in both the liquid and solid phases. Even though KC is able to dominate the organisms suspended in the liquid phase, will KC be able to compete with the organisms attached to the solid phase? As it turned out, KC was able to persist in the aquifer over the time

frame of the bioaugmentation project. It was previously demonstrated (in the laboratory) that KC preferentially attaches to solids. Liquid phase KC concentrations in the field decreased in one sampling location two to three weeks after inoculation. This liquid phase decrease could be attributed to solid phase attachment. If so KC was able to colonize the solid phase as well. Analysis of solids removed from the aquifer should indicate whether this is an accurate conclusion. The subsistence of strain KC in the aquifer suggests that, at least in some cases, the observed dominance of an organism in the liquid phase demonstrates that it can persist when a biologically colonized solid phase is present.

CHAPTER 8

CONCLUSIONS

1) *Pseudomonas stutzeri* KC is most competitive with the groundwater flora at pH 8.2. Strain KC's highest $\mu_{\max}$ and lowest $S_{\min}$ values occur at pH 8.2. Strain KC achieved the greatest density in mixed-strain experiments at pH 8.2.

2) Washing a starter culture probably stresses it, causing a decrease in its competitiveness. Washing the KC starter cultures used in the $\mu_{\max}$ determination experiments may have affected their outcome. Strain KC may actually have a higher maximum specific growth rate than is reported here.

3) Single nutrient competition theory is not a viable tool for predicting the outcome of all batch competition experiments. For example, if the inoculum density is high enough, strain KC can maintain cell densities comparable to the groundwater flora even when strain KC's $S_{\min}$ is greater.

4) Strain KC does not experience rapid decay after growth has ceased in mixed-strain experiments. In batch, pure culture experiments, it was previously shown that the density of strain KC decreased rapidly after the cessation of growth. This suggests that a factor present in the mixed-strain experiments helps stabilize the strain KC population. This stabilizing factor could be the groundwater flora. Utilization of nitrite by the indigenous flora may protect KC from nitrite toxicity and as a result stabilize the KC population.

5) The minimum inoculum size of KC required to dominate the groundwater flora is approximately 10^5 CFU/mL when the groundwater flora concentration is less than 10^5 CFU/mL.

6) Of the starter media tested, SGW medium proved to be the best for promoting the proliferation of KC in mixed-strain experiments. KC inocula grown in SGW medium may be in log phase growth at inoculation and may have access to all the nutrients it requires. KC inocula grown in the other media may not have these two distinct advantages.

7) 100 ppm of bleach proved to be an effective groundwater disinfectant. Pre-inoculation disinfection could be used to create niches in situations where the indigenous population is too dense to overcome by competition.

FUTURE WORK RECOMMENDATIONS

1) Another mixed-strain competition experiment in groundwater amended with nitrate would be useful. Ion and cell concentrations as well as CT transformation capacity could be measured. This experiment would be similar to the experiment where ion concentrations were assayed, but adding nitrate would provide additional information about the relationship between strain KC and the groundwater flora.

2) Significant differences in the competitive fitness of strain KC are observed when it is grown in SGW versus it being acclimated to amended groundwater.

Identifying the chemical differences between the two growth media should increase the understanding of strain KC growth requirements.

3) Samples from the mixed strain experiment in which ion concentrations were quantified were collected and frozen for genetic analysis. Genetic assays could provide information about the initial community structure and changes in the community during experiments.

4) Chemostat studies involving strain KC and the groundwater flora could be used to demonstrate the applicability of single nutrient competition theory to interactions of groundwater bacteria. Maximum specific growth rates for strain KC and the groundwater community could also be measured more accurately. Maximum specific decay rates as a function of pH could be recorded. If strain KC was not the dominant species under certain conditions, samples could be collected and the remaining species could be evaluated. Many interesting experiments (in addition to those mentioned here) could be conducted in a chemostat.

LIST OF REFERENCES

1. Alexander, M. 1982. A.L. Page (ed.), Most Probable Number Method for Microbial Populations, ASA-SSSA. Madison, Wisconsin. 815-820.
2. Atlas, R.M., R. Bartha. 1993. Microbial Ecology. The Benjamin/Cummings Publishing Company, Inc. Redwood City, CA
3. Bailey, J.E., D.F. Ollis. 1986. Biochemical Engineering Fundamentals, McGraw-Hill, Inc.: New York, New York.
4. Brock, T.D. and M.T. Madigan. 1991. Biology of Microorganisms, Prentice Hall: Englewood Cliffs, New Jersey.
5. Criddle, C.S., L.A. Alvarez, and P.L. McCarty. 1991. J. Bear and M.Y. Corapcioglu (eds.), Microbial Processes in Porous Media, Kluwer Academic Publishers. The Netherlands, 639-691.
6. Criddle, C.S., J.T. DeWitt, D. Grbić-Galić, and P.L. McCarty. 1990. Transformation of carbon tetrachloride by *Pseudomonas* sp. strain KC under denitrification conditions. *Appl. Environ. Microbiol.* **56**:3240-3246.
7. de Wit, C.T. 1960. On competition. *Versl. Landbouw. Onderz.* **66**: 1-82.
8. Dybas, M.J., G.M. Tatara, C.S. Criddle. 1995. Localization and characterization of the carbon tetrachloride transformation activity of *Pseudomonas stutzeri* KC. *Appl. Environ. Microbiol.* **61**: 758-762.
9. Firbank, L.G., A.R. Watkinson. 1990. On the effects of competition: from monocultures to mixtures, p. 165-192. *In* J.B. Grace and D. Tilman (eds.), Perspectives on plant competition. Academic Press, San Diego, Calif.

10. Halvorson, H.O., N.R. Ziegler. 1933. Application of statistics to problems in bacteriology: I. A means of determining bacterial populations by the dilution method. *J. Bacteriol.* 25:101-121.
11. Hansen S.R., S.P. Hubbell. 1980. Single-nutrient microbial competition: qualitative agreement between experimental and theoretically forecast outcomes. *Science.* 207:1491-1493.
12. Hsu, S.B., S.P. Hubbell, P. Waltman. 1977. A mathematical theory for single-nutrient competition in continuous cultures of microorganisms. *Society of Industrial and Applied Mathematics, Journal of Applied Mathematics.* 32:366-383.
13. Jensen, H.L. 1963. Carbon nutrition of some microorganisms decomposing halogen-substituted aliphatic acids. *Acta Agr. Scand.* 13: 404-412.
14. Harper, J.L. 1977. *The population biology of plants.* Academic Press, London.
15. Keshtacher-Liebson, E., Y. Hadar, Y. Chen. 1995. Oligotrophic bacteria enhance algal growth under iron-deficient conditions. *Appl. Environ. Microbiol.* 61: 2439-2441.
16. Knoll, W.H. 1994. M.S. Thesis. Michigan State University. East Lansing, Mich.
17. Lewis, T.A., R.L. Crawford. 1993. Physiological factors affecting carbon tetrachloride dehalogenation by denitrifying bacterium *Pseudomonas* sp. Strain KC. *Appl. Environ. Microbiol.* 59: 1635-1641.
18. Lawrence, A. W., P.L. McCarty. 1970. Unified basis for biological treatment design and operation. *Jour. Sanitary Engineering Division, Amer. Soc. of Civil Engineers*, 96/SA3. 757-778.
19. Lehman, J.T., D.B. Botkin, and G.E. Likens. 1975. The assumptions and rationales of a computer model of phytoplankton population dynamics. *Limnology and Oceanography.* 20: 343-364.

20. Leon, J.A., D.B. Tumpsen. 1975. Competitions between two species for two complementary or substitutable resources. *Journal of Theoretical Biology*. **50**: 185-201.
21. Monod, J. 1942. *Recherches sur la croissance des bacteriennes*. Hermann and Cie (eds.) Rue de la Sorbonne, Paris.
22. Novick, A., L. Szilard. 1950. Description of a chemostat. *Science*. **112**: 715-716.
23. Petersen, R. 1975. The paradox of the plankton: an equilibrium hypothesis. *American Naturalist*. **109**: 35-49.
24. Rittmann, B.E., P.L. McCarty. 1980. Model of steady-state biofilm kinetics. *Biotechnology and Bioengineering*. **22**: 2243-2357.
25. Semprini, L., G.D. Hopkins, P.L. McCarty, P.V. Roberts. 1992. In-situ transformation of carbon tetrachloride and other halogenated compounds resulting from biostimulation under anoxic conditions. *Env. Sci. Tech.* **26**: 2454-2461.
26. Snaydon, R.W. 1991. Replacement or additive designs for competition studies? *J. Appl. Ecol.* **28**: 930-946.
27. Stewart, F.M., B.R. Levin. 1973. Partitioning of resources and the outcome of interspecific competition: a model and some general considerations. *American Naturalist*. **107**: 171-198.
28. Stumm, W., J.J. Morgan. 1981. Aquatic Chemistry, 2nd ed.; John Wiley & Sons: New York, 248.
29. Tatara, G.M., M.J. Dybas, and C.S. Criddle. 1993. Effects of medium and trace metals on the kinetics of carbon tetrachloride transformation by *Pseudomonas* sp. strain KC. *Appl. Environ. Microbiol.* **59**:2126-2131.
30. Taylor, P. P. Williams. 1975. Theoretical studies on the coexistence of competing species under continuous flow conditions. *Canadian Journal of Microbiology*. **21**: 90-98.
31. Tiedje, J.M. 1994. Denitrifiers, ASA-SSSA. Madison, Wisconsin. 815-820.

32. Tilman, D. 1977. Resource competition between planktonic algae: an experimental and theoretical approach. *Ecology*. **58**:338-348.
33. Tilman, D. 1980. Resources: a graphical-mechanistic approach to competition and predation. *American Naturalist*. **116**: 362-393.
34. Tilman, D. 1981. Tests of resource competition theory using four species of Lake Michigan algae. *Ecology*. **62**: 802-815.
35. van Uden, N. 1967. Transport-limited growth in the chemostat and its competitive inhibition; a theoretical treatment. *Archiv fur Mikrobiologie*. **58**: 145-154.
36. Vogel, T.M., C.S. Criddle, P.L. McCarty. 1987. Transformations of halogenated aliphatic compounds. *Env. Sci. Tech.* **21**: 722-736.
37. Warren, T.M., V. Williams, M. Fletcher. 1992. Influence of Solid Surface, Adhesive Ability, and Inoculum Size on Bacterial Colonization in Microcosm Studies. *Appl. Environ. Microbiol.* **58**: 2954-2959.
38. Wilson, M., S.E. Lindow. 1994. Ecological similarity and Coexistence of Epiphytic Ice-Nucleating (Ice⁺) *Pseudomonas syringae* Strains and a Non-Ice-Nucleating (Ice⁻) Biological Control Agent. *Appl. Environ. Microbiol.* **60**: 3128-3137.
39. Zhu, D. 1994. M.S. Thesis. Michigan State University. East Lansing, Michigan.

APPENDICES

APPENDIX A

MAXIMUM SPECIFIC GROWTH RATE RESULTS

Table A-1: Data used to calculate $\mu_{\max}$ of PKC at pH 7.0

Time (hours)	PKC, pH 7.0					
	Replicate #1		Replicate #2		Replicate #3	
	O.D	u (days ⁻¹)	O.D	u (days ⁻¹)	O.D	u (days ⁻¹)
0	0.001	3.5176	0.002	2.5670	0	—
17.5	0.013	0.6621	0.013	0.6621	0.013	0.5281
49	0.031	-0.0593	0.031	0.2909	0.026	0.3604
76	0.029	0.1591	0.043	-0.2348	0.039	0.0500
100	0.034	0.0730	0.034	0.1661	0.041	0.0887
169.5	0.042	0.0221	0.055	-0.0176	0.053	0.0176
220	0.044	0.1396	0.053	-0.0396	0.055	0.0180
267.5	0.058		0.049		0.057	

Table A-2: Data used to calculate $\mu_{\max}$ of PKC at pH 7.5

Time (hours)	PKC, pH 7.5					
	Replicate #1		Replicate #2		Replicate #3	
	O.D	u (days ⁻¹)	O.D	u (days ⁻¹)	O.D	u (days ⁻¹)
0	-0.006	—	-0.008	—	0.005	2.296
15	0.018	4.349	0.014	6.613	0.021	0.372
18	0.031	0.443	0.032	0.000	0.022	2.090
23	0.034	1.776	0.032	0.224	0.034	2.097
29.5	0.055	0.988	0.034	2.466	0.060	1.250
39.5	0.083	0.353	0.095	-1.589	0.101	-1.538
45	0.090	-2.603	0.066	2.301	0.071	2.660
48	0.065	0.588	0.088	-0.377	0.099	0.091
63.5	0.095	0.083	0.069	0.361	0.105	-0.352
72.5	0.098		0.079		0.092	

Table A-3: Data used to calculate $\mu_{\max}$ of PKC at pH 8.0

Time (hours)	PKC, pH 8.0					
	Replicate #1		Replicate #2		Replicate #3	
	O.D	u (days ⁻¹)	O.D	u (days ⁻¹)	O.D	u (days ⁻¹)
0	0.001	2.639	0.001	2.996	0.002	2.251
24	0.014	0.932	0.020	0.234	0.019	1.121
29	0.017	0.373	0.021	0.500	0.024	0.393
84	0.040	0.231	0.066	0.049	0.059	-0.086
98.5	0.046	0.093	0.068	0.032	0.056	0.566
120	0.050	0.396	0.070	0.210	0.093	0.134
150	0.082	-0.110	0.091	0.053	0.110	0.055
169.5	0.075	0.156	0.095	0.103	0.115	0.104
190.5	0.086		0.104		0.126	

Table A-4: Data used to calculate $\mu_{\max}$ of PKC at pH 8.2

Time (hours)	PKC, pH 8.2					
	Replicate #1		Replicate #2		Replicate #3	
	O.D	u (days ⁻¹)	O.D	u (days ⁻¹)	O.D	u (days ⁻¹)
0	0	----	0.001	3.078	0.001	2.877
20	0.009	0.120	0.013	-0.300	0.011	0.498
41	0.010	0.662	0.010	0.392	0.017	0.172
77	0.027		0.018		0.022	

Table A-5: Data used to calculate $\mu_{\max}$ of Groundwater Flora at pH 7.0

Groundwater Flora, pH 7.0							
Time (hours)	Replicate #1		Time (hours)	Replicate #2 ^A		Replicate #3 ^A	
	O.D.	$\mu(\text{days}^{-1})$		O.D.	$\mu(\text{days}^{-1})$	O.D.	$\mu(\text{days}^{-1})$
0.0	0.001	0.853	3.0	-0.002	—	-0.001	—
19.5	0.002	—	22.5	0.002	-2.218	0.001	—
27.0	0.000	—	30.0	0.001	3.197	0.000	—
45.0	0.003	0.739	48.0	0.011	0.739	0.006	1.564
67.5	0.006	0.619	70.5	0.022	0.314	0.026	0.201
103.0	0.015	0.463	106.0	0.035	0.121	0.035	0.000
129.5	0.025	-0.292	132.5	0.040	0.000	0.035	0.127
140.0	0.022	0.118	143.0	0.040	0.000	0.037	-0.107
166.0	0.025	0.251	190.0	0.040	-0.105	0.030	0.236
211.0	0.040	0.362	214.0	0.036	0.133	0.038	-0.055
234.5	0.057	-0.029	237.5	0.041	-0.011	0.036	0.012
264.5	0.055	-0.079	290.5	0.040	0.062	0.037	0.380
287.5	0.051	-0.550	309.5	0.042	0.024	0.050	
306.5	0.033	0.141	333.5	0.043			
330.5	0.038						

Note A: Data recorded at different time scale 3.0, 22.5, 30, 48, 70.5, 106, 132.5, 143, 190, 214, 237.5, 290.5, 309.5, 333.5.

Table A-6: Data used to calculate $\mu_{\max}$ of Groundwater Flora at pH 7.5

Groundwater Flora, pH 7.5							
Time (hours)	Replicate #1		Replicate #3		Time (hours)	Replicate #2 ^A	
	O.D.	μ (days ⁻¹)	O.D.	μ (days ⁻¹)		O.D.	μ (days ⁻¹)
0.0	0.000	—	0.000	—	6.0	0.007	—
17.0	0.004	1.024	0.000	—	19.0	-0.002	0.000
26.5	0.006	—	0.002	2.931	22.0	-0.002	—
43.0	0.000	—	0.015	-3.353	27.0	0.003	—
47.5	0.013	0.209	0.008	0.630	33.5	-0.001	—
56.0	0.014	3.718	0.010	4.015	43.5	0.013	4.322
65.5	0.061	-3.467	0.049	2.331	49.0	0.035	-1.785
68.3	0.041	0.944	0.064	0.513	52.0	0.028	1.073
77.5	0.059	0.070	0.078	-0.027	67.5	0.056	1.353
89.0	0.061	0.266	0.077	0.025	76.5	0.093	0.100
113.5	0.080	-0.459	0.079	-0.551	121.0	0.112	0.064
122.0	0.068	-0.288	0.065	-0.185	137.5	0.117	-0.063
146.0	0.051	—	0.054	—	157.5	0.111	—

Note A: Data recorded at different time scale: 6,17,26.5,43,47.5,56 hours.

Table A-7: Data used to calculate $\mu_{\max}$ of Groundwater Flora at pH 8.0

Groundwater Flora, pH 8.0						
Time (hours)	Replicate #1 ^A		Replicate #2		Replicate #3	
	O.D.	μ (days ⁻¹)	O.D.	μ (days ⁻¹)	O.D.	μ (days ⁻¹)
0	-0.002	0.000	0.001	—	0	—
19	-0.002	-2.559	0	—	0.004	—
32	-0.001	—	0.001	2.944	0	—
56	0.013	-0.729	0.019	1.787	0.021	1.652
63	0.011	1.538	0.032	0.137	0.034	0.105
88.5	0.036	—	0.037	—	0.038	—

Note A: Data recorded at different time scale: 0, 17, 26.5, 43, 47, 56 hours.

Table A-8: Data used to calculate $\mu_{\max}$ of Groundwater Flora at pH 8.5

Groundwater Flora, pH 8.5							
Time	Replicate #1		Time	Replicate #2 ^A		Replicate #3 ^A	
(hours)	O.D.	μ (days ⁻¹)	(hours)	O.D.	μ (days ⁻¹)	O.D.	μ (days ⁻¹)
3.0	0.001	0.853	0.0	0.001	0.000	-0.001	—
22.5	0.002	—	19.0	0.001	1.344	0.001	1.040
30.0	0.000	—	51.0	0.006	1.677	0.004	1.180
48.0	0.004	0.666	67.5	0.019	0.255	0.009	0.556
106.0	0.020	0.241	116.5	0.032	0.212	0.028	0.141
143.0	0.029	-0.214	144.5	0.041	0.000	0.033	0.028
169.0	0.023	0.225	170.0	0.041	-0.014	0.034	0.063
190.0	0.028	-0.074	212.5	0.040		0.038	
214.0	0.026	-0.082					

Note A: Data recorded at different time scale: 0, 19, 51, 67.5, 116.5, 144.5, 170, 212.5 hours.

APPENDIX B

RESULTS FROM MIXED STRAIN COMPETITION EXPERIMENTS

Table B-1: Mixed-strain experiment, initial pH 7.5, KC starter medium: SGW medium, initially 150:1 (PKC:Schoolcraft)

Initially 150:1 (PKC:Schoolcraft)

148.89

Time (hrs)	PKC	Geo Mean (CFU/mL)	StdDev	Ground- water	Geo Mean (CFU/mL)	StdDev	
0	1.11E+07			1.25E+05			Rep. 1
0	1.07E+07	1.79E+07	2.25E+07	1.11E+05	1.20E+05	8.09E+03	Rep. 2
0	4.82E+07			1.25E+05			Rep. 3
25.5	1.22E+07			1.00E+05			Rep. 1
25.5	1.40E+07	1.21E+07	1.75E+06	2.00E+05	1.26E+05	5.84E+04	Rep. 2
25.5	1.05E+07			1.00E+05			Rep. 3
74	2.50E+07			5.00E+05			Rep. 1
74	2.30E+07	2.31E+07	1.85E+06	1.20E+06	8.14E+05	3.57E+05	Rep. 2
74	2.13E+07			9.00E+05			Rep. 3
95	1.35E+07			1.90E+06			Rep. 1
95	2.33E+07	1.40E+07	7.58E+06	1.10E+06	1.40E+06	4.19E+05	Rep. 2
95	8.70E+06			1.30E+06			Rep. 3
168	1.06E+07			1.36E+07			Rep. 1
168	5.60E+06	7.70E+06	2.53E+06	9.00E+05	3.40E+06	7.43E+06	Rep. 2
168	7.70E+06			3.20E+06			Rep. 3
217							Rep. 1
217	2.34E+06	3.32E+06	1.69E+06	7.50E+05	1.28E+06	1.06E+06	Rep. 2
217	4.70E+06			2.20E+06			Rep. 3

Table B-2: Mixed-strain experiment, initial pH 8.0, KC starter medium: SGW medium, initially 18:1 (PKC:Schoolcraft)

Initially 18:1 (PKC;Schoolcraft)

17.92

Time (hours)	PKC (CFU/mL)	Geo Mean	StdDev	Schoolcraft (CFU/mL)	Geo Mean	StdDev	
0	1.40E06			1.44E05			Rep.1
0	2.30E06	2.74E06	2.77E06	1.39E05	1.53E05	2.18E04	Rep.2
0	6.40E06			1.79E05			Rep.3
22.5	4.10E06						Rep.1
22.5	7.70E06	6.74E06	2.89E06		1.00E00	1.22E00	Rep.2
22.5	9.70E06						Rep.3
46	9.10E06			1.00E00			Rep.1
46	1.17E07	1.11E07	1.99E06	1.00E00	1.00E00	0.00E00	Rep.2
46	1.30E07			1.00E00			Rep.3
70	1.51E07			1.00E05			Rep.1
70	1.84E07	1.61E07	1.94E06	2.00E05	2.15E05	2.17E05	Rep.2
70	1.50E07			5.00E05			Rep.3
117	1.38E07						Rep.1
117	1.17E07	1.67E07	9.60E06	2.00E05	7.75E05	2.30E06	Rep.2
117	2.90E07			3.00E06			Rep.3
168	7.10E06			1.00E05			Rep.1
168	7.40E06	8.15E06	1.77E06	1.00E05	1.26E05	5.84E04	Rep.2
168	1.03E07			2.00E05			Rep.3
204	5.40E06			5.00E05			Rep.1
204	5.60E06	6.78E06	2.80E06	1.00E05	2.15E05	2.17E05	Rep.2
204	1.03E07			2.00E05			Rep.3

Table B-3: Mixed-strain experiment, initial pH 8.2, KC starter medium: SGW medium, initially 100:1 (PKC:Schoolcraft)

Initially 100:1 (PKC:Schoolcraft)

96.14

Time (hours)	PKC	Geo Mean (CFU/mL)	StdDev	Ground- water	Geo Mean (CFU/mL)	StdDev	
0	4.00E06			3.00E04			Rep. 1
0	1.00E06	2.52E06	1.83E06	3.00E04	2.62E04	5.80E03	Rep. 2
0	4.00E06			2.00E04			Rep. 3
22	2.00E08			1.00E00			Rep. 1
22	3.00E08	2.36E08	5.31E07	1.00E00	1.00E00	0.00E00	Rep. 2
22	2.20E08			1.00E00			Rep. 3
96	1.00E07			1.00E00			Rep. 1
96	1.40E07	1.25E07	2.32E06	1.00E00	1.00E00	0.00E00	Rep. 2
96	1.40E07			1.00E00			Rep. 3
120	1.70E07			1.00E06			Rep. 1
120	3.20E07	2.90E07	1.43E07	2.00E06	1.41E06	7.17E05	Rep. 2
120	4.50E07						Rep. 3
144	1.65E07			2.90E06			Rep. 1
144	1.12E07	1.44E07	3.02E06		2.90E06	2.33E-09	Rep. 2
144	1.63E07						Rep. 3

Table B-4: Mixed-strain experiment, initial pH 8.2, KC starter medium: SGW medium, initially 160:1 (PKC:Schoolcraft)

Initially 160:1 (PKC;Schoolcraft)

158.30

Time (hrs)	PKC (CFU/mL)	GeoMean (CFU/mL)	StdDev	Ground water (CFU/mL)	GeoMean (CFU/mL)	StdDev	
0	4.00E+06			3.00E+04			Rep.1
0	5.10E+06	4.15E+06	8.21E+05	3.00E+04	2.62E+04	5.80E+03	Rep.2
0	3.50E+06			2.00E+04			Rep.3
48	5.20E+07			6.00E+05			Rep.1
48	4.10E+07	4.86E+07	7.01E+06	6.00E+05	4.76E+05	1.76E+05	Rep.2
48	5.40E+07			3.00E+05			Rep.3
72	2.70E+07			0 @ 10 ⁻⁶			Rep.1
72	6.30E+07	4.51E+07	1.91E+07	0 @ 10 ⁻⁶	1.00E+00	1.00E+00	Rep.2
72	5.40E+07			0 @ 10 ⁻⁶			Rep.3
96	5.10E+07			2.00E+06			Rep.1
96	4.50E+07	4.86E+07	3.22E+06	1.00E+06	1.26E+06	5.84E+05	Rep.2
96	5.00E+07			1.00E+06			Rep.3
144	4.50E+07			0 @ 10 ⁻⁶			Rep.1
144	4.30E+07	4.53E+07	2.52E+06	1.00E+06	1.00E+06	6.59E-10	Rep.2
144	4.80E+07			1.00E+06			Rep.3
168	1.60E+07			1.00E+06			Rep.1
168	2.10E+07	1.82E+07	2.52E+06	0 @ 10 ⁻⁶	1.00E+06	1.00E+00	Rep.2
168	1.80E+07			0 @ 10 ⁻⁶			Rep.3

Table B-5: Mixed-strain experiment, initial pH 8.2, KC starter medium: SGW medium, initially 7.2:1 (PKC:Schoolcraft)

Initially 7.2:1 (PKC:Schoolcraft)

7.18

Time	PKC	GeoMean	StdDev	Ground- water	GeoMean	StdDev	
(hrs)	(CFU/mL)			(CFU/mL)			
0	2.60E+05			9.00E+04			Rep.1
0	2.99E+05	3.12E+05	6.69E+04	1.30E+04	4.34E+04	4.36E+04	Rep.2
0	3.90E+05			7.00E+04			Rep.3
22	1.62E+07			2.00E+05			Rep.1
22	2.25E+07	1.89E+07	3.19E+06		1.41E+05	7.17E+04	Rep.2
22	1.86E+07			1.00E+05			Rep.3
45	4.70E+07			1.20E+07			Rep.1
45	5.50E+07	4.95E+07	4.62E+06	2.00E+06	4.58E+06	5.57E+06	Rep.2
45	4.70E+07			4.00E+06			Rep.3
68	7.30E+07			2.00E+06			Rep.1
68	6.40E+07	6.62E+07	5.86E+06	4.00E+06	3.63E+06	2.05E+06	Rep.2
68	6.20E+07			6.00E+06			Rep.3
92	6.20E+07			2.90E+06			Rep.1
92	5.70E+07	6.06E+07	3.22E+06	3.00E+06	2.97E+06	5.77E+04	Rep.2
92	6.30E+07			3.00E+06			Rep.3
120	5.30E+07			5.00E+06			Rep.1
120	6.80E+07	5.93E+07	7.65E+06	1.00E+06	2.71E+06	2.22E+06	Rep.2
120	5.80E+07			4.00E+06			Rep.3

Table B-6: Mixed-strain experiment, initial pH 8.2, KC starter medium: SGW medium, washed inoculum, initially 11:1 (PKC:Schoolcraft)

Initially 11:1(PKC:Schoolcraft)

11.18689

Time (hrs)	PKC (CFU/mL)	GeoMean (CFU/mL)	StdDev	Ground- water (CFU/mL)	GeoMean (CFU/mL)	StdDev	
0	2.00E+05			3.00E+04			Rep.1
0	4.20E+05	2.93E+05	1.11E+05	3.00E+04	2.62E+04	5.80E+03	Rep.2
0	3.00E+05			2.00E+04			Rep.3
22	5.00E+03			3.60E+05			Rep.1
22	1.00E+03	2.24E+03	2.66E+03	6.00E+05	3.46E+05	2.11E+05	Rep.2
22				1.91E+05			Rep.3
51				3.10E+06			Rep.1
51	4.00E+05	4.00E+05	1.00E+00	4.00E+06	3.51E+06	4.52E+05	Rep.2
51				3.50E+06			Rep.3
72	1.00E+00			7.90E+06			Rep.1
72	1.00E+00	1.00E+00	1.00E+00	2.65E+07	9.66E+06	1.26E+07	Rep.2
72	1.00E+00			4.30E+06			Rep.3

Table B-7: Mixed-strain experiment, initial pH 8.2, KC starter medium: SGW medium, washed inoculum, initially 15:1 (PKC:Schoolcraft)

Initially 15:1(PKC:Schoolcraft)

15.04

Time (hours)	PKC (CFU/mL)	GeoMean (CFU/mL)	StdDev	Groundwater (CFU/mL)	GeoMean (CFU/mL)	StdDev	
0	3.60E+06			7.00E+05			Rep.1
0	4.40E+06	3.98E+06	5.66E+05	1.00E+05	2.65E+05	4.65E+05	Rep.2
22	4.50E+05			2.60E+05			Rep.1
22	3.30E+05	3.85E+05	8.51E+04	1.50E+05	1.97E+05	7.85E+04	Rep.2
51	4.00E+05			3.10E+06			Rep.1
51	1.00E+05	2.00E+05	2.24E+05	3.50E+06	3.29E+06	2.83E+05	Rep.2
72	3.00E+05			7.90E+06			Rep.1
72	1.10E+06	5.74E+05	5.93E+05	8.10E+06	8.00E+06	1.41E+05	Rep.2

**Table B-8: Mixed-strain experiment, initial pH 8.2, KC starter medium:
Schoolcraft medium (acclimated culture) , initially 32:1 (PKC:Schoolcraft)**

Initially 32:1 (PKC:Schoolcraft)

32.1

Time (hrs)	KC (CFU/mL)	GeoMean (CFU/mL)	StdDev	Ground- water (CFU/mL)	GeoMean (CFU/mL)	StdDev	
0	2.88E+05			3.00E+03			Rep.1
0	2.80E+05	3.10E+05	4.99E+04	3.00E+04	9.65E+03	1.51E+04	Rep.2
0	3.70E+05			1.00E+04			Rep.3
19	4.00E+03			4.20E+04			Rep.1
19		3.46E+03	7.09E+02	7.00E+04	5.31E+04	1.44E+04	Rep.2
19	3.00E+03			5.10E+04			Rep.3
42	1.00E+00			5.40E+05			Rep.1
42	1.00E+00	1.00E+00	1.00E+00	4.20E+05	4.90E+05	6.44E+04	Rep.2
42	1.00E+00			5.20E+05			Rep.3
66				3.90E+06			Rep.1
66	2.40E+04	1.55E+04	1.01E+04	1.41E+06	2.31E+06	1.29E+06	Rep.2
66	1.00E+04			2.25E+06			Rep.3
88				7.70E+06			Rep.1
88	5.00E+04	3.16E+04	2.17E+04	7.20E+06	7.22E+06	4.51E+05	Rep.2
88	2.00E+04			6.80E+06			Rep.3
109	1.00E+00			5.40E+06			Rep.1
109	1.00E+00	1.00E+00	1.00E+00	1.06E+07	8.82E+06	3.53E+06	Rep.2
109	1.00E+00			1.20E+07			Rep.3
170	1.00E+00			1.97E+07			Rep.1
170	1.00E+00	1.00E+00	1.00E+00	1.58E+07	1.46E+07	4.87E+06	Rep.2
170	1.00E+00			1.01E+07			Rep.3

**Table B-9: Mixed-strain experiment, initial pH 8.2, KC starter medium:
Schoolcraft medium (acclimated culture) , initially 308:1 (PKC:Schoolcraft)**

Initially 308:1(PKC:Schoolcraft)

308

Time (hours)	PKC GeoMean (CFU/mL)	StdDev	Ground- water GeoMean (CFU/mL)	StdDev	
0	2.70E+06		1.00E+04		Rep.1
0	3.60E+06	3.08E+06	1.00E+04	1.00E+04	Rep.2
0	3.00E+06		1.00E+04		Rep.3
19	1.00E+05				Rep.1
19		1.00E+05	1.00E+05	1.00E+05	Rep.2
19					Rep.3
42	1.00E+00		4.00E+05		Rep.1
42	1.00E+00	1.00E+00	2.00E+05	2.60E+05	Rep.2
42	1.00E+00		2.20E+05		Rep.3
66			1.70E+06		Rep.1
66		8.00E+04	3.80E+06	3.38E+06	Rep.2
66	8.00E+04		6.00E+06		Rep.3
88	1.20E+05		5.00E+06		Rep.1
88	8.00E+04	7.83E+04	6.70E+06	6.80E+06	Rep.2
88	5.00E+04		9.40E+06		Rep.3
109			1.09E+07		Rep.1
109	2.20E+05	2.20E+05	5.80E+06	8.29E+06	Rep.2
109			9.00E+06		Rep.3
170			1.43E+07		Rep.1
170	3.00E+05	1.73E+05	6.20E+06	7.47E+06	Rep.2
170	1.00E+05		4.70E+06		Rep.3

Table B-10: Mixed-strain experiment, initial pH 8.0, KC starter medium: Medium D, washed Inoculum, initially 3:1 (PKC:Schoolcraft)

Initially 3:1 (PKC:Aquifer Flora)

3.05

Time (hrs)	PKC (CFU/mL)	GeoMean (CFU/mL)	StdDev	Ground- water	GeoMean (CFU/mL)	StdDev	
0	1.60E+06			3.10E+05			Rep.1
0	6.00E+05	1.42E+06	1.26E+06	7.00E+05	4.66E+05	2.81E+05	Rep.2
0	3.00E+06						Rep.3
22	1.20E+05			1.80E+05			Rep.1
22	4.00E+04	8.32E+04	4.78E+04	3.20E+05	2.70E+05	8.81E+04	Rep.2
22	1.20E+05			3.40E+05			Rep.3
51	7.00E+04			7.00E+06			Rep.1
51	1.00E+04	3.66E+04	3.83E+04	4.20E+06	4.55E+06	1.99E+06	Rep.2
51	7.00E+04			3.20E+06			Rep.3
72	1.00E+05			1.04E+07			Rep.1
72	1.00E+04	3.16E+04	5.07E+04	7.60E+06	7.14E+06	2.94E+06	Rep.2
72				4.60E+06			Rep.3

Table B-11: Mixed-strain experiment, initial pH 8.0, KC starter medium: Medium D, washed inoculum, initially 10:1 (PKC:Schoolcraft)

Initially 10:1 (PKC:Schoolcraft)

9.6

Time (hrs)	PKC (CFU/mL)	GeoMean (CFU/mL)	StdDev	Ground- water	GeoMean (CFU/mL)	StdDev	
0							Rep.1
0	4.00E+05	9.59E+05	1.34E+06		1.00E+05	1.00E+00	Rep.2
0	2.30E+06			1.00E+05			Rep.3
72				4.50E+06			Rep.1
72		1.00E+00	1.00E+00	6.70E+06	4.63E+06	1.74E+06	Rep.2
72				3.30E+06			Rep.3
96				1.33E+07			Rep.1
96		1.00E+00	1.00E+00	7.10E+06	1.16E+07	4.86E+06	Rep.2
96				1.65E+07			Rep.3

Table B-12: Mixed-strain experiment, initial pH 8.0, KC starter medium: Medium D, washed inoculum, initially 12:1 (PKC:Schoolcraft)

Initially 12:1 (PKC:Groundwater)

12

Time (hrs)	PKC (CFU/mL)	GeoMean (CFU/mL)	StdDev	Ground- water (CFU/mL)	GeoMean (CFU/mL)	StdDev	
0				1.00E+04			Rep.1
0	2.00E+04	1.20E+05	1.00E+05	1.00E+04	1.00E+04	1.58E-11	Rep.2
0	1.20E+05			1.00E+04			Rep.3
72				2.37E+06			Rep.1
72		3.00E+04	1.00E+00	2.48E+06	2.88E+06	6.48E+05	Rep.2
72	3.00E+04			2.88E+06			Rep.3

Table B-13: Mixed-strain experiment, initial pH 7.5, KC starter medium: SGW medium, initially 8.5:1 (PKC:Schoolcraft)

Time (hrs)	Sample	Acetate	Nitrate	Phos.	Nitrite		(CFU/mL)	
							PKC	Aquifer
0	75-1	144.1	65.1	8.3	0.0		4.10E+07	3.00E+06
0	75-2	149.0	68.2	5.1	0.0		3.80E+07	4.00E+06
0	75-3	148.6	70.0	5.0	0.0		4.30E+07	9.00E+06
0	Avg	147.2	67.8	6.1	0.0	G.Mean	4.06E+07	4.76E+06
0	StdDv	2.721	2.48	1.877	0.0	StdDev	2.52E+06	3.29E+06
24	75-1	78.3	0.0	4.0	0.0		1.56E+07	1.60E+06
24	75-2	82.4	0.0	6.4	0.0		1.44E+07	8.00E+05
24	Avg	80.4	0.0	5.2	0.0	G.Mean	1.50E+07	1.13E+06
24	StdDv	2.899	0.0	1.697	0.0	StdDev	8.49E+05	5.74E+05
48	75-3	77.9	0.0	4.1	0.0		4.00E+07	2.00E+06
48	75-4	76.1	0.0	4.0	0.0		2.52E+07	2.00E+06
48	Avg	77.0	0.0	4.1	0.0	G.Mean	3.17E+07	2.00E+06
48	StdDv	1.273	0.0	0.071	0.0	StdDev	1.05E+07	9.88E-10
96	75-5	80.2	0.0	4.4	0.0		9.60E+06	0.00E+00
96	75-6	88.0	0.0	4.2	0.0		1.80E+07	1.20E+06
96	Avg	84.1	0.0	4.3	0.0	G.Mean	1.31E+07	1.20E+06
96	StdDv	5.515	0.0	0.141	0.0	StdDev	6.01E+06	2.33E-10
140	75-7	76.4	0.0	5.0	0.0		0.00E+00	0.00E+00
140	75-8	78.2	0.0	6.4	0.0		1.20E+06	0.00E+00
140	Avg	77.3	0.0	5.7	0.0	G.Mean	1.20E+06	0.00E+00
140	StdDv	1.273	0.0	0.990	0.0	StdDev	1.20E+06	0.00E+00

Table B-14: Mixed-strain experiment, initial pH 8.0, KC starter medium: SGW medium, initially 9.2:1 (PKC:Schoolcraft)

Time (hrs)	Sample	Acetate	Nitrate	Phos.	Nitrite		(CFU/mL)	
							PKC	Aquifer
0	80-1	152.3	66.7	3.8	0.0		3.40E+07	2.00E+06
0	80-2	149.9	66.2	4.4	0.0		4.10E+07	5.00E+06
0	80-3	150.7	65.9	4.0	0.0		2.80E+07	5.00E+06
0	Avg	151.0	66.3	4.1	0.0	G.Mean	3.39E+07	3.68E+06
0	StdDv	1.222	0.404	0.306	0.0	StdDv	6.53E+06	1.77E+06
24	80-1	104.1			10.2		2.36E+07	8.00E+05
24	80-2	102.9			13.8		1.40E+07	4.00E+05
24	Avg	103.5			12.0	G.Mean	1.82E+07	5.66E+05
24	StdDv	0.849			2.546	StdDv	6.85E+06	2.87E+05
48	80-3	80.2	0.0	4.9	0.0		1.84E+07	2.00E+06
48	80-4	88.0	0.0	4.0	0.0		2.90E+07	2.00E+06
48	Avg	84.1	0.0	4.5	0.0	G.Mean	2.31E+07	2.00E+06
48	StdDv	5.515	0.0	0.636	0.0	StdDv	7.54E+06	9.88E-10
96	80-5	85.1	0.0	3.7	0.0		3.40E+06	2.00E+05
96	80-6	81.5	0.0	5.1	0.0		3.36E+06	3.20E+05
96	Avg	83.3	0.0	4.4	0.0	G.Mean	3.38E+06	2.53E+05
96	StdDv	2.546	0.0	0.990	0.0	StdDv	2.83E+04	8.54E+04
140	80-7	84.6	0.0	6.4	0.0		1.00E+05	1.00E+05
140	80-8	80.9	0.0	6.3	0.0		2.00E+05	0.00E+00
140	Avg	82.8	0.0	6.4	0.0	G.Mean	1.41E+05	1.00E+05
140	StdDv	2.616	0.0	0.071	0.0	StdDv	7.17E+04	1.46E-11

Table B-15: Mixed-strain experiment, initial pH 8.2, KC starter medium: SGW medium, initially 6.8:1 (PKC:Schoolcraft)

Time (hrs)	Sample	Acetate	Nitrate	Phos.	Nitrite		(CFU/mL)	
							PKC	Aquifer
0	82-1	151.0	66.4	4.8	0.0		6.00E+07	7.00E+06
0	82-2	150.2	66.1	3.5	0.0		2.40E+07	2.80E+06
0	82-3	149.3	66.9	3.7	0.0		3.90E+07	9.00E+06
0	Avg	150.2	66.5	4.0	0.0	G.Mean	3.83E+07	5.61E+06
0	StdDv	0.850	0.404	0.700	0.0	StdDv	1.84E+07	3.27E+06
24	82-1	93.6	0.0	5.5	5.9		1.36E+07	4.00E+05
24	82-2	86.9	0.0	4.4	0.0		2.72E+07	8.00E+05
24	Avg	90.3	0.0	5.0	3.0	G.Mean	1.92E+07	5.66E+05
24	StdDv	4.738	0.0	0.778	4.17	StdDv	9.76E+06	2.87E+05
48	82-3	85.3	0.0	3.3	0.0		1.12E+07	1.00E+05
48	82-4	87.4	0.0	3.5	0.0		1.88E+07	3.20E+06
48	Avg	86.4	0.0	3.4	0.0	G.Mean	1.45E+07	5.66E+05
48	StdDv	1.485	0.0	0.141	0.0	StdDv	5.42E+06	2.68E+06
96	82-5	85.5	0.0	3.1	0.0		8.20E+06	1.20E+06
96	82-6	85.6	0.0	3.0	0.0		3.50E+06	2.00E+05
96	Avg	85.6	0.0	3.1	0.0	G.Mean	5.36E+06	4.90E+05
96	StdDv	0.071	0.0	0.071	0.0	StdDv	3.40E+06	7.67E+05
140	82-7	89.4	0.0	5.2	0.0		3.00E+05	0.00E+00
140	82-8	83.0	0.0	6.0	0.0		3.00E+05	1.00E+05
140	Avg	86.2	0.0	5.6	0.0	G.Mean	3.00E+05	1.00E+05
140	StdDv	4.525	0.0	0.566	0.0	StdDv	1.16E-10	1.00E+05

APPENDIX C

DATA RECORDED FROM CT TRANSFORMATION ASSAYS

Table C-1: Percent CT remaining, Schoolcraft acclimated culture, pH 8.2

Time (hours)	Control		308:1		32:1	
	Average	StdDev	Average	StdDev	Average	StdDev
0.0	100.0	12.5	100.0	9.2	100.0	10.8
12.0			90.4	9.6	94.1	6.4
21.5	66.8	7.6				
42.5			81.0	12.6	88.9	14.3
50.0	51.8	6.7				
65.5	51.5	5.3				
77.0			67.2	15.6	83.2	3.0
156.5	30.2	9.3				
171.5	26.7	11.8				

Table C-2: Percent CT remaining, SGW, pH 8.2

Time (hours)	Control		160:1	
	Average	StdDev	Average	StdDev
0.00	100.00	4.15	100.00	3.33
0.75	90.86	4.33	87.45	3.87
3.58	104.90	8.51	86.37	2.81
5.08	93.96	3.79	76.74	3.52
115.20			1.98	1.98
120.00	60.10	3.95		

Table C-3: Percent CT remaining, SGW, pH 8.0

Time (hours)	Control		18:1	
	Average	StdDev	Average	StdDev
0	100.00	5.89	100.00	5.98
21	93.56	4.92	6.43	6.19
46	83.79	4.20	3.62	4.54

APPENDIX D

DATA RECORDED FROM DISINFECTION EXPERIMENTS

Table D-1: Groundwater disinfection with hydrogen peroxide (CFU/mL)

Time (min)	No H ₂ O ₂	Geo Mean	Std Dev	Geo 1.5 % Mean	Std Dev	Geo 3 % Mean	Std Dev	Geo 0.1% Mean	Std Dev
0	354000 322000 273000	314539	40855	354000 322000 273000	314539 40855	354000 322000 273000	314539 40855	354000 322000 273000	314539 40855
30				1 1 1	1 0			26000 29000 24000	26254 2518
60	156000	156000		1 1	1	1 1	1 0	10800 7600 3400	10059 2457
120	203000	203000		1 1 1	1 0	1 1 1	1 0	6900 5900 1370	5173 1824
180	187000	187000		1 1	1 0	1 1	1 0	2370 2760	2077 725

Table D-2: Groundwater disinfection with bleach (CFU / mL)

Time (min)	No Bleach	Geo Mean	Std Dev	2 ppm	Geo Mean	Std Dev	10 ppm	Geo Mean	Std Dev
0	234000			234000			120000		
0	237000	230213	9075	237000	230213	9075	143000	118918	22569
0	220000			220000			98000		
5				256000			95000		
5				216000	227166	24352	98000	104337	14821
(6.5 for 50ppm)				212000			122000		
10									
10	196000	196000	1						
10									
15				210000			99000		
15				212000	207266	6430	89000	129064	88655
15				200000			244000		
20									
20	199000	199000	1						
20									
30							101000		
30	189000	189000	1		100000	1	78000	93573	14255
30							104000		
60							117000		
60	213000	213000	1		100000	1	54000	88308	34845
60							109000		

Table D-2 (cont)

Time (min)	100 ppm	Geo Mean	Std Dev	25 ppm	Geo Mean	Std Dev	50 ppm	Geo Mean	Std Dev
0	234000			148000			115000		
0	237000	230213	9075	146000	141817	8721	53600	83368	31621
0	220000			132000			94000		
5	6900			22900			9300		
5	5500	6003	758	23300	23099	23100	9900	10367	1476
(6.5 for 50ppm)		5700						12100	
10	5600								
10	5600	5463	231						
10	5200								
15	4800			19300			9600		
15	5700	4823	804	18700	18414	1027	16500	12819	3474
15	4100			17300			13300		
20	8100								
20	5800	5728	2076						
20	4000								
30	4100			24000			7600		
30	4200	4099	100	20800	21746	1909	11800	9135	2221
30	4000			20600			8500		
60				24900			11000		
60				22000	22996	1620	17200	12882	3513
60				22200			11300		

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