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The influence of moisture on microbial transport, survival and 2,4-D biodegradation with a genetically marked *Burkholderia cepacia* in unsaturated soil columns

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Abstract

The influence of moisture on the survival, movement and degradation activity of a 2,4-D degrading bacterium, *Burkholderia cepacia* strain BRI6001L, genetically engineered to contain bioluminescent and lactose utilization genes, was studied in unsaturated soil columns. The distance traveled by BRI6001L was dependent on the clay content of the soil, higher clay contents being responsible for higher filtration coefficients. Long term survival, in excess of one year, was attributed to strain BRI6001L's ability to survive dry conditions. Changes in the 2,4-D biodegradation rate showed a better correlation with the BRI6001L population density than with the total viable bacterial population. At moisture levels between field capacity and 40% moisture (−33 kPa to −100 kPa) 2,4-D degradation was attributed mainly to BRI6001L. At moisture levels between 6 and 15%, 2,4-D disappearance was attributed to the indigenous microbial population, with no degradation occurring at moisture levels below 6%. Returning the moisture to above 40% led to an increase of 4 orders of magnitude in the BRI6001L population density and to a 10-fold increase in the 2,4-D degradation rate. The ability to monitor a specific microbial population using reporter genes has demonstrated the importance of controlling moisture levels for maximizing biodegradation rates in unsaturated soil environments.

Introduction

The degree of water saturation is a major factor controlling microbial activity in, and the transport of microorganisms through, soil. Bacteria are transported over much greater distances in saturated soils, and the general lack of movement and limited survival of pathogens in soil is associated with unsaturated conditions (Bitton & Harvey 1992). Straining, which occurs within soil pores smaller than the limiting dimension of the cell, and adsorption onto particles control the movement of bacteria through soils. Bacteria are negatively charged biocolloids which interact with soil particles, and adsorption is favoured by the presence of cations, clay minerals, low concentrations of soluble organics and low pH (Bitton & Harvey 1992). An additional factor limiting colloidal movement in unsaturated soils is the presence of a gas-water interface, creating a zone of colloidal accumulation (Wan & Wilson 1994a, b). Rainfall, which lowers the ionic strength of the pore fluid, and preferential flow paths created by plant roots and soil invertebrates promote microbial transport by percolating water (Zyman & Sorber 1988; Duboise et al. 1976; Trevors 1990; Madsen & Alexander 1982). Bacterial sedimentation has also been shown to be a significant transport mechanism under hydrostatic conditions (Wan et al. 1995).

Moisture has also been shown to play a major selective role in determining whether bacteria or fungi are the dominant organisms in soil respiration (Griffin 1981). For effective biodegradation of PCP by *Phanaerochaete* spp. in contaminated soils, water potential was monitored daily and maintained near the soil water-holding capacity at approximately −50 kPa (Lamar & Dietrich 1990). On the other hand, optimal biodegradation of oily sludges required moisture con-

tents between 30% and 90% of the soil water-holding capacity (Dibble & Bartha 1979).

To better understand the influence of soil moisture on the transport, fate and biodegradation activity, a reporter gene marked bacterium was introduced into unsaturated non-sterile soil columns, the soils were periodically leached with simulated rainwater to simulate wet/dry cycles, and population levels of the marked bacterium were monitored for a period in excess of one year. The value of soil columns for assessing the transport and fate of microbial species has been well documented (Bitton et al. 1979; Huysman & Verstraete 1993a, b). Column studies have shown that soil drying prevented the release of sorbed microbes after rainwater application (Lance et al. 1976). The bacterium used in this study, *Burkholderia cepacia* strain BRI6001 was previously isolated by enrichment culture using 2,4-D as sole carbon source (Greer et al. 1990). Further characterization indicated that the genes encoding 2,4-D degradation were located on the chromosome and not on a plasmid (Beaumier et al. 1990). The bacterium was marked with a *lacZY-luxAB* reporter gene construct which was stably integrated into the chromosome resulting in the bioluminescent, lactose-utilizing strain, BRI6001L (Masson et al. 1993). Bacterial bioluminescence genes have been employed in a variety of applications (Meighen 1991), and the utility of strains marked with these genes, to evaluate their fate following release into the environment, has been demonstrated by several research groups (De Weger 1991; Shaw et al. 1992; Silcock et al. 1992; Blackburn et al. 1994).

Materials and methods

Soil and bacterial strain

The soil used was collected from the upper 6 inches of a forest soil having no known 2,4-D pre-exposure history. The soil was sieved (2 mm) without air drying and the organic matter content and density were measured as 15.5% (combustion at 600 °C according to a modified method of Nelson & Sommers (1982), and 2.39 g ml^{-1}, respectively. Granulometry results for the fractions 0–2, 2–53 and > 53 μm were 0.03, 0.29 and 0.68, respectively, indicated a sandy loam soil (Gee & Bauder 1986). For the control experiments, sterile soil was obtained by autoclaving at 121 °C for 30 minutes twice, the second time following a 3-day incubation at room temperature.

The organism used in this study was *Burkholderia* (*Pseudomonas*) *cepacia* BRI6001L, a 2,4-dichlorophenoxyacetic acid (2,4-D) biodegradative soil strain harboring a chromosomally integrated *lac-lux* reporter gene cassette (Masson et al. 1993). The soil inoculum was prepared by growing two 500 ml cultures of BRI6001L in minimal salts medium (MSM) containing 3 mM 2,4-D (2,4-D/MSM) with 5% (dry weight) of soil, at 30 °C and 200 rpm. Preparation of the inoculum in batch culture as a soil slurry, and use of the resulting soil as inoculum, was previously shown to enhance biodegradation rates and promote biomass activity (Otte et al. 1994). MSM was prepared as previously described (Greer et al. 1990) except that NaH_2PO_4 was used instead of KH_2PO_4 and the amount of phosphate in the 2,4-D solution was doubled. After 5 days, each culture was centrifuged at 4500 × *g* for 5 min, and the two soil pellets were gently washed in MSM, centrifuged and resuspended in 75 ml of either MSM or 2,4-D/MSM. BRI6001L cell counts for the soil pellet and liquid fractions, were determined by dilution and spread plating prior to inoculation. The soil pellet, which contained 99% of the BRI6001L viable cells (2×10^7 cfu g^{-1} soil) was used as the inoculum to the columns.

Microbial analysis

The spread plate technique was used to quantify cell populations in the soil phase. Soil samples of 0.5–1.0 g from each of the 3 sampling ports were transferred to a pre-weighed 50 ml culture tube containing approximately 2.5 g of glass beads (3 mm diameter). The weight of soil was determined and a volume of sterile 0.1% (w/v) tetrasodium pyrophosphate ($Na_4P_2O_710H_2O$) at pH 7, equivalent to 3 times the weight (g) of soil sample was added. The samples were kept on ice, then vortexed for 2 min, and serially diluted in sterile pyrophosphate solution. Aliquots of 0.1 ml were immediately spread plated in duplicate onto solid MSM medium containing 3 mM 2,4-D as sole carbon source and 8 mg l^{-1} X-Gal (5-bromo-4-chloro-indolyl-β-D-galacto-pyranoside) for the detection of BRI6001L as blue colonies. Starting on day 43, cycloheximide (50 mg l^{-1}) was added to the 2,4-D/X-Gal agar plates to prevent interfering fungal growth, which resulted in a small but appreciable increase in BRI6001L cell counts. Soil samples were also spread plated on MSM/YTS-250 agar plates (250 mg l^{-1} each of yeast extract, tryptone and soluble starch) for the determination of total viable cell counts. The plates

were incubated at 30 °C for 7 days before counting X-Gal positive blue colonies. Bioluminescence was determined by lifting the colonies with Biotrans nylon membranes (ICN, Irvine, CA), sealing the membranes in blotting bags in the presence of 100 μl of n-decanal (Sigma, D-7384), and exposing to Kodak X-AR2 film for 20 min at 30 °C. The use of these two reporter gene systems (*lac* and *lux*) facilitated the specific detection of the introduced strain, allowing a potential detection limit of 10 cfu g^{-1} soil. The *tfd*C gene probe (361 bp) was prepared by the polymerase chain reaction (PCR) using primers derived from the *tfd*C gene, encoding chlorocatechol dioxygenase from *Alcaligenes eutrophus* JMP134 (Perkins et al. 1990). The probe was labeled at its 5′ end with [^{32}P]dATP using T4 polynucleotide kinase as recommended (Pharmacia). Colony lifts and DNA hybridization were performed using standard protocols (Greer et al. 1993).

Chemical analysis

The soil samples were extracted with MSM for 2,4-D and chloride analyses. 2,4-D extraction from soil was performed by mixing 0.6 g samples with a volume of MSM (ml) equivalent to 5 times the weight (g) of the soil sample. Samples were vigorously agitated (30 min) on a Wrist Action Shaker (Burrell), filtered (0.45 μm pore size, Millex HV, Millipore), and 750 μl of filtrate were transferred into 1.8 ml vials containing 15 μl of 1 N H_2SO_4 for HPLC analysis using 280 nm as the absorbance maximum. The remaining filtrate (approx. 1.5 ml) was frozen at -20 °C for subsequent chloride analysis. Inorganic chloride was determined colorimetrically according to Bergmann & Sanik (1957).

Transport and fate in soil columns

The apparatus consisted of two 9 cm diameter glass columns provided with sampling ports at 2, 9 and 16 cm height. A 2 l batch of the sandy loam was mixed with 1 l of distilled water simulating rainwater (Duboise et al. 1976; Lance et al. 1976) and the resulting slurry was poured into each of the two columns. The base of the columns was filled with glass wool. The soil particles filled the column, while excess liquid was allowed to percolate through. A soil gradient was established with finer particles at the top and larger particles at the bottom of the columns. Media separation could be inferred from the differing moisture contents (A.I. values, Table 1) at field capacity (Foth 1990) indicating a clay loam (55–56% moisture) in the top (2 cm port) and a sandy loam (23–29% moisture) in the bottom (16 cm port) of the columns. Moisture contents at different matric potentials were obtained using a MA30 moisture analyzer (Sartorius AG, Göttingen, Germany). The water potential/soil moisture curve was established using a pressure membrane extractor (Soil Moisture Equipment Co, Santa Barbara, CA).

Microbial transport through unsaturated soil has been shown to follow the colloidal filtration theory (McDowell-Boyer et al. 1986):

$$dN/dL = -\lambda \times N$$

and after integration and logarithmic transformation:

$$log_{10}N = log_{10}N_0 - \lambda \times L$$

with N representing the number of bacteria at different depths (cfu g^{-1}), N_0 the number of bacteria at the surface, λ the filtration coefficient (cm^{-1}) and L (cm) the depth under the soil surface. The depth for a 90% reduction in the number of bacteria (D_{90} value) is equal to the inverse of the filtration coefficient ($D_{90} = \lambda^{-1}$) (Huysman & Verstraete 1993a). The inoculum slurries were uniformly distributed over the top of the columns. To avoid the effect of drying and accumulation of BRI6001L in the first one or two centimeters of soil, irrigation began immediately after inoculation and was continued for 2 hours at a flow rate of 60 ml h^{-1} with a peristaltic pump (LKB-Pharmacia, Microperpex Model 2132). The 2,4-D amended column received 3 mM 2,4-D in MSM (conductivity, κ = 9.1 mS cm^{-1}) in the irrigating solution while the unamended column ($-$2,4-D) received MSM only (κ = 8.70 mS cm^{-1}).

The columns were initially irrigated at monthly intervals with 400 ml of the appropriate solution sprinkled over the top, to simulate irrigation practices. Subsequent irrigation followed longer drying periods (3–4 months), as indicated in the figures. Both amended and non-amended columns also received distilled water (κ = 30 μS cm^{-1}) on day 430 to test the effect of low ionic strength irrigating solution on microbial transport and survival. It is known that reducing the ionic strength in the irrigating solution promotes the desorption and movement of pathogens through saturated soil (Lance et al. 1976). Soil samples were withdrawn aseptically from each port using a small stainless steel sampling rod fabricated in-house. Viable cell counts of BRI6001L were obtained by the spread plate technique. After each irrigation, soil samples from each

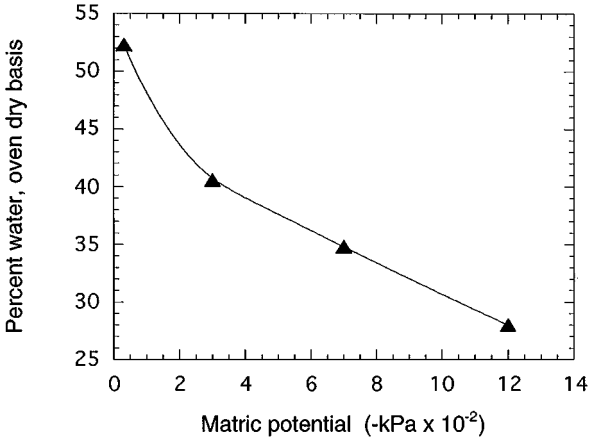

Figure 1. Water potential curve showing the relation between matric potential ψ_m and percent water for the surface layer (2 cm depth) of the amended soil column.

Table 1. Soil moisture fluctuations (wt%) in unamended and 2,4-D amended columns

Depth (cm)	(−2,4-D)			(+2,4-D)		
	A.I.[a]	B.I.[b]	P.I.[c]	A.I.[a]	B.I.[b]	P.I.[c]
2	55	52	42	56	39	26
9	43	39	46	45	38	38
16	23	16	16	29	20	24

Notes:
[a] A.I. = After irrigation, day 103.
[b] B.I. = Between irrigations, day 118.
[c] P.I. = Prior to irrigation, day 124.

port were taken at consecutive time intervals and monitored for moisture content.

Results

In unsaturated soil the matric potential ψ_m is the main contributor to the total water potential, whereas the gravitational potential plays a minor role in water movement (Foth 1990). A water potential curve versus soil moisture for the soil representative of the upper 2 cm of the 2,4-D amended soil column is shown in Figure 1. Moisture levels at field capacity were generally higher at the top than at the bottom of the columns (Table 1), where field capacity refers to the water content of an unsaturated soil measured at -30 kPa (-0.3 bars) soon after the removal of gravitational water (Foth 1990). The decreasing moisture contents at field capacity provided indirect evidence of the decreasing clay content of the soil with increasing depth. Water

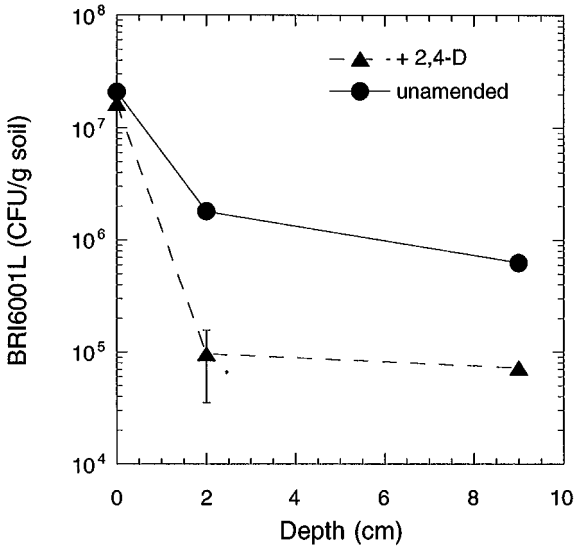

Figure 2. Population distribution of BRI6001L as a function of depth immediately following inoculum application and subsequent irrigation.

content measurements from the literature, which were performed on a variety of soils (Foth 1990), suggested that the top 2 cm of soil were indicative of a clay loam while the deeper layer was a sandy loam.

Inoculation of the surface of the columns with BRI6001L followed by immediate irrigation led to a decrease in BRI6001L bacterial counts with depth as shown in Figure 2. BRI6001L penetrated the column to the 9 cm depth in less than 2 hours after irrigating with 3 cm of liquid at a rate of 1 cm hr^{-1}. The logarithmic rate of decrease of cell counts with depth was not constant as predicted by the filtration theory. There was a large decrease in the population density of BRI6001L in the first 2 cm of the column, and very little decrease in cell counts between 2 and 9 cm. In addition, BRI6001L was not detected at the 16 cm depth.

BRI6001L and total viable bacterial population densities were monitored for 15 months in both the unamended and the 2,4-D amended soil columns (Figure 3). BRI6001L cell counts changed periodically as a function of irrigation frequency, with highs occurring immediately after irrigation. In addition, BRI6001L cell counts were seen to make up a greater portion of the total bacterial population upon addition of 2,4-D in the irrigating solution in the amended column. At the 16 cm depth in the unamended column (Figure 3C), the BRI6001L cell count in the soil phase was zero, despite finding BRI6001L in the outflow during irriga-

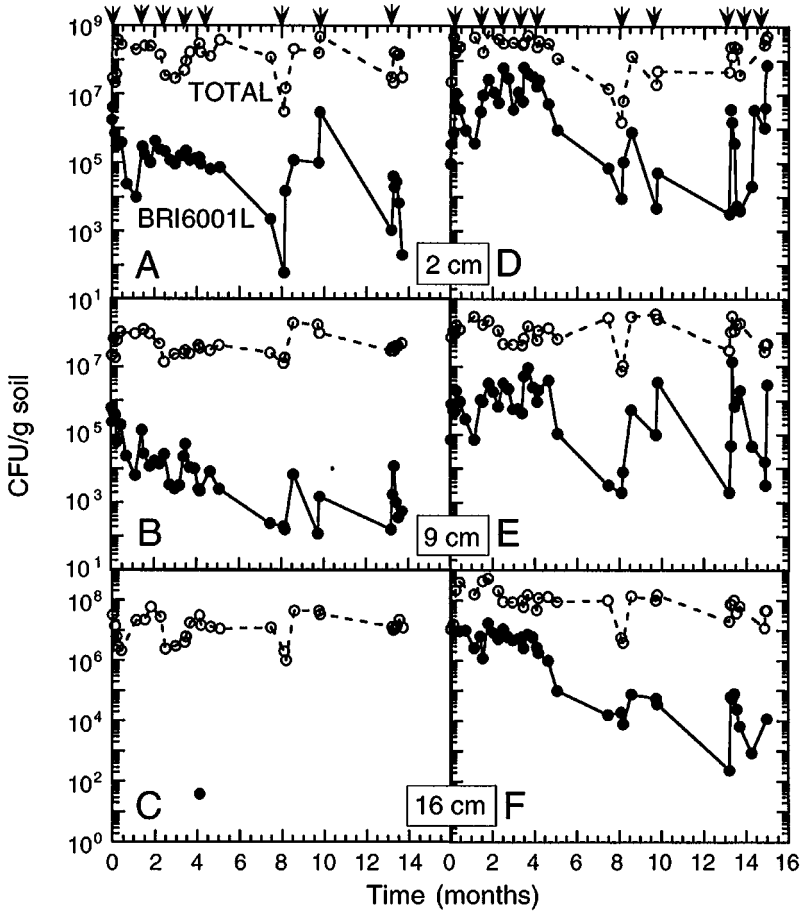

Figure 3. Bacterial population densities at three different depths (2, 9 and 16 cm) for unamended (graphs A–C) and 2,4-D amended (graphs D–E) soil columns. BRI6001L (filled circles); total bacterial population (open circles); and irrigation frequency (arrows at the top of graphs A and D).

tion at day 103 (3.2×10^2 cfu ml^{-1}). A single detection of BRI6001L in the soil at this depth was also found at day 124, however the magnitude (40 cfu g^{-1} soil) was probably too low to induce colonization (Comeau et al. 1993). The inability to find BRI6001L at the 16 cm depth was not due to detection deficiencies, since the advantage of bioluminescence over other detection methods lies in its excellent sensitivity. Compared to other methods such as gene probes with detection limits of the order of 10^3–10^4 cfu g^{-1} soil, the detection limit for bioluminescence is 10 cfu g^{-1} soil (Masson et al. 1993). In the amended column, the BRI6001L population was initially zero when, after the second week, the BRI6001L cell count increased to levels comparable with the rest of column (Figure 3F). Repetitive irrigation at monthly intervals produced a relatively constant BRI6001L population density. At the 2 cm depth in the non-amended column (Figure 3A), the BRI6001L population showed an initial decline from 1.8×10^6 to 1×10^4 cfu g^{-1} soil during the first month. Exposing the column to a 4 month dry cycle starting at month 4, which decreased the moisture levels to less than 5%, resulted in a significant drop in the BRI6001L population from 9.3×10^4 to 60 cfu g^{-1} soil. Subsequent irrigation showed a dramatic increase in the BRI6001L cell count to 1.2×10^5 cfu g^{-1} soil. For the 2,4-D amended column, the BRI6001L population showed similar patterns, except that the addition of a selectable substrate in the irrigating solution increased the BRI6001L population from 0.1 to 10 % of the total viable bacterial population (Figure 3D).

With time, BRI6001L colonies from the unamended column plated on selective medium were generally easier to identify than colonies from the 2,4-D amend-

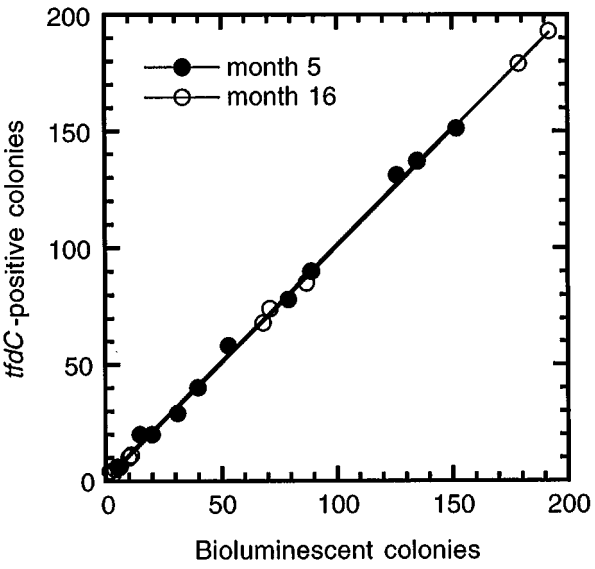

Figure 4. Linear relationship between *tfd*C probe-positive and bioluminescent colonies on solid 3 mM 2,4-D medium.

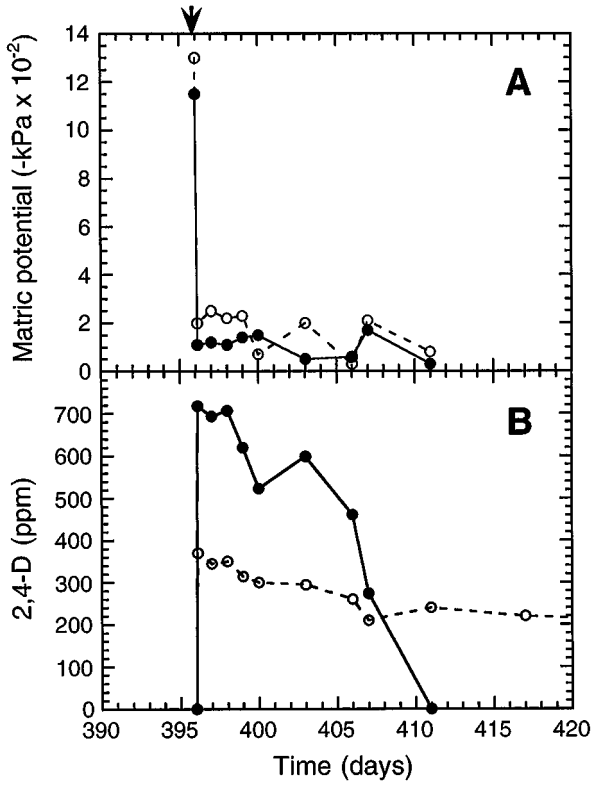

Figure 5. Relationship between matric potential (A) and 2,4-D disappearance (B) for the original soil used to pack the soil columns (filled circles) and for the sterile control soil (open circles).

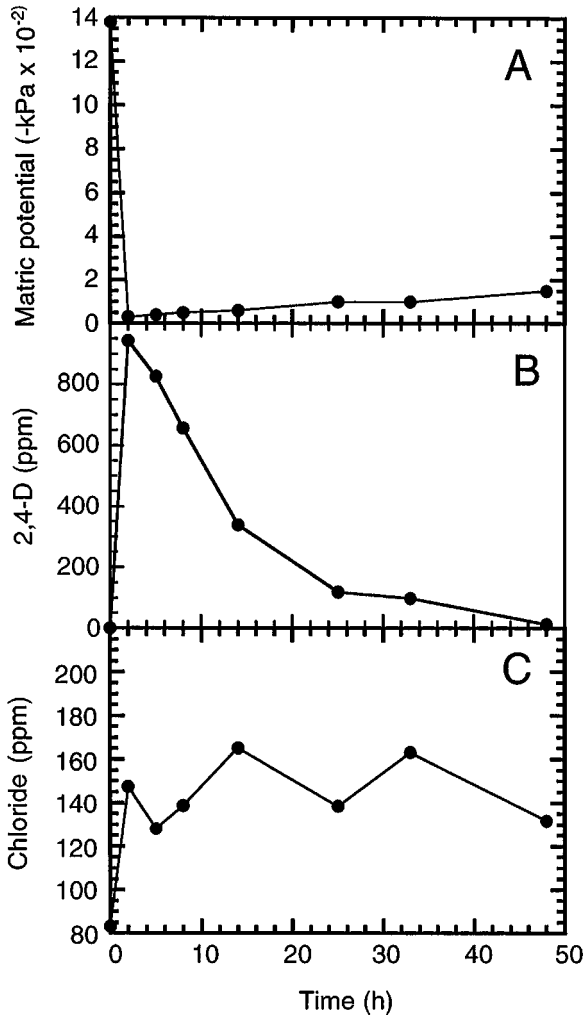

Figure 6. Effect of moisture on the degradation of 2,4-D in soil from the surface 2 cm of the 2,4-D amended column at day 124. Moisture expressed as matric potential (A); 2,4-D degradation (B); and chloride release (C).

ed column. Unlike during wet conditions, where positive identification of BRI6001L could be determined by counting blue and bioluminescent colonies on the same selective medium, during dry conditions positive identification of BRI6001L was only possible by bioluminescence detection. Fungal colonies, which were able to break down X-Gal and be identified as blue colonies on selective media, clearly dominated the microbial population when moisture was below 10%. Few BRI6001L colonies were detected under these conditions and none were detected at the surface of the unamended column. Bioluminescent colonies were also probed with a *tfd*C gene probe at two consecutive

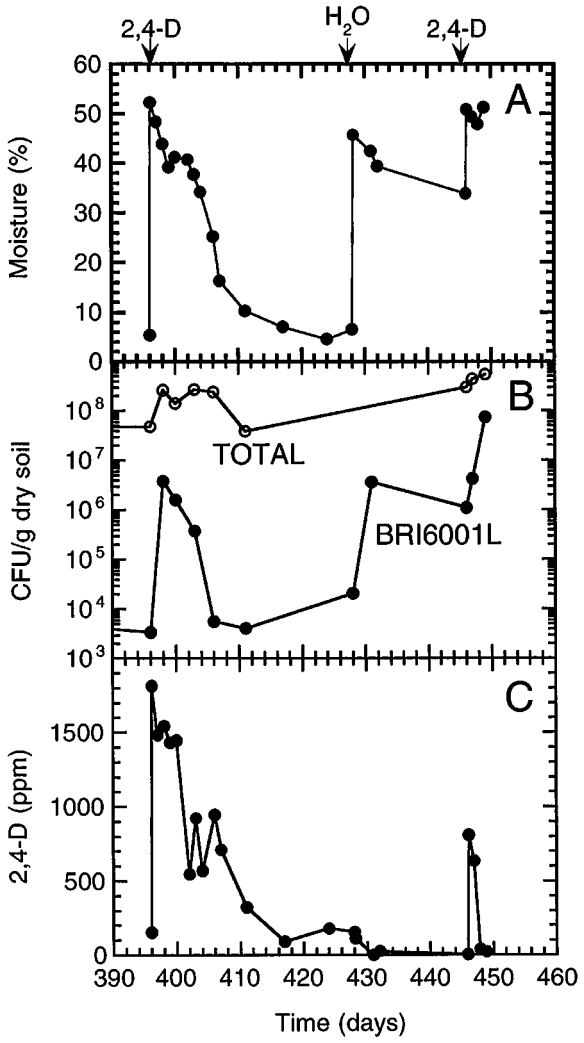

Figure 7. Effect of moisture on BRI6001L population density and 2,4-D degradation for the top 2 cm of soil of the 2,4-D amended column. Moisture (A), BRI6001L population density (B), and 2,4-D degradation (C).

time intervals, 5 and 16 months, as shown in Figure 4. The one to one relationship between *tfd*C and bioluminescent colonies demonstrated the stability of the chromosomally integrated construct.

The concentration of 2,4-D was measured to test the biodegradation potential at different penetration depths. The original, non-bioaugmented soil was used as a control and its moisture content was kept near field capacity, as shown in Figure 5. The presence of indigenous 2,4-D degraders in the non-bioaugmented soil was confirmed by adding 2,4-D and noting its disappearance at rates around 3 ppm hr^{-1}. The slow but detectable decrease in 2,4-D concentration from 360 ppm to 210 ppm in the sterile control soil may be due to sequestration, since 2,4-D measurements in soil involved mild extraction with MSM. This phenomenon has often been observed with a multitude of organic compounds after aging in a soil matrix (Alexander 1995).

The results presented in Figure 6 represent data corresponding to the 2 cm depth at day 124. A rapid decrease in 2,4-D concentration and an increase in chloride ion concentration was measured soon after irrigation. The liberation of chloride is a measure of 2,4-D degradation. The lack of further increase in chloride levels after approximately 10 hrs may be due to the effect of 2,4-D sorption, rendering the substrate less bioavailable.

The moisture content was varied and monitored for the upper 2 cm of soil in the 2,4-D amended column (Figure 7). As the moisture decreased from 52% to 40% (-10 to -300 kPa) the BRI6001L population decreased by one order of magnitude from 5×10^6 to 5×10^5 cfu g^{-1} soil. This moisture content was characterized by a 2,4-D disappearance rate of 20–30 ppm hr^{-1}, similar to the rate found at day 124, as reported in Figure 6. Moisture levels below 40% (<-300 kPa) resulted in a 6-day halt to 2,4-D degradation and in a 2-orders of magnitude decrease in the BRI6001L population from 5×10^5 to 5×10^3 cfu g^{-1} soil. Further reductions in moisture, to below 15%, resulted in a decrease of one order of magnitude in the 2,4-D degradation to 3 ppm hr^{-1}, similar to when 2,4-D was added to the control soil in the absence of BRI6001L, as previously shown in Figure 5. Since the control soil was maintained at field capacity, the 2,4-D degradation rates found here were similar to those in the BRI6001L amended soil at moisture contents below 15%, suggesting that the indigenous microbial population depended less on moisture content than BRI6001L. A moisture level below 6% abrogated 2,4-D degradation from day 417 to day 428, but subsequently increasing the moisture level to field capacity raised the 2,4-D degradation rate back to the 20–30 ppm hr^{-1} range.

Discussion

In this work the effect of moisture on bacterial transport, survival and ability to degrade 2,4-D was studied in repacked soil columns. In undisturbed soil columns there is significant water movement through macropores and soil cracks (van Elsas et al. 1991; Thomas

& Phillips 1979). Bacterial transport has been reported to be greater in undisturbed compared to repacked soil columns (Huysman & Verstraete 1993a). The use of repacked soil columns reduced the interference of irregularities, such as soil macropores in the soil structure, on the transport of bacteria in unsaturated soil. In addition, Verstraete's group indicated that microbial transport behaved according to a colloidal filtration theory model only when the irrigation solution was applied to the soil surface immediately after the inoculum. The success of this method was probably a result of minimizing the effect that drying time would have on microbial movement (Lance et al. 1976).

The logarithmic rate of decrease of cell counts with depth was not constant as predicted by the filtration theory. The top 2 cm of the column had a higher logarithmic cell drop with depth than the deeper 2–9 cm layer. This was probably due to media separation in the soil column. Because of the way the columns were prepared as a wet slurry, clay particles remained at the top and larger sand particles settled to the bottom of the columns, thus providing a diminishing clay gradient with increasing depth.

B. cepacia is a known hydrophilic bacterium (Wan & Wilson 1994; Koeppel et al., unpubl.). The filtration coefficient of hydrophilic bacteria is known to increase significantly with an increase in clay content of a sandy soil (Huysman & Verstraete 1993a). The higher adhesion of hydrophilic bacteria to clay is due to charge interactions between negatively charged bacteria and clay particles, and the presence of bivalent cations such as Ca^{2+} (Bitton & Harvey 1992). Our data shows higher drops in cell counts, hence higher filtration coefficients, for the first 2 cm depth followed by a more gradual drop at greater depths. The estimated value of the filtration coefficient for the first 2 cm (λ = 0.5–1 cm^{-1}, D_{90} = 1–2 cm) is comparable to values obtained by Verstraete for a hydrophilic bacterium transported through a sandy soil with 20% clay loam content (Huysman & Verstraete 1993a). The more gradual drop in cell counts between 2 cm and 9 cm gives rise to a lower filtration coefficient indicative of transport of hydrophilic bacteria through sandy soil. Air pockets, present in the unsaturated column during the initial microbial application, may have interfered with the transport of BRI6001L to the 16 cm depth. Gas-water interfaces are known to severely limit colloidal transport in unsaturated porous media (Wan & Wilson 1994a, b).

The initial lack of BRI6001L in the soil phase at the 16 cm depth for both 2,4-D amended and non-amended columns may have been due to an insufficient cell number to promote soil colonization (Comeau 1993). However, one week following inoculum application, bacterial sedimentation, known to play a role in bacterial transport under hydrostatic conditions (Wan et al. 1995), may have played a role in promoting colonization of the 2,4-D amended column at the 16 cm depth. In addition, the presence of a selectable substrate was instrumental in ensuring colonization at this greater depth, perhaps by facilitating metabolism, growth and attachment to soil particles. Unlike previous reports (Jacobsen & Pedersen 1992) the BRI6001L population in the non-amended soil never died off completely during the 15-month study. Raising the moisture content of the soil after a prolonged dry period always resulted in an increase in the BRI6001L population density.

Reducing the ionic strength of the irrigation solution, which is known to increase bacterial transport (Bitton & Harvey 1992; Lance et al. 1976) did not have significant effects. Irrigating with an equivalent volume of minimal salts medium or distilled water (300-fold lower ionic strength), produced similar cell counts in soil. This is in agreement with previous studies indicating that soil drying is the primary factor responsible for microbial desorption from soil, irrespective of changes in ionic strength of the eluting solution (Lance et al. 1976).

This study also shows that successful colonization, long-term survival and the ability to biodegrade pollutants is strongly related to environmental conditions, especially moisture. In the matric potential range between -100 to -1500 kPa, relative competitive advantage between bacterial and fungal species is of great significance. Griffin (1981) reported that as ψ_m declined to between -300 and -600 kPa, bacterial respiration fell rapidly and was negligible at -2000 kPa, whereas fungi were responsible for continued respiration between -800 and -3000 kPa. Similarly, this study showed that BRI6001L represented 10% of the total microbial population at moisture contents near field capacity while fungal species dominated in drier soil. In addition, rates of 2,4-D degradation declined rapidly as the matric potential fell below -300 kPa.

Conclusions

The use of a bioluminescently-marked bacterium in soil columns provided useful insight on several factors that could affect bioremediation in the field. Microbial transport was dependent on the clay content of the

soil, higher clay contents being responsible for higher filtration coefficients. Long term survival in excess of one year was related to strain BRI6001L's ability to survive dry conditions. The addition of 2,4-D affected BRI6001L's ability to colonize a non-sterile soil environment. The bacterium used, *Burkholderia cepacia*, could reach population densities in soil that suggested a strong competitive advantage relative to the indigenous microflora. The effect of moisture on the BRI6001L population density was more pronounced than it was on the indigenous microbial population. On the other hand, the ability of the indigenous population to biodegrade 2,4-D was similar at high and low moisture contents. This study indicated that proper control of water potential in unsaturated soils is necessary for achieving optimal 2,4-D biodegradation.

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